

# Current Trends in Quality Science – design, quality and safety of products



Ministerstwo  
Edukacji i Nauki

EDITOR: Inga Klimczak

Program:  
**DOSKONAŁA  
NAUKA**



**Łukasiewicz**

**Current trends in Quality Science  
– design, quality and safety  
of products**

**EDITOR: Inga KLIMCZAK**

**Institute of Quality Science**  
**Poznań University of Economics & Business**

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Editor: Inga Klimczak

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Lukasiewicz Research Network – Publishing House of Institute for Sustainable Technologies  
K. Pułaskiego Street 6/10, 26-600 Radom, phone: 48 364 42 41  
e-mail: [instytut@itee.lukasiewicz.gov.pl](mailto:instytut@itee.lukasiewicz.gov.pl) <http://www.itee.lukasiewicz.gov.pl>

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# Introduction

The global demand for safe and high quality food and non-food products is still growing. The control of quality and safety is an emerging issue requiring attention as one of sustainable development goals. Designing safe and sustainable products require a new approach not only to raw materials technology, but also what is extremely important to environment.

The Covid-19 pandemic has led to unprecedented challenge to public health and food systems, all over the world. Particular attention lately has been paid to personal health, including physical and emotional well-being. Consumers started to look for products, both food and non-food, tailored for their specific needs to achieve a higher quality of life.

The monograph: *Current Trends in Quality Science – Design, Quality and Safety of Products*, is a collection of 33 original and creative studies, covering three basic subject areas, in the field of Management and Quality Sciences. The first one is designing the quality of the product, concerning the influence of raw materials properties. The second group of studies focuses on monitoring and improving final products and packaging quality by selecting appropriate methods and techniques to ensure product safety and consumer protection. The last but not least group of studies covering area of food and non-food product design, selection of ingredients, materials and appropriate technology, following the rules of sustainable development.

*Inga Klimczak*

Part I

DESIGN,  
QUALITY  
AND SAFETY  
OF FOOD  
PRODUCTS

# 1

## HERBAL MEDICINE IN THE TREATMENT OF COVID-19

Alfred Błaszczuk<sup>1</sup>

### Abstract

Currently, the number of confirmed cases and deaths of COVID-19 worldwide continues to rise, receiving great concern from the international community. Laboratories around the world have been involved in the fight against the pandemic to develop vaccines and drugs. During the first COVID-19 pandemics, in the absence of drugs to prevent and treat COVID-19, China and South Korea widely used Traditional Chinese Medicine (TCM) recipes to treat infected patients. The benefits of TCM herbal formulations in the treatment of COVID-19 are mainly reflected in the following three aspects: relieving symptoms, delaying disease progression from mild and moderate to severe and critical, and reducing mortality in severe ill patients. There are a few TCM prescriptions for COVID-19, including Maxing Shigan (MXSGD), Xue Bijing (XBJ), Qingfei Paidu (QFPDD), Dayuan (DYD), Huashi Baidu (HSBD), Shufeng Jiedu (SFJD), Lianhua Qingwen (LHQW), Huoxiang Zhengqi (HXZQ), Jinhua Qinggan (JHQG), Xuan Feibaidu (XFBD) and Toujie Quwen Granules (TJQW). TCM herbal mixtures are also used in the prevention of viral infection and the recovery of patients after illness. This review highlights the latest advances of traditional Chinese medicine, focusing on the active compounds and potential mechanisms of herbal composition applied for the treatment of COVID-19.

**Keywords:** herbal recipes, SARS-Cov-19, TCM

<sup>1</sup> The Poznań University of Economics and Business, Institute of Quality Science, Department of Natural Science and Quality Assurance, Poland; [alfred.blaszczuk@ue.poznan.pl](mailto:alfred.blaszczuk@ue.poznan.pl)

## Introduction

The first cases of coronavirus infections were reported on December 1, 2019 in Wuhan, Hubei Province, China. Subsequently, the epidemic has spread throughout the country as well as in the rest of the world. It was soon later recognized that the mode of transmission of COVID-19 is through inhalation of droplets from sneezing and coughing, or physical contact with the mucus secretions of infected individuals. Due to the sudden increase in COVID-19 cases, most countries in the world have immediately introduced strict national guidelines for the prevention and control of the epidemic.

During the COVID-19 pandemic, in the absence of drugs to prevent and treat COVID-19, China and South Korea widely promoted the use of traditional Chinese medicine (TCM). Chinese central government formulated series of TCM diagnosis and treatment plans for COVID-19 and recommended a batch of Chinese herbal medicines for the prevention and treatment of COVID-19. A total of 166 modified herbal recipes consisting of 179 individual herbal medicines for the treatment of COVID-19 have been tested in China. TCM has accumulated rich experience in the long-term practice of epidemic prevention and treatment, and it is characterized by broad-spectrum immunity, universal adaptability, foresight etc. The unique advantages of TCM have attracted more and more attention to the epidemic prevention and treatment of COVID-19 (Yang, *et al.* 2020a). TCM has been utilized to effectively reduce symptoms of COVID-19 patients and inhibit disease progression from mild to severe, bringing remarkable clinical response. It has been shown that over 70,000 COVID-19 patients in China have received TCM treatment, with the total effective rate over 90 % (Huang, *et al.* 2020).

This review highlights the latest advances of traditional Chinese medicine, focusing on the active compounds and potential mechanisms, clinical application of herbal compositions applied for the treatment of COVID-19.

## Features of COVID-19

COVID-19 is caused by SARS-CoV-2, which has a diameter of 60–140 nm and obvious spike proteins ranging from 9 to 12 nm, giving the virion the appearance of a solar corona (Goldsmith, *et al.* 2004). According to clinical data, SARS-CoV-2 has several main features, such as a strong infectious potential, eliciting a weak immune response and inducing severe disease when complicated with comorbidities. SARS-Cov-2 is made of four structural proteins:

- S is spike protein responsible for interaction with the receptor on the cell surface,
- E (envelope) is the coat protein responsible for forming virions,

- M (membrane) is the main matrix protein of the virus,
- N is a nucleocapsid protein protecting a large RNA molecule and participating in the modification of cellular processes and viral replication.

The spike protein is responsible for binding to the host cell's membrane (Wu, *et al.* 2020). The S glycoprotein consists of S1 and S2 subunits. The S1 subunit mediates binding to the host cell's surface receptor, and the S2 subunit mediates fusion with its cell membrane and the virus then enters the cell via endocytosis (Lu, *et al.* 2020). Protein S has an appropriate affinity for the receptor protein of the angiotensin-converting enzyme 2 (ACE2), which is used as a mechanism for penetration into host cells (Xu, *et al.* 2020a). SARS-CoV-2 enters 293/hACE2 cells mainly by endocytosis. ACE2 is a receptor found in the tissues of the lungs and other organs of the human body. Therefore, coronavirus can multiply not only within the lungs, but also in intestinal epithelial cells, blood vessels and kidneys (Lamers, *et al.* 2020). The ACE2 protein is not the only receptor that the virus uses for cell fusion (Shang, *et al.* 2020). In addition to the ACE2 protein, an important factor in the pathogenesis of CoV-2 is the enzyme serine 2 (TMPRSS2), which along with ACE2, has been recognized by scientists as a key factor in the penetration into host cells. The TMPRSS2 protease activates the cell fusion process with the S protein of the SARS-CoV-2 virus. SARS-CoV-2 may also use the CD147 protein receptor as an additional route of invasion of host cells. CD147 receptors are found on olfactory and cerebral neurons, red blood cells, epithelial and endothelial cells, leukocytes, monocytes, lymphocytes, neutrophils and platelets. The lungs, kidneys, heart, brain, digestive system, skin and blood vessels are affected. There is also increased blood clotting in the body but in the brain, it is the source of mild to severe strokes.

The incubation period for COVID-19 is approximately 7–14 days, but may be 24 days in some cases. The clinical features of COVID-19 are fever, cough, and fatigue. However, a small number of patients had a stuffy nose, runny nose, sore throat, muscle aches and diarrhea. At worst, severe patients usually have shortness of breath and/or hypoxaemia after one week of infection, in which some cases may develop acute respiratory distress syndrome (ARDS), septic shock, refractory metabolic acidosis, bleeding and coagulation disorders, and multiple organ failure (HEMMATI, *et al.* 2020).

Currently, this disease is divided into five stages on the basis of different severity: mild stage, moderate stage, severe stage, critical stage and recovery stage (Table 1). In the mild stage SARS-Cov-2 replication is occurring in the trachea. Patients with mild symptoms are characterized by low-grade fever, dry cough and mild fatigue. In addition, some patients also exhibit runny nose, sore throat and diarrhea, which disappear spontaneously within 6–10 days. The mild stage concerns about 80% of infected patients. About 20% patients develop viral infection from trachea to lungs. In the moderate stage, replication occurs in the lungs and the immune



system, with the most common symptoms such as blood saturation below 95%. Virus binds with receptors such as ACE2 and TMPRSS2 and induces apoptosis response associated with vascular leakage (Dong, *et al.* 2020). It causes the first wave of local inflammation and recruits immune cells from the blood into the lungs, thereby eliminating extracellular viruses and destroying infected cells. In the severe stage, the increased proinflammatory cytokines accelerate the local inflammatory by increased release of proinflammatory cytokines in lungs such as IL-6, IL-1B, IP-10, IFN- $\gamma$ , MCP-1 etc (Ackermann, *et al.* 2020). When cytokine storm appears, the disease is rapidly developing into a severe stage manifested as acute respiratory distress syndrome (ARDS), acute lung injury, bleeding and coagulation dysfunction, multiple organ failure and septic shock and blood saturation below 90% (Perico, *et al.* 2021). It has been observed that, the levels of G-CSF, IP-10, MCP-1, MIP-1A and TNF- $\alpha$  in the serum of severe patients are higher than those of mild patients. In the last stadium (recovery stage) a decreased count of natural killer T cells is observed in patient's blood. Some patients still have symptoms such as cough, poor appetite, fatigue or abnormal mood, which demand more time for complete recovery (He, *et al.* 2020).

**Table 1. Clinical stages of COVID-19 with TMC herbal recipes**

Stage of illness	Symptoms	Symptoms in TCM	Reaction in organism	TMC herbal recipe
<b>Mild</b>	Low-grade fever, dry cough and mild fatigue. In addition: runny nose, sore throat and diarrhea	Cold-dump accumulation lung	appearance of replication in the trachea	JHQG LHQW QFPDD MXSGD DYD TJQW
<b>Moderate</b>	fever, dry cough SpO <sub>2</sub> < 95% Chest imaging – abnormal Lymphopenia Pulmonary edema	Cold-dampness blocking lung	appearance of replication in the lungs and the immune system	XFBD QFPDD MXSGD DYD TJQW
<b>Severe</b>	SpO <sub>2</sub> < 93% Tachypnea RR > 30 times/min PaO <sub>2</sub> /FiO <sub>2</sub> < 300 mmHg Chest imaging – significant progress of the lesion within 24–48 hours > 50%	Epidemic toxin closing lung	increased proinflammatory cytokines in lungs	HSBD QFPDD SFJD XBJ
<b>Critical</b>	Respiratory failure Mechanical ventilation required, Septic shock Multiorgan dysfunctions	Inner blocking causing collapse	cytokine storm	QFPDD SFJD XBJ

Source: based on (Wu, *et al.* 2021; An, *et al.* 2021).

## Traditional Chinese medicine in the treatment of COVID-19

Traditional Chinese medicine (TCM) is a treasure of ancient Chinese medicine with a history of over 2000 years. It is a natural medicine system that uses herbal medicine, acupuncture, gua sha massage, qi gong exercises and diet therapy. TCM can effectively treat many chronic diseases, such as hypertension, diabetes (Hao, *et al.* 2017) or H1N1 flu infection (Wang, *et al.* 2011). During the current COVID-19 pandemic, in the absence of drugs and to prevent and treat COVID-19, China and South Korea have widely promoted the use of TCM. The benefits of TCM in the treatment of COVID-19 are mainly reflected in the following three aspects: it is effective in relieving symptoms, slowing disease progression from mild and moderate to severe and critical, and reducing mortality in severe and critical patients. According to TCM theory COVID-19 belongs to a category of phytophthora blight (Li, *et al.* 2020a). However, there are different understandings of COVID-19 including damp-toxin epidemic, cold-damp epidemic, and damp-heat epidemic. TCM divides the disease into four stages: early stage, advanced stage, critical stage, and recovery stage. In the early stage, the common syndromes of COVID-19 are the syndrome of cold-dampness repressing the defensive qi of the lungs or of wind heat attacking the lungs, spleen and stomach. At this stage, the disease is characterized by cold-dampness, qi-stasis and qi-deficiency. In the advanced stage, the common syndromes are the syndrome of dampness blocking the lungs and stomach or pathogenic heat accumulating in the lungs. The main treatment is increasing clear qi and lowering adverse qi. Finally, the late stage characterized with symptoms of cold-dampness evils closing lung and injuring spleen. At this stage, the disease is located in lung, spleen, stomach, liver, kidney and heart. The treatment should allow the Yang to recover and open the orifices to induce resuscitation. The recovery stage is characterized with qi-deficiency of lung and spleen. Attention should be paid to qi and Yin in the later period. In addition, in the treatment of plague, both evil and positive are emphasized by TCM (Zhao, *et al.* 2021).

Generally, there are four TCM treatment principles:

- dissipating cold and dispelling dampness, ventilating lung and relieving superficialities are used to restore homeostasis and regulate immunity to prevent further evaluation of COVID-19 syndromes;
- clearing heat and resolving dampness, ventilating lung and detoxifying are applied to block virus replication and enhance immune function;
- clearing away heat and toxic substances are used to inhibit inflammation and cell differentiation, anti-apoptotic pathways;
- replenishing energy and increasing Yang-qi are applied to enhance immunity and being beneficial for recovery (Ren, *et al.* 2021).

## TCM herbal recipes in the treatment of COVID-19

Herbal recipes often combine different botanicals, sometimes containing even up to 50 species and thousands of chemical substances. However, only a part of them exhibit favorable pharmacokinetics (the absorption, distribution, metabolism, and excretion properties of a drug) with potential of a biological effect (Li, *et al.* 2012). Moreover, the therapeutic effects of these herbal products might arise from cooperate actions of the herbal ingredients.

There are a few TCM herbal recipes recommended from the Diagnosis and Treatment Protocol for COVID-19 of China. Among clinical tested are Maxing Shigan (MXSGD), Xue Bijing (XBJ), Qingfei Paidu (QFPDD), Dayuan (DYD), Huashi Baidu (HSBD), Shufeng Jiedu (SFJD), Lianhua Qingwen (LHQW), Huoxiang Zhengqi (HXZQ), Jinhua Qinggan (JHQG), Xuan Feibaidu (XFBD) and Toujie Quwen (TJQW) (Table 2). Among them, HXZQ is suggested for patients with fatigue and gastrointestinal discomfort. In contrast, JHQG, LHQW and SFJD are used for patients with fatigue and fever. In turn, QFPDD is used in the treatment of mild and severe coronavirus disease stages. MXSGD, DYD and TJQW are also recommended for patients with symptoms of early and advanced stage (Table 1 and 2). Based on the reported components of herbal Chinese recipes, several research groups have adopted the method of network pharmacology, molecular docking, and computer-aided drug design to provide data and clues for the multi-directional exploration of the material basis and pharmacodynamic mechanism of these herbal prescriptions in the treatment of COVID-19.

**Maxing Shigan** decoction (MXSGD) consists of 4 ingredients *Herba Ephedrae* (*Ephedra sinica* Stapf), *Semen Armeniacae Amarum* (*Prunus armeniaca* L.), *Gypsum fibrosum* and *Radix Glycyrrhizae* (*Glycyrrhiza glabra* L.). It has been shown that the main active compounds of this herbal mixture quercetin, kaempferol, isoramnetin, naringenin and wogonin, effectively inhibit SARS-CoV-2 replication and reduce the cytokine storm. It was found that MXSGD can control disease progress by regulating multiple targets, including AKT1, MAPK3, IL-6, TP53, TNF, CASP3, EGFR and MARK1.

After application of MXSGD the levels of IL-6, TNF, MAP-1 and CRP in serum are significantly reduced, suggesting a weakened inflammation (Zhang, *et al.* 2020). MXSGD is currently uses by clinicians to control radio-chemotherapy induced lung injury, asthma, viral pneumonia and influenza infection (Li, 2020).

**Xuebijing (XBJ)** as an injection is a commercialized product offered by Tianjin Hongri Pharmaceutical Co., Ltd. with national medicine approval Z20040033. It is used in managing severe cases effectively. This injection is suggested the use of intravenous infusion of 50 mL XBJ injection plus 100 mL saline to be administered within 30–40 min, twice per day. Its extract is from 5 components *Flos Carthami* (*Carthamus tinctorius* L.), *Radix Paeoniae Rubra* (*Paeonia lactiflora* Pallas

and *Paeonia veitchii* Lynch), *Chuanxiong Rhizome* (*Ligusticum chuanxiong* Hort), *Salvia Miltiorrhizae Radix et Rhizoma* (*Salvia miltiorrhiza* Bunge), *Radix Angelicae sinensis* (*Angelica sinensis*). It was used to treat infections caused by H7N9, H1N1, Ebola virus, MERS and dengue virus (Tong, *et al.* 2020). XBJ reduced levels of inflammatory mediators of IL-6 and TNF and alleviated lung injury (Ma, *et al.* 2015). It has been recommended by China's National Health Commission to treat severe and critical cases of COVID-19 with systematic inflammatory response syndrome and multi-organ failure (Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia, 2020). XBJ was predicted to treat SARS-CoV-2 infection via modulation of arachidonic acid metabolic pathway, which is used to synthesize inflammatory cytokines such as: TNF, IFN, MCP-1 etc. The molecular docking analyses have shown that chlorogenic acid, salvianolic acid B and hydroxysafflor yellow A present in XBJ have high affinity to SARS-CoV-2 S-protein (He, *et al.* 2020).

**Dayuan decoction (DYD)** is herbal mixture composed with 7 ingredients: *Semen Arecae* (*Arecae Pericarpium*), *Cortex Magnoliae* (*Magnoliae Officinalis*), *Fructus Tsaoko* (*Amomum Tsaoko*), *Rhizoma Anemarrhenae* (*Anemarrhenae asphodeloides*), *Radix Paeoniae* (*Paeoniae Alba*), *Radix Scutellariae* (*Scutellariae baicalensis*), *Radix Glycyrrhizae* (*Glycyrrhiza glabra* L.) in the mass ratio of 10:10:15:10:6:6:10:10:10 (Zhang, *et al.* 2020c). It is found that DYD can relieve symptoms of cough, dry throat and asthma, improve prognosis of COVID-19 patients, and shorten disease progression. In the treatment of COVID-19, DYD also decreases the severity of ARDS by acting on cytokine storm. By using molecular docking analysis, it has been found that the active compounds present in DYD (quercetin, naringenin, kaempferol and fomononetin) have a high affinity to cytokines (IL-6, IL-1 $\beta$ , CCL<sub>2</sub>) (Ruan, *et al.* 2020).

Next Chinese herbal prescription used in the treatment of COVID-19 is **Shufeng Jiedu (SFJD)** in form of capsules and it is composed of 8 botanicals including *Polygonum cuspidatum*, *Forsythia suspensa*, *Isatis indigotica*, *Bupleurum chinense*, *Patrinia scabiosifolia*, *Verbena officinalis*, *Phragmites communis* and *Glycyrrhiza glabra*. It was patented by the Chinese Food and Drug Administration in 2009. SFJD showed clinical effectiveness for the treatment of infectious diseases such as influenza A (H1N1) (Yuan, *et al.* 2018). It can effectively reduce the inflammation and immune-regulatory activity during lipopolysaccharide (LPS)-induced acute lung injury. SFJD increases the partial pressure of oxygen in the lung tissue, reduces the lactic acid level and inhibits IL-1 $\beta$ , and TNF- inflammatory factors. Apart from that these herbal capsules can regulate proteins and key inflammatory pathways, e.g. MAPK/NF- $\kappa$ B signaling pathway (Tao, *et al.* 2014). It has been shown in clinical evaluation that SFJD capsules combined with Arbidol have significantly decreased IL-6 levels and CRP (C-reactive protein present in blood as factor of inflammatory), suggesting that inflammatory response is weakened (Chen, *et al.* 2020). It has been found that quercetin, wogonin and polydatin could directly bind to the key protease of SARS-CoV-2 (Xia, *et al.* 2020).

**Table 2. Herbal recipes used by TCM for the treatment of COVID-19**

Name of recipes	Composition	Active substances	Pharmacological effects	Potential targets	Literature
<b>Maxing Shigan (MXSGD)</b>	<i>Herba Ephedrae</i> ( <i>Ephedra sinica</i> Stapf), <i>Semen Armeniacae Amarum</i> ( <i>Prunus armeniaca</i> L.), <i>Gypsum Fibrosum</i> and <i>Radix Glycyrrhizae</i> ( <i>Glycyrrhiza uralensis</i> Fisch)	quercetin, kaempferol, herbacetin, delphinidin, resivrit, estrone, stigmasterol, sitosterol, isotrifoliol, inflacoumarin A, kanzonol F	inhibiting SARS-CoV-2 replication, reducing cytokine storm	AKT1, MAPK3, IL-6, TP53, TNF, CASP3, EGFR, MAPK1	Zhang, et al. 2020a; Zhang, et al. 2020b; Wang, et al. 2020a
<b>Xuebijing (XBJ)</b>	<i>Flos Carthami</i> ( <i>Carthamus tinctorius</i> L.), <i>Radix Paeoniae Rubra</i> ( <i>Paeonia lactiflora</i> Pallas and <i>Paeonia veitchii</i> Lynch), <i>Chuanxiong Rhizome</i> ( <i>Ligusticum chuanxiong</i> Hort), <i>Salviae Miltiorrhizae Radix et Rhizoma</i> ( <i>Salvia miltiorrhiza</i> Bunge), <i>Angelicae sinensis Radix</i> ( <i>Angelica sinensis</i> )	chlorogenic acid, salvanolic acid B, hydroxysafflor yellow A	high affinity to SARS-CoV-2 S-protein	TNF, IFN, MCP-1	Ren, et al. 2020
<b>Dayuan (DYD)</b>	<i>Semen Arecae</i> ( <i>Arecae Pericarpium</i> ), <i>Cortex Magnoliae</i> ( <i>Magnoliae Officinalis</i> ), <i>Fructus Tsaoko</i> ( <i>Amomum Tsaoko</i> ), <i>Rhizoma Anemarrhenae</i> ( <i>Anemarrhenae asphodelioides</i> ), <i>Radix Paeoniae</i> ( <i>Paeoniae Alba</i> ), <i>Radix Scutellariae</i> ( <i>Scutellariae baicalensis</i> ), <i>Radix Glycyrrhizae</i> ( <i>Glycyrrhiza glabra</i> L.)	kaempferol, quercetin, 7-methoxy-2-methyl isoflavone, naringenin, formononetin	anti-inflammatory, antiviral, immunomodulatory	L6, MAPK3, MAPK8, CASP3, IL10, IL1B, CXCL8, MAPK1, CCL2, IFNG, IL4	Li, et al. 2020b; Zong, et al. 2020
<b>Shufeng Jiedu (SFJD)</b>	<i>Polygonum cuspidatum</i> , <i>Forsythia suspensa</i> , <i>Isatis indigotica</i> , <i>Bupleurum chinense</i> , <i>Patrinia scabiosifolia</i> , <i>Verbena officinalis</i> , <i>Phragmites communis</i> , <i>Glycyrrhiza glabra</i>	quercetin, wogonin, polydatin	anti-inflammatory, antiSARS-CoV-2	MAPK14, TNF, IL-6, IL-10, PTGS2	Cao, et al. 2020; Xiao, et al. 2020; Xia, et al. 2021

<p><b>Huoxiang Zhengqi (HXZQ)</b></p>	<p><i>Pericarpium Arecae, Radix Angelicae, Dahurica, Perillae, Poria, Rhizoma, Pinelliae, Rhizoma Atractylodis, Macrocephalae, Pericarpium Citri Reticulatae, Cortex Magnoliae Officinalis, Radix Platycodonis, Herba Pogostemonis, Radix Glycyrrhizae</i></p>	<p>quercetin, isornamentin</p>	<p>anti-inflammatory, antiSARS-CoV-2</p>	<p>IL-6, IL-1<math>\beta</math>, TNF, IL-10, PTGS2, AR</p>	<p>Deng, <i>et al.</i> 2020; Yang, <i>et al.</i> 2020b</p>
<p><b>Jinhua Qinggan (JHQG)</b></p>	<p><i>Arctii Fructus, Artemisiae Annuae Herba, Menthae Haplocalycis Herba, Forsythia suspensa, Lonicerae Japonicae Flos, Armeniacae Semen Amarum, Gypsum fibrosum, Glycyrrhizae Radix et Rhizoma, Scutellariae Radix, Ephedrae Herba, Fritillariae Thunbergii Bulbus, Anemarrhenae Rhizoma</i></p>	<p>kaempferol, oroxilin A, baicalein</p>	<p>anti-inflammatory, antiSARS-CoV-2</p>	<p>IL-6, IL-1<math>\beta</math>, CXCL8, CCL-2, IL-2, IL-4, ICAM1, IL 10, IL-1</p>	<p>Lin, <i>et al.</i> 2020d; Duan, <i>et al.</i> 2020</p>
<p><b>Lianhua Qingwen (LHQW)</b></p>	<p><i>Forsythia suspensa (Thunb.) Vahl., Lonicera japonica Thunb., Ephedra sinica Stapf, Armeniacae Amarum Semen, Gypsum Fibrosum, Isatis tinctoria L., Dryopteridis Crassirhizomatis Rhizoma, Houltuyenia cordata Thunb., Pogostemon cablin Benth., Rheum palmatum L., Rhodiola rosea Linn., Mentha haplocalyx Briq., Glycyrrhiza uralensis Fisch</i></p>	<p>amygdalin, prunasin, neochlorogenic acid, rutin, forsythoside A</p>	<p>anti-SARS-CoV-2, anti-inflammatory</p>	<p>IL-6, TNF, MAPK1, IL-1<math>\beta</math>, MIAPK8</p>	<p>Chen &amp; Li, 2020; Wang, <i>et al.</i> 2020b</p>

<p><b>Xuanfeibaidu (XFBD)</b></p>	<p>Herba Ephedrae (Ephedra sinica Stapf), Armeniaca Semen Amarum, raw Gypsum, Semen Coici, Rhizoma Atractylodes (Atractylodes lancea Thunb. DC), Herba Patchouli (Pogostemon cablin Blanco) Benth.), Herba Artemisiae (Artemisia annua L.), Rhizoma Polygonum cospidatum (Reynoutria japonica), Herba Verbena (Verbena officinalis), Radix Coughgrass (Phragmites australis), Semen Lepidium (Lepidium sativum L.), pummelo peel (Citrus grandis L.), Radix Glycyrrhizae (Glycyrrhiza glabra L.)</p>	<p>quarctetin, glabridin, gallic acid</p>	<p><i>inhibits viral infection and replication mainly by binding to ACE2 and 3CL<sup>pro</sup> of SARS- CoV-2</i></p>	<p>IL-6, TNF</p>	<p>Niu, et al. 2020</p>
<p><b>Huashi Baidu (HSBD)</b></p>	<p>Ephedra Herba, Amygdalus Communis Vas, Gypsum Fibrosum, Ilicice, Pogostemon Cablin Benth., Magnolia Offcinalis Rehd Et Wils, Atractylodes Lancea (Thunb.) Dc., Amomum Tsao-Ko Crevostet, Pinelliae rhizoma preparata, Poria Cocos (Schw.) Wolf., Radix Rhei Et Rhizome, Hedyсарum Multijugum Maxim., Lepidii Semen Descurainiae Semen, Radix Paeoniae Rubra</p>	<p>luteolin, quercetin, baicalein, kaempferol</p>	<p>anti-inflammatory, inhibiting SARS-CoV-2 replication</p>	<p>IL-6, MAPK8, MAPK1, IL 1B</p>	<p>Lai, et al. 2020; Yang, 2020</p>
<p><b>Toujie Quwen (TIQW)</b></p>	<p>Lonicerae Japonicae Flos, Pseudostellariae Radix, Artemisia Annua L, Peucedani Radix, Forsythiae Fructus, Scutellariae Radix, Hedysarum Multijugum Maxim, Isatidis Folium, Radix Bupleuri, Fritillariae Irriosae Bulbus, Ciadae Periostracum, Poria Cocos Wolf, Pseudobulbus Cremastrae Seu Pleiones, Mume Fructus, Figwort Root, Fritillariae Thunbrgii Bulbus</p>	<p>quercetin, rutin, kaempferol, luteolin, isoquercitin, isorhamnetin</p>	<p>anti-inflammatory, antiSARS-CoV-2, improving immune</p>	<p>PTGS2, IL-6, TNF</p>	<p>Ma, et al. 2020; Ye, et al. 2020; Fu, et al. 2020</p>

<p><b>Qingfei Paidu (QFPDD)</b></p>	<p><i>Herba Ephedrae</i> (Ephedra sinica Stapf),  <i>Radix Glycyrrhizae</i> (Glycyrrhiza uralensis Fisch.), <i>Semen Armeniacae Amarum</i> (Prunus armeniaca L.),  <i>Raw Gypsum</i>, <i>Ramulus Cinnamomi</i> (<i>Cinnamomum cassia</i> (L.) J. Presl), <i>Rhizoma Alismatis</i> (<i>Alisma plantago-aquatica</i> Linn.), <i>Polyporus Umbellatus</i> (<i>Polyporus umbellaru</i> (Pers.) Fr.), <i>Rhizoma Atractylodis Macrocephalae</i> (<i>Atractylodes macrocephala</i> Koidz.),  <i>Portia</i> (<i>Portia cocos</i> (Schw.) Wolf.), <i>Radix Bupleuri</i> (<i>Bupleurum chinensis</i> DC.), <i>Radix Scutellariae</i> (<i>Scutellaria baicalensis</i> Georgi),  <i>Rhizome Pinelliae Preparata</i> (<i>Pinellia ternata</i> (Thunb.) Breit.), <i>Rhizoma Zingiberis Recens</i> (<i>Zingiber officinale</i> Roscoe),  <i>Radix Asteris</i> (<i>Aster tataricus</i> Linn.),  <i>Flos Farfarae</i> (<i>Tussilago farfara</i> Linn.),  <i>Rhizoma Belamcandae</i> (<i>Iris domestica</i> (L.) Goldblatt &amp; Mabb.), <i>Herba Asari</i> (<i>Asarum sieboldii</i> Miq.), <i>Rhizoma Dioscoreae</i> (<i>Dioscorea oppositifolia</i> L.),  <i>Fructus Aurantii Immaturus</i> (<i>Citrus sinensis</i> Osbeck), <i>Pericarpium Citri Reticulatae</i> (<i>Citrus aurantium</i> L.), <i>Herba Pogostemonis</i> (<i>Pogostemon cablin</i> (Blanco) Benth)</p>	<p>quercetin, kaempferol, naringenin, isorhamnetin</p>	<p>anti-inflammatory, protecting lung injury, inhibiting virus adsorption and replication</p>	<p>AKT1, MAPK1, MAPK14, IL-6, TNF, CASP3, DPP4</p>	<p>Tan, Zou &amp; Zou, 2020; Xu, He &amp; Yang, 2020; Wang, et al. 2020d</p>
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Source: based on (An, et al. 2021; Huang, et al. 202; Ren, et al. 2021).



**Huoxinag Zhengqi (HXZQ)** is a Chinese herbal prescription consisting of 11 herbs, including *Semen Arecae* (*Arecae Pericarpium*, 120 g), *Radix Angelicae* (*Angelicae Dahuricae*, 120 g), *Poria* (*Poria cocos* (Schw.) Wolf., 120 g), *Rhizoma Atractylodis Macrocephalae* (*Atractylodes macrocephala* Koidz., 80 g), *Cortex Magnoliae* (*Magnoliae Officinalis*, 80 g), *Pericarpium Citri Reticulatae* (*Citrus aurantium* L., 80 g), *Rhizome Pinelliae* (*Pinellia ternata* (Thunb., 80 g), *Radix Platycodonis* (*Platycodon grandiflorum*, 80 g), *Herba Pogostemonis* (*Pogostemon cablin* Blanco, oli 0.8 ml), *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch., extract 10 g), *Perilla frutescens* L. Britt (oil 0.4 ml) (Liu, *et al.* 2014). It comes in many dosage forms such as: pills, capsules, boluses and liquids. HXZQ is manufactured by Taiji Group Chongqing Fuling Pharmaceutical Co. (Chongqing, China) following the approved Good Manufacturing Practice of the China's State Food and Drug Administration, according to criteria of Chinese pharmacopeia 2010. It is applied to relieve the abdominal distention and pain, vomiting and diarrhea induced by exogenous *wind-cold* and endogenous damp stagnation. HXZQ liquid has therapeutic effects on a variety of virus infections such as H5N1, Norovirus, Rotavirus, etc. HXZQ may have therapeutic effects for patients infected with COVID-19 because it decreases levels of various pro-inflammatory cytokines such as IL-6, IL-1 $\beta$ , IL-2 and TNF- $\alpha$ . It's also responsible for increasing level of IL-10 as well as regulating NF-kB pathways (Huo, *et al.* 2020).

Another Chinese herbal prescription used in treatment of COVID-19 patients is **Jinhua Qinggan (JHQG)**. It is a commercial product sold by Juxiechang Pharmaceutical Co. Ltd., (Beijing) with national medicine approval Z20160001. Its suggested use is one dose per day, boiled with water. The treatment course includes six doses. This multi-herbal granules are composed of 12 ingredients, including *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch.), *Herba Ephedrae* (*Ephedra sinica* Stapf), *Radix Scutellariae* (*Scutellariae baicalensis*), *Gypsum fibrosum*, *Fructus Forsythiae* (*Forsythia suspense*), *Herba Menthae* (*Menthae Haplocalycis*), *Flos Lonicerae* (*Lonicerae Japonicae*), *Semen Armeniacae Amarum* (*Prunus armeniaca* L.), *Fructus Arctii* (*Arctium lappa* L.), *Herba Artemisiae* (*Artemisiae annuae* L.), *Bulbus Fritillariae* (*Fritillariae Thunbergii*), *Rhizoma Anemarrhena* (*Anemarrhena asphodeloides*). JHQG was formulated for treating H1N1 influenza and in clinical study showed that JHQG markedly reduced duration of fever and alleviated symptoms (Qi, Qi & Wang, 2016). Recently appeared that it significantly alleviated clinical symptoms of mild COVID-19 patients, such as fever, fatigue, cough, and expectoration, and can relieve anxiety of the patients (Duan, *et al.* 2020). It was reported that the active ingredients of JHQG, 3-methoxy-glycerol, crude-glycerin and glycyrrhizin B, have strong binding activity for 3CLpro and ACE2 by network pharmacology and high throughput molecular docking analyses (Shen, *et al.* 2020). The core active compounds present in JHQG have strong affinity for 3CLpro, S protein, ACE2, and SOCS1, thereby inhibiting viral replication

and binding to target cells, reducing host inflammation and activating antiviral immunity (Ren, *et al.* 2020).

**Lianhua Qingwen (LHQW)** capsules are a commercial product sold by Shijiazhuang Yiling Pharmaceutical Co. with national medicine approval Z20040063. It is suggested to use four pills three times per day, oral administration for 6 days. The herbal capsules are a TCM prescription composed of 13 ingredients: *Gypsum fibrosum*, *Fructus Forsythia* (*Forsythia suspense*), *Semen Armeniacae Amarum* (*Prunus armeniaca* L.), *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch.), *Herba Menthae* (*Menthae Haplocalycis*), *Flos Lonicerae* (*Lonicerae Japonicae*), *Herba Ephedrae* (*Ephedra sinica* Stapf), *Rhizoma Dryopteridis* (*Dryopteridis Crassirhizomatis*), *Radix Isatis* (*Isatis tinctoria* L.), *Herba Houttuynia* (*Houttuynia cordata* Thunb.), *Herba Patchouli* (*Pogostemon patchouli*), *Rheum palmatum* L., *Radix Rhodiola* (*Rhodiola rosea* Linn.). LHQW capsules were patented in 2003 in China. It has been proven to have broad spectrum antiviral effects on a number of influenza viruses with immune regulatory effects (Ding, *et al.* 2017). It was found that LHQW decreased viral replication, inflammation and lung lesions in the case of mice with influenza A virus infection (Gao, *et al.* 2020). In addition, LHQW also blocked the early stages of viral infection, suppressed virus-induced NF- $\kappa$ B activation and alleviated virus-induced gene expression of IL-6, IL-8, TNF- $\alpha$ , IP-10, and MCP-1 in a dose-dependent manner (Ding, *et al.* 2017). It has been reported that LHQW can also significantly inhibit the SARS-Cov-2 replication and reduce generation of proinflammatory cytokines such as IL-6, TNF- $\alpha$ , CCL-2/MCP-1 and CXCL-10/IP-10 (Li, *et al.* 2020c). Clinical research of 42 mild cases with COVID-19 has shown that LHQW has positive effects on improving symptoms of fever, sputum, cough and polypnea by effectively decreasing 1.5 days of fever reduction time and proportion turning to severe cases (Lv, Wang & Li, 2020). It has been proven that neochlorogenic acid, amygdalin, prunasin, forsythoside I, rutin, forsythoside A, glycyrrhizin and rhein from LHQW have the most potential targets for treating COVID-19 because they exhibit good binding affinity to ACE2 (Chen, *et al.* 2020).

**Huashi Baidu (HSBD)** is multi-herbs Chinese prescription consisting of 14 ingredients, including *Herba Ephedra* (*Ephedra sinica* Stapf, 6 g), *Amygdalus Communis Vas* (9 g), *Gypsum fibrosum* (15 g), *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch., 3 g), *Herba Pogostemonis* (*Pogostemon cablin* (Blanco) Benth., 10 g), *Cortex Magnoliae* (*Magnoliae Officinalis*, 10 g), *Rhizoma Atractylodis Lancea* (*Atractylodes Lancea* Thunb., 15 g), *Amomum Tsao-Ko Crevostet* (10 g), *Rhizoma preparate Pinelliae* (*Pinellia ternate* (Thunb.) Breit, 9 g), *Poria* (*Poria cocos* (Schw.) Wolf., 15 g), *Radix & Rhizome Rhei* (*Rheum officinale*, 5 g), *Hedysarum Multijugum Maxim.* (10 g), *Semen Lepidii* (*Descurainia sophia* L., 10 g), *Radix Paeoniae* (*Paeoniae Rubra*, 10 g). Suggested use: 1–2 doses per day, boiled with 100–200 mL water, two or four times per day. HSBD has been

specifically formulated for treating COVID-19, and not previously used to treat other viral infections (Pan, *et al.* 2020). It has been used for the treatment of mild, moderate and severe stages. It has been shown that HSBD can effectively relieve symptoms of severe COVID-19 and reduce the conversion rate of mild/moderate to moderate/severe stage and decrease mortality rate of critical illness (Pan, *et al.* 2020). Additionally, HSBD can decrease the average length of hospitalization and significantly improve clinical symptoms and pulmonary CT findings. The molecular docking and network pharmacology analyses has shown that quercetin, kaempferol and luteolin present in HSBD have strong binding activities for 3CL hydrolase, S protein and ACE2, associated to key targets such as IL-6, MAPK8, MAPK1 and IL-1 $\beta$  (Lai, *et al.* 2020a).

**Toujie Quwen (TJQW)** is composed of 16 ingredients, including *Flos Lonicerae* (*Lonicerae Japonicae*), *Radix Pseudostellariae* (*Pseudostellariae heterophylla*), *Herba Artemisiae* (*Artemisiae annuae* L.), *Radix Peucedani* (*Peucedanum praeruptorum* dunn), *Fructus Forsythia* (*Forsythia suspense*), *Radix Scutellariae* (*Scutellariae baicalensis*), *Hedysarum Multijugum Maxim*, *Folium Isatis* (*Isatis tinctoria* L.), *Radix Bupleuri* (*Bupleurum chinense* DC & *Bupleurum scorzonerifolium* Willd.), *Bulbus Fritillariae Cirrhosae*, *Cicadae Periostracum*, *Poria* (*Poria cocos* (Schw.) Wolf.), *Pseudobulbus Cremastrae Seu Pleiones*, *Fructus Mume* (*Prunus mume* Siebold&Zucc), *Root Figwort* (*Scrophularia ningpoensis*), *Bulbus Fritillariae* (*Fritillariae Thunbergii*). This prescription has function of clearing lung-heat and detoxification, dissipating phlegm and resolving masses. It has been shown that TJQW can have therapeutic effects on COVID-19 by regulating viral infection, immune and inflammation related targets and pathways. The molecular docking results have shown that some active compounds such as quercetin and isoquercitrin present in TJQW have high affinity to SARS-CoV-2 S-protein and rutin and astragaloside IV can bind with ACE2 rather than with S-protein (Ye, *et al.* 2020). Whereas in the clinical investigation, TJQW prescription in combination with Arbidol (antiviral drug) improved symptoms of patients infected with SARS-CoV-2 with a total effective rate of 89.2% (Fu, *et al.* 2020).

**Qingfei Paidu (QFPDD)** is composed out of 21 ingredients, including *Radix Glycyrrhizae* (*Glycyrrhiza uralensis* Fisch., 6 g), *Herba Ephedrae* (*Ephedra sinica* Stapf, 9 g), *Semen Armeniacae Amarum* (*Prunus armeniaca* L., 9 g), *Rhizoma Alismatis* (*Alisma plantago-aquatica* Linn., 9 g), *Raw Gypsum* (15–30 g), *Ramulus Cinnamomi* (*Cinnamomum cassia* L., 9 g), *Polyporus Umbellatus* (*Polyporus umbellaru* (Pers.) Fr., 9 g), *Rhizoma Atractylodis Macrocephalae* (*Atractylodes macrocephala* Koidz., 9 g), *Radix Bupleuri* (*Bupleurum chinensis* DC., 16 g), *Poria* (*Poria cocos* (Schw.) Wolf., 15 g), *Radix Scutellariae* (*Scutellaria baicalensis* Georgi, 6 g), *Rhizome Pinelliae Preparata* (*Pinellia ternata* (Thunb.) Breit., 9 g, processed with ginger), *Radix Asteris* (*Aster tataricus* Linn. f., 9 g), *Flos Farfarae* (*Tussilago farfara* Linn., 9 g), *Rhizoma Zingiberis Recens* (*Zingiber*

*officinale* Roscoe, 9 g), *Rhizoma Belamcandae* (*Iris domestica* L., 9 g), *Herba Asari* (*Asarum sieboldii* Miq., 6 g), *Fructus Aurantii Immaturus* (*Citrus sinensis* Osbeck, 6 g), *Rhizoma Dioscoreae* (*Dioscorea oppositifolia* L., 12 g), *Herba Pogostemonis* (*Pogostemon cablin* (Blanco) Benth., 9 g) and *Pericarpium Citri Reticulatae* (*Citrus aurantium* L., 6 g). Its suggested use is: 1 dose per day, boiled with water, twice per day in the morning and evening. Three doses are a course of treatment. QFPDD is a combination of several classical Chinese prescriptions for the treatment of exogenous febrile disease caused by pathogenic cold, including MXSGD (removing lung fever), Chaihu (regulating the stomach), Shegan Mahauang (relieving cough and asthma) and Wuling (warming Yang and activating Qi). This prescription has the functions of dispelling cold and dampness, eliminating heat and turbidity, promoting and nourishing lung and spleen, detoxifying and removing pathogenic factors, etc. Therefore, this formula is suitable for the pathogenesis of COVID-19, affecting cold, dryness, damp toxin and dampness, and can effectively improve the symptoms. QFPDD was selected and recommended by the National Administration of TCM as a general prescription for treating different stages of COVID-19. It has been reported that QFPDD had an inhibitory effect on cytokine storm in the treatment of COVID-19 by reducing excessive immune response and eliminating inflammation by targeting to AKT1, MAPK1, MAPK14, IL-6 and TNF (Zhou, *et al.* 2020). The molecular docking analysis showed that active substances (quercetin, luteolin, kaempferol, isorhamnetin, naringenin and  $\beta$ -sitosterol) present in QFPDD have a good affinity with 2019-nCoV 3C-like protease and ACE2 to form complexes with stable conformations and high binding energy, indicating that QFPDD might treat COVID-19 through RAS signaling pathway (Yan, *et al.* 2020). In clinical study, till February 2020 QFPDD has been used to treat 701 confirmed patients in 57 hospitals. As a result, 130 cases were cured with asymptomatic, 51 cases with disappeared symptoms, 268 cases with improved symptoms, 212 cases with stable symptoms, which reached to the effective rate of 94.29% (Lai, *et al.* 2020b). This herbal prescription is also called **Lung Cleansing and Detoxifying Decoction (LCDD)** and is recommended for the treatment of severe and non-severe patients.

**Xuanfeibaidu (XFBD)** granulates are recommended for treating moderate stage of COVID-19. It consists 13 ingredients including *Herba Ephedrae* (*Ephedra sinica* Stapf, 6 g), *Armeniacae Semen Amarum* (15 g), *raw Gypsum* (30 g), *Semen Coicis* (30 g), *Rhizoma Atractylodis* (*Atractylodes lancea* Thunb. DC, 10 g), *Herba Patchouli* (*Pogostemon cablin* (Blanco) Benth., 15 g), *Herba Artemisiae* (*Artemisia annua* L., 12 g), *Rhizoma Polygonum cospidatum* (*Reynoutria japonica*, 20 g), *Herba Verbena* (*Verbena officinalis*, 30 g), *Radix Couchgrass* (*Phragmites australis*, 30 g), *Semen Lepidium* (*Lepidium sativum* L., 15 g), *pummelo peel* (*Citrus grandis* L., 15 g), *Radix Glycyrrhizae* (*Glyzyrrhiza glabra* L., 10 g). Its suggested use: 1 dose per day, boiled with 400 ml water, twice a day at morning and evening. This herbal prescription is a new recipe specifically formulated for the treatment of COVID-19.

Among the ingredients *Rhizoma Polygonum cospidatum* has the strongest anti-coronavirus effect. Apart from that *Herba Verbena* has a strong activity on lung damage caused by coronavirus (Zhang, 2020). It has been found, that XFBD significantly reduce fever, cough, fatigue and other symptoms in mild and moderate stages of COVID-19 (Pan, *et al.* 2020). According to molecular docking analyses and network pharmacology XFBD inhibits viral infection and replication mainly by binding to ACE2 and 3CL<sup>pro</sup> of SARS-CoV-2 with active substances present in the recipe (Wang, *et al.* 2020c).

**Table 3. The most frequently used herbal plants applied for treatment of Covid-19**

Name of herbal plant	Part of plant	Therapeutic properties	Active substances
<i>Glycyrrhiza glabra</i> L./ <i>Glycyrrhiza uralensis</i> Fisch	Radix & Rhizome	Qi-tonic	glycyrrhetic acid, glycyrrhizin, licoricidin, glabridin, glycyrrhiza polysaccharides, glutathione, isoangustone A, dehydroglyasperin C, licochalcone A, B, C, D, E, liquiritigenin
<i>Scutellariae baicalensis</i>	Radix	Heat-clearing	baicalein, phenylethanoid glycosides, iridoid glycosides, diterpenes, triterpenoids, alkaloids, phytosterols, polysaccharides
<i>Prunus armeniaca</i> L.	Semen	Expectorant & cough-suppressing	amygdalin
<i>Lonicerae Japonicae</i>	Flos	Heat-clearing	chlorogenic acid, flavonoids, iridoids, organic acids, saponins, chlorogenic acid, luteolin, loganin, lonicerin A, hexadecanoic acid, octadecadienoic acid, ethyl palmitate, dihydrocarveol
<i>Forsythia suspense</i>	Fructus	Heat-clearing	forsythiaside, forsythin, rutin
<i>Ephedra sinica</i> Stapf	Herba	Exterior-relieving	ephedrine, pseudoephedrine, N-methylephedrine, N-methylpseudoephedrine, norephedrine, norpseudoephedrine
<i>Poria cocos</i> (Schw.) Wolf.	Herba	Dampness-resolving	pachymic acid, triterpenes, polysaccharides
<i>Pogostemon cablin</i> (Blanco) Benth.	Herba	Dampness-resolving	patchouli alcohol
<i>Citrus aurantium</i> L.	Folium & Cortex	Qi-regulating	hesperidin, alkaloids, flavonoids, essential oils
<i>Platycodon grandiflorum</i>	Radix	Expectorant & cough-suppressing	platycodin D, platycodonate, polygalacic acid, flavonoids, polysaccharides, sterols, phenols, polyacetylenes, fatty acids, essential oils

Source: based on (Luo, *et al.* 2020).

Luo, *et al.* (2020) have found 10 most frequently applied botanicals in 166 herbal prescriptions collected in China against COVID-19 among them Glycyrrhizae Radix et Rhizome, Scutellariae Radix, Armeniacae Semen Amarum, Lonicerae Japonicae Flos, Forsythiae Fructus, Ephedrae Herba, Poria, Pogostemon Cablin, Citri Reticulatae Pericarpium and Platycodonis Radix (Table 3).

*Glycyrrhiza glabra* L., root and rhizome, raw or honeyed is the most often herb in the prescriptions against COVID-19. In TCM it is used for the treatment of cough and influenza virus. Several reports showed that the active compounds (glycyrrhizin, 18  $\beta$ -glycyrrhetic acid) present in this herb have anti-inflammatory activities (Ramalingam, *et al.* 2018). It is claimed that this herb decreases adverse effects of Ephedra sinica Stapf., which is effective for allaying asthma and for inducing diaphoresis and diuresis (Wei, *et al.* 2016). The major active compounds present in Ephedrae Herba enhance the release of norepinephrine from sympathetic neurons. *Scutellariae baicalensis radix* has broad therapeutic effects including anti-inflammatory and antioxidative effects which are related to cytokine inhibition and growth factor production in macrophages (Lee, *et al.* 2000; Yoon, *et al.* 2009). *Prunus armeniaca* L. (fried fruits) is used as an antiasthmatic, a mucolytic, an expectorant, and a laxative agent. The herb has been used to reduce fever, relieve cough and quench thirst. Amygdalin is the major compound metabolized to produce hydrocyanic acid in human body, which could inhibit the respiratory center in the brain to render smoother breathing, thereby gradually reducing cough and asthma (Shi & Liu, 2018).

## Conclusions

At present, there is good evidence that TCM herbal recipes can improve the symptoms of patients with COVID-19 at different stages, delay the progression of the disease, and reduce the mortality rate. All TCM herbal recipes are complex system acting on multi-targets at multi-pathways. Some of TCM prescriptions have been shown to be more effective in treating patients with COVID-19. To the most often used and clinical tested herbal recipes belong Maxing Shigan (MXSGD), Xue Bijing (XBJ), Qingfei Paidu (QFPDD), Dayuan (DYD), Huashi Baidu (HSBD), Shufeng Jiedu (SFJD), Lianhua Qingwen (LHQW), Huoxiang Zhengqi (HXZQ), Jinhua Qinggan (JHQG), Xuan Feibaidu (XFBD) and Toujie Quwen (TJQW). Based on the network pharmacology and molecular docking analyses it has been found that active compounds present in the Chinese recipes may exert therapeutic effects on COVID-19 via targeting of ACE2, 3CLpro and IL-6. However, there is a lack of extended research with larger number of clinical research samples.



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# 2

## HERBS AND SPICES WITH IMPORTANT ANTI-INFLAMMATORY PROPERTIES

Joanna Newerli-Guz<sup>1</sup>

### Abstract

Inflammatory processes in the human body are a normal and healthy response to injuries or attacks by different factors. Very important in this case is a diet rich in anti-inflammatory products. Special in this group are herbs and spices, especially in case of the table salt replacement. They also fit in a plant-based diet, opting for seasonal, local, organic products with special health benefits, important in ESFC.

The main goal of study was to select and indicate herbs and spices with anti-inflammatory effects with special attention to the country of origin and the possibility of local sourcing.

Polish spices – oregano, fenugreek, basil, common thyme, rosemary and onion and garlic have anti-inflammatory properties and their use in the treatment of different illnesses is known. Foreign spices with anti-inflammatory properties are ginger, turmeric cinnamon, nutmeg, cloves, chilli and bay leaf. In Polish folk medicine oak bark, feverfew, comfrey are those with anti-inflammatory potential. Now popular on Polish market are anti-inflammatory Ayurveda herbs: Sallaki, Ashwagandha and Sophora japonica.

Presented herbs and spices are helpful in the fight against the effects of chronic inflammation, They are very close related to ESFC, because of their use, which makes it easier to reject refined, processed and manufactured food.

**Keywords:** spices, herbs, anti-inflammatory properties

<sup>1</sup> Gdynia Maritime University; Department of Commodity and Quality Sciences; [j.newerli-guz@wzsj.umg.edu.pl](mailto:j.newerli-guz@wzsj.umg.edu.pl)

## Introduction

The term inflammation come from the Latin *inflammare* – it means burn. Inflammatory process, called inflammation or inflammatory response which takes place in human body is a normal and healthy response to injury or attack by different damaging factors of chemical, biological and physical origin. The inflammatory process destroys homeostasis in the body, which responds with heat, pain, redness and swelling. Degree of intensity divides inflammation into short-term and long-lasting inflammation. Short-term inflammation ends with removal of the cause of inflammation like bacteria or virus. Long-lasting chronic states deals with the problem with removing the inflammatory factor from the body.

Diseases associated with chronic inflammation are allergies and autoimmune diseases like type 1 diabetes, rheumatoid arthritis, lupus or Hashimoto's thyroiditis. Whole-body inflammation refers to chronic, imperceptible, low-level inflammation. Mounting evidence suggests that over time this kind of inflammation sets the foundation for many serious, age-related diseases including heart disease, cancer and neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. Recent evidence indicates that whole-body inflammation may also contribute to psychological disorders, especially depression (Wail, 2011). Currently, drugs used to treat chronic inflammatory diseases are mainly various nonsteroidal drugs, which may exert side effects (Cuevas-Rodriguez, *et al.* 2010). There is a wide range of drugs, including those of plant origin, with this anti-inflammatory effect (Reis Nunes, *et al.* 2020). Therefore, the development of natural sources of anti-inflammatory food products has gained increasing attention. Selected food products have anti-inflammatory properties, which are advisable to achieve an anti-inflammatory.

The main goal of this study was to select and indicate herbs and spices with anti-inflammatory effects with special attention to the country of origin and the possibility of local sourcing.

## Food products rich in anti-inflammatory ingredients

Many plant raw materials have strong anti-inflammatory properties due to their strong antioxidant effect and the content of essential oils and other bioactive ingredients. Among them special attention should be paid on herbs and spices. The richest in antioxidants are green vegetables, red and orange fruits, dark berries and citrus. Among fruits those with anti-inflammatory properties are: pomegranate (Ghavipour, *et al.* 2017), forest fruits – especially berries (Johnson, *et al.* 2013; Ya Li, *et al.* 2016), and fruits with enzymes like papain and bromelain

(Muller, *et al.* 2013): pineapple (Hale, *et al.* 2010; Hossain, Akhtar & Anwar, 2015), papaya (Pandey, *et al.* 2016). Bromelain has demonstrated significant anti-inflammatory effects, reducing swelling in inflammatory conditions such as acute sinusitis, sore throat, arthritis and gout and speeding recovery from injuries and surgery. Pineapple enzymes have been used with success to treat rheumatoid arthritis and to speed tissue repair as a result of injuries, diabetic ulcers and general surgery. Pineapple reduces blood clotting and helps remove plaque from arterial walls (Hossain, Akhtar & Anwar, 2015).

To anti-inflammatory vegetables belongs onions, garlic, broccoli, brussels sprouts, cauliflower and cabbage (Fengmei Zhu, Bin Du & Baojun Xu. Zhu, 2018). Oily sea fish such as mackerel, herring, salmon, tuna and sardines are rich in EPA and DHA fatty acids from the omega-3 family (Olgunoglu, 2017). Many popular diets already adhere to anti-inflammatory principles, e.g. the Mediterranean and DASH diet (Matsumoto, *et al.* 2018). They included fresh fruits, vegetables, whole grains, and products especially rich in omega3 polyunsaturated fatty acids like fish and olive oil. Now very popular is anti-inflammatory diet which must consist of a range of antioxidants, healthful fats and nutrients. One of the most important advantage of this diet is balancing of omega-3 and omega-6 fatty acids amounts (Sears, 2015) Too many people nowadays consume an excess of omega-6 fatty acids, which one the body uses to synthesize compounds promoting inflammation. Omega 6 polyunsaturated fatty acids come from high processed, refined food, fast food and sweet and savoury snacks, whereas omega-3 fatty acids come from oily fish, seafood, walnuts, flax, hemp (Wail, 2011). These „good” products can be used in design of anti-inflammatory diet, such studies are already carried out and the diets are used (Berbert, *et al.* 2005; Bustamantea, *et al.* 2020).

An anti-inflammatory diet should be made of the following ingredients:

- green leafy vegetables, such as spinach, kale, and collards,
- nuts like almonds and walnuts,
- fatty fish like salmon, mackerel, tuna and sardines,
- fruits such as: strawberries, blueberries, cherries, and oranges,
- green tea,
- olive oil (Sears & Ricordi, 2011; Sears, 2015).

Other vegetable oils like rapeseed oil, linseed and avocado oil, due to the presence of monounsaturated fatty acids and omega-3 acids have a strong anti-inflammatory, anti-atherosclerotic and protective effect on the cardiovascular system and can by also used in this diet (Lunn & Theobald, 2006).

From beverages with anti-inflammatory ingredients special attention should be paid on green tea and red wine. Green tea – belongs to the group of anti-inflammatory products due to gallic acid content (Ohishi, *et al.* 2016), red wine



with its resveratrol counteract cancer and silences inflammatory processes (Birrell, *et al.* 2005; Kopeć, *et al.* 2011; Shaito, *et al.* 2020).

Plant products with a high pro-inflammatory effect include highly refined flours, gluten and of course trans- and saturated-fatty acids. Products of animal origin with anti-inflammatory effects are dairy products especially cheeses (Demmer, *et al.* 2016) and red meat (Ley, *et al.* 2014; Samraj, *et al.* 2015).

## Place of anti-inflammatory spices and herbs in ESFC

Environmentally Sustainable Food Consumption (ESFC) is defined as the use of food products “that respond to basic needs and bring a better quality of life, while minimizing the use of natural resources, toxic materials and emissions of waste and pollutants over the life cycle, so as not to jeopardize the needs of future generations” (Oslo Roundtable, 1994). Environmentally Sustainable Food Consumption insist on increasing consumption of plant-based food (Lea, Crawford & Worsley, 2006) insect-based foods (Bakhsh, *et al.* 2021) and therefore also decreasing meat consumption (Hoek, *et al.* 2004). ESFC placed great emphasis on the seasonal, locally and/or organically produced products (Macdiarmid, 2014). Special in this group are herbs and spices. They can be useful in anti-inflammatory diet, especially in case of table salt replacement and their unique position in healthy eating pyramid.

Presented herbs and spices are very helpful in the fight against the effects of chronic inflammation influenced by genetics, a sedentary, stressed lifestyle, and exposure to environmental toxins. They contain bioflavonoids, polyphenols, essential oils that reduce inflammation and limit free radical production. The correlation between the presence of phenolic compounds and their anti-inflammatory activity is widely reported in the available literature. Herbs and spices are very close related with Environmentally Sustainable Food Consumption (ESFC), because of their use, which makes it easier to abandon refined, processed and manufactured foods. They also very good fit in plant-based diet, opting for seasonal, locally produced and organically grows products with special health benefits, very important in ESFC. In addition to the nutritional use of spices, they may be used as medicinal products e.g. herbal spices. Plants with effective health promoting effects are known as herbs, and these have a long history of being used as medicine to cure several diseases.

## Foreign spices with anti-inflammatory properties

The most important foreign spices with anti-inflammatory properties are ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma* L.), cinnamon (*Cinnamomum verum*), nutmeg (*Myristica fragrans* Houtt.), cloves (*Syzygium aromaticum*), chilli (*Capsicum*) and bay leaf (*Laurus nobilis*). Now popular in different herbs mixtures available on Polish market are those recommended as anti-inflammatory herbs in Ayurveda: Sallaki (*Boswellia serrata*) and Ashwagandha (*Withania somnifera*). Also used is Sophora japonica (*Styphnolobium japonicum* L.), and its dried flower and buds (*Huaihua*). They are rich in rutin, reduces inflammation and are popular in Chinese, Japanese and Korean medicine. Presence of pungent phenolics such as shogaols and gingerols make **ginger** (*Zingiber officinale* Rosc.) very good known for its therapeutic properties (Karegar, 2019; Ramadan, Al-Kahtani & El-Sayed, 2011; Ramadan & El-Menshawey, 2013).

**Turmeric** (*Curcuma* L.), rich in phenolic curcuminoids, has also proved its beneficial effects against several malignancies (Ramadan, Al-Kahtani & El-Sayed, 2011; Ramadan & El-Menshawey, 2013; Smith, Oertle & Prato, 2015). Curcumin the basic ingredients of turmeric also shows a potent anti-inflammatory effect by blocking the expression of IL-1 and IL-6 in an *in vitro* study with RA patient-derived fibroblast-like synoviocytes (Kloesch, *et al.* 2013).

**Cinnamon bark** (*Cinnamomum zeylanicum*) is widely used in South-East Asian dishes. The anti-inflammatory activity of cinnamon and their components has been reported in several studies. Various studies reported the anti-inflammatory activity of cinnamon and its essential oils (Chao, *et al.* 2005; Lee, *et al.* 2018). There are several flavonoid compounds (e.g., gossypin, gnaphalin, hesperidin, hypolaetin and quercetin) in cinnamon which have anti-inflammatory activities (Stoner & Wang, 2013).

**Nutmeg** (*Myristica Fragrans* Houtt) is special spice, which produces two distinct spices, nutmeg and mace. Nutmeg has sweet, spicy and nutty taste and is used in most of the Indian kitchens, where its culinary value is well-known. The main properties of nutmeg is that it is helpful in stimulating the brain, is a great heart and liver tonic. Nutmeg oil is also recommended for inflammation of bladder and urinary passage and it has anti-inflammatory properties and can be used as pain reliever (Agbogidi & Azagbaekwe, 2013).

*Syzygium aromaticum* (synonym: *Eugenia caryophyllata*) commonly known as **clove**-dried flower bud is rich in flavonoids, especially gallic acid and tannins. Clove and their ingredients have antioxidant and antimicrobial activity (Cortés-Rojas, 2014). The clove essential oil (CEO) is typically used for a variety of health purposes, it has the anti-inflammatory and anticancer properties. The activity of CEO was checked in a dermal fibroblast system, showed robust antiproliferative

effects on human dermal fibroblasts. It significantly inhibited the increased production of several proinflammatory biomarkers. CEO also significantly inhibited tissue remodelling protein molecules e.g. collagen-I, collagen-III (Han & Parker, 2017).

Pungency and color are the two main characteristics of **chili** pepper fruits that determine their quality. Chili (*Capsicum*) has strong anti-inflammatory properties confirmed by many authors (Villa-Rivera & Ochoa-Alejo, 2020). Carotenoids present in chilli have antioxidant, anti-inflammatory, and photoprotective properties. They might help improve cardiovascular health through the maintenance of blood pressure baseline levels, the reduction of pro-inflammatory cytokines and markers of inflammation and correction of dyslipidemia (Gammone, Riccioni & D’Orazio, 2015). Hernández-Ortega *et al.* reported that a carotenoid extract from guajillo chili pepper containing violaxanthin,  $\beta$ -cryptoxanthin and  $\beta$ -carotene showed analgesic activity, probably produced by inhibition of the local prostaglandins. Furthermore, high doses of a carotenoid extract were capable of significantly inhibiting the formation and progression of edema in mice (Hernández-Ortega, *et al.* 2012).

As already mentioned above, strong anti-inflammatory properties have herbs often used in Ayurveda. **Sallaki** (*Boswellia serrata*) is widely recommended as an anti-inflammatory herb as prescribed in Ayurveda (Ammon, 2001). It contains boswellic acid from pentacyclic triterpene family, which inhibits the expression of lipoxygenase-5 (Wang, *et al.* 2014).

**Ashwagandha** (*Withania somnifera*) is a potent anti-inflammatory plant described in Ayurveda. It is rich in Withaferin A, a steroidal phytochemical which has shown inhibitory properties against breast, colon, prostate, lung, brain cancers (Dutta, 2019).

The best known and most frequently used action is its toning effect on the nervous system, ashwaganda is also used in immunomodulatory, antidiabetic, neurological inflammatory disorders, hemopoietic and Parkinson’s disease. It is also useful as antibiotic, antioxidant, deobstruent, diuretic and sedative agent (Połumackanycz, 2020).

**Sophora japonica** (*Styphnolobium japonicum* L.) is a plant belonging to the 50 most important medicinal plants of Chinese folk medicine. Anti-inflammatory properties of its flowers come from the high content of rutin, a substance with a strong anti-inflammatory effect. *Sophora japonica* also has anti-edematous, antibacterial and regenerating properties. It also strengthens blood vessels and prevents their excessive fragility (Chen & Hsieh, 2010).

## Polish sources of anti-inflammatory ingredients-spices' products and herbs

Also among the spices and herbs grown in Poland, we can find those with a significant anti-inflammatory effect. They combine both, these characteristics and the possibility of self-cultivation or the possibility of purchasing on the local market. Polish spices with anti-inflammatory properties are: oregano (*Origanum vulgare*), fenugreek (*Trigonella foenum-graecum* L.), basil (*Ocinum basilicum*) and common thyme (*Thymus vulgaris*) and especially rosemary (*Rosmarinus officinalis*). Polish vegetables used as spices – onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) are included in those with anti-inflammatory potential. In Polish folk medicine, herbs such as tan bark – in polish oak bark (*Quercus* L. *cortex*), feverfew (*Tanacetum parthenium*), comfrey (*Symphytum officinale* L.) are good known as those with big anti-inflammatory potential.

Use of **oregano** (*Origanum vulgare*), bay leaf (*Laurus nobilis*), basil (*Ocinum basilicum*) and common thyme (*Thymus vulgaris*) in the treatment of rheumatoid arthritis has been indicated by Kardas and co-authors (Kardas, Toczyńska & Grochowska-Niedworok, 2017).

**Fenugreek** (*Trigonella foenum-graecum* L.) is not one of the most popular Polish spices, its availability on the market is limited. Fenugreek seeds are used for seasoning vegetable and meat dishes. In addition to its spice properties, it is a valuable medicinal plant, due to its hypoglycemic properties. Fenugreek extracts help regulate cholesterol and have an anti-inflammatory effect.

**Basil** (*Ocinum basilicum*), **thyme** (*Thymus vulgaris*), **rosemary** (*Rosmarinus officinalis*) belong to the *Lamiaceae* (*Labiatae*) family which has a cosmopolitan distribution. They are also cultivated successfully in Poland. Those leafy spices due to high total phenolic content (the phenolic acids and flavonoids mainly the flavone and flavonol sub groups) have good anti-inflammatory properties. Chohan, *et al.* reported that at amounts used in food preparation, uncooked, cooked, and cooked and digested rosemary, thyme elicited an anti-inflammatory effect via the inhibition of, and also protection against, the action of pro-inflammatory agents (Chohan, *et al.* 2012).

Rosemary have many ingredients which are responsible for its antioxidant and anti-inflammatory properties. Antioxidant activity of rosemary come from the presence of rosmarinic and carnosic acid. Carnosic acid and carnosol are anti-inflammatory compounds which helps in the fight against pain and prevent carcinogens from binding to DNA, and stimulates liver detoxification of carcinogens (Gad & Sayd, 2015). The thyme (*Thymus vulgaris*) improved the activity of the enzyme superoxide dismutase (which acts as an anti-inflammatory agent (Opara & Chohan, 2014).

**Onion** (*Allium cepa* L.) and **garlic** (*Allium sativum* L.) are vegetables with high seasoning abilities due to containing organosulphur compounds. In this vegetable they scavenge oxidizing agents, inhibit the oxidation of fatty acids, thereby preventing the formation of pro-inflammatory messengers, and inhibit bacterial growth, via interaction with sulphur-containing enzymes (Wilson & Demming-Adams, 2007). Garlic prevents heart disease and atherosclerosis, and treats bronchitis, asthma and whooping cough.

Onion and its constituents such polyphenolics and flavonoids mainly quercetin play crucial role in inflammatory disorders of cardiovascular, gastrointestinal, neuronal respiratory and urogenital systems. Quercetin can help cure asthma and as an antioxidant acts like an antihistamine and an anti-inflammatory agent. The sulphur compounds content makes onion and garlic products which prevent the activation of inflammatory enzymes in the body. Their presence causes antioxidant effect by decreasing lipid peroxidation and reduction of swelling (Marefati, *et al.* 2021).

**Oak bark** (*Quercus* L. *cortex*) is a natural medicinal product, has anti-hemorrhagic, antibacterial and fungicidal properties, helps in the fight against inflammation of the body. Oak bark is prepared in the form of a decoction or rinse. Is ideal for the treatment of, among others, inflammations of the throat, skin, as well as genitals and hemorrhoids (Taib, *et al.* 2020)

There are more than 30 substances in **feverfew** (*Tanacetum parthenium*) composition that support the fight of our body with inflammation, which provokes a headache. Feverfew named Maruna gold has anti-inflammatory and antipyretic properties, so it is worth using it in case of fever, during colds and ear ache (Pareek, 2011).

**Comfrey** (*Symphytum officinale* L.) is anti-inflammatory herb for joints and fractures. The powdered root of this plant mixed with oil was used to treat broken bones and dislocated joints. Comfrey is suitable for the treatment of inflammation of tendons, rheumatic and back pain. It is a product especially recommended for sportsmen who are exposed to injuries and people with rheumatism. Most often it is used in the form of comfrey ointment, which is applied to the affected places in the form of compresses (Seigner, *et al.* 2019).

## Conclusions

Many factors, of both external and internal origin, affect the occurrence of inflammation in human body. Chronic inflammation is influenced by genetics, used diet with bad and good eating habits and behaviour. Sedentary lifestyle, too much stress, different environmental toxins, too much alcohol and tobacco

also influenced on body condition. In the fight against inflammation, a diet rich in ingredients with anti-inflammatory properties, especially spices, is indicated.

On the Polish market we can find many valuable anti-inflammatory plant products of foreign origin, but in our climate also the valuable raw materials with this effect are cultivated and collected from natural sites. Among them are very popular in Polish cuisine basil, oregano and vegetables like onion and garlic. It is important to remember that these plants, originating from Poland, are used for years in our homes. It is possible to cultivate them at home, in the garden or buy them from a local store. We can use fashionable, exotic products, but we shouldn't forget about their Polish counterparts.

Additionally, it is recommended to abandon eating refined, processed and manufactured foods and try to cook at home using different ingredients.

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# 3

## ANTIOXIDANT ACTIVITY OF *CENTELLA ASIATICA* EXTRACTS

Dobrawa Kwaśniewska<sup>1</sup>, Justyna Kiewlicz<sup>1</sup>

### Abstract

*Centella asiatica* plant known and used for its properties for thousands of years, it was first used in Indian Ayurvedic practice. It is described in literature as a rich source of flavonoids: quercetin, kaempferol, catechin, rutin, apigenin and naringin. The richness of the active ingredients caused noticed that the healing properties of *Centella asiatica* such as the ability to learn and memory, lowers blood glucose levels, and has cardioprotective effects. In addition, the reduction of transepidermal water loss, improved hydration of the epidermis and antioxidant properties make *Centella asiatica* increasingly used in cosmetic products. All this meant that the authors made an attempt to assess the antioxidant activity of cosmetic components from *Centella asiatica* available on the Polish market. Total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) of three commercially available cosmetic components from *Centella asiatica* for self-production of formulations were evaluated in this study. The obtained results show that the highest TPC value had water-glycerin liquid *Centella asiatica* extract, and it was 416 mg GAE/L. The highest ferric reducing properties exhibited both water-glycerin liquid *Centella asiatica* extract and powdered *Centella asiatica* extract (respectively, 2.3 mmole Fe<sup>2+</sup>/L and 1.89 mmole Fe<sup>2+</sup>/L). The lowest antioxidant activity demonstrated infusion of *Centella asiatica*.

**Keywords:** antioxidant activity, *Centella asiatica*, cosmetics, phenolic compounds

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Technology and Instrumental Analysis, Poland; [dobrawa.kwasniewska@ue.poznan.pl](mailto:dobrawa.kwasniewska@ue.poznan.pl)

## Introduction

*Centella asiatica* herb which is also known as Gotu kola or Tiger Grass. Usually grows up to 15 cm in height. Its leaves resemble a small fan, and the flowers are white to purple. The flowering period is from April to June, while the fruits appear throughout the growing season. This plant is found in Southeast Asia, Sri Lanka, China, Indonesia, Malaysia, India but also South Africa and Madagascar. Depending on the origin of *Centella asiatica*, it may differ in appearance, currently there are several morphotypes (Chandrika & Kumara, 2015). However, it seems that Gotu kola was first used in Indian Ayurvedic practice, and the literature reports that this plant has also been used for thousands of years in China and Indonesia. In prehistoric times, it was used to heal wounds, as well as an internal therapy, it was also used in the treatment of leprosy. The therapeutic effect of leprosy was confirmed in the 19th century, with the first clinical report from 1852. In China, the aerial part of *Centella asiatica* was used to treat fever, urinary tract infections, dysentery and jaundice. The healing effect of Gotu kola is also known to the inhabitants of East Africa, the plant is used there to treat stomach problems, fever, tuberculous affecting of the lymph nodes (Bandara, Lee & Thomas, 2011).

Phytochemical analysis allowed to state that *Centella asiatica* contains over 70 active ingredients (Bandara, Lee & Thomas, 2011). It seems that the most important group of compounds are triterpenes and their derivatives. *Centella asiatica* is rich in: asiatic acid, madecassic acid, asiaticoside, madecassoside, brahmoside, brahmic acid, brahminoside, thankinaside, isothankuniside, centelloside, madasiatic acid, centic acid, and cenellicacid (Seevaratnam, *et al.* 2012). Discussed the herb is attributed to the ability to treat wounds. Scientific evidence indicates that *Centella asiatica* owes this ability to the presence of: asiatic acid, madecassic acid and asiaticoside (Bylka, *et al.* 2013). The literature indicates that *Centella* extracts containing asiatic acid, madecassic acid and asiaticoside stimulated fibroblasts to synthesize collagen (Hashim, *et al.* 2011). In addition, it was proven that the *Centella* extract containing asiatic acid, madecassic acid and asiaticoside even by 20–35% stimulated the synthesis of collagen and fibronectin (Tenni, *et al.* 1988). The presence of asiaticoside in *Centella* extracts is attributed to biocidal activity against both Gram positive and negative bacteria. The bactericidal activity of this triterpene is based on the mechanism of weakening the cell wall (Das, 2011). Other studies have confirmed this activity indicating that *Centella asiatica* extracts showed their activity against Gram positive bacteria: *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis* and *Sarcinalutea* and Gram negative bacteria: *Shigelladysenteriae*, *Escherichia coli*, *Salmonella paratyphi*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigellaboydii*, *Vibrio mimicus* and *Vibrio parahemolyticus* (Zahara, Bibi & Tabassum, 2014). Currently, the society has realized the importance of the fight against microorganisms, numerous studies on the activity of *Centella asiatica* extracts (alcohol, ether, water) have

confirmed its antifungal activity. Activity has been found against: *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* (Dash, *et al.* 2011; Seevaratnam, *et al.*, 2012).

Chemical analysis made it possible to conclude that *Centella asiatica* is also a rich source of flavonoids such as: quercetin, kaempferol, catechin, rutin, apigenin and naringin. It is also estimated that most of these compounds are responsible for the antioxidant activity of *Centella asiatica* (Seevaratnam, *et al.* 2012; Zainol, *et al.* 2003). Gotu kola is also considered a source of vitamins: C, B1, B2, niacin and A (Seevaratnam, *et al.* 2012). It is therefore no surprise that it contains carotenoids that are vitamin A precursors, including the presence of  $\beta$ -carotene, lutein, neoxanthin and violaxanthin (Chandrika, *et al.* 2011).

Research shows that *Centella asiatica* contains about 36% volatile and fatty oils, usually glycerides of palmitic, oleic, stearic, linoleic, linolenic and lignoceric acids. In the essential oil of *Centella asiatica* also occur: terpenic acetate,  $\beta$ -caryophellene, farnesene, trans  $\beta$ -farnesene, gemacrene-D,  $\alpha$ -humulene, bicyclogermacrene, sesquiterpene and p-cymol (Chandrika, *et al.* 2011; Joshi & Chaturvedi, 2013). In addition to the above-mentioned main groups of compounds found in *Centella asiatica*, the presence of amino acids, sugars, resins and tannins was also found (Chandrika, *et al.* 2011; Rahman, *et al.* 2013).

The richness of active ingredients present in *Centella asiatica* meant that this plant has found numerous not mentioned before medical applications. Studies in rats have shown that ethanolic and methanolic extracts of *Centella asiatica* lowered blood glucose levels in diabetic rats induced by alloxan (Seevaratnam, *et al.*, 2012). Also the hypoglycemic effects of the glycosides (bhramoside and brahminoside) present in *Centella asiatica* are known (Joshi & Chaturvedi, 2013). Pragada conducting a study for cardioprotective activity against myocardial infarction caused by ischemia-reperfusion in rats, concluded that *Centella asiatica* exhibits a cardioprotective effect (Pragada, *et al.* 2004).

The research of Kumar and Gupta led to interesting observations, namely that they found that water extracts of *Centella asiatica* improved memory and learning ability in rats. An increase in the concentration of endogenous antioxidant enzymes in the brain was also observed (Kumar & Gupta, 2002).

*Centella asiatica* extractions can also be useful in dermatology and cosmetology. Their properties stimulating wound healing have been known for a long time (Maquart, *et al.* 1990). Research is being carried out on the use of *Centella asiatica* extract in the process of renewing the epidermal surface after laser treatments (Damkerngsuntorn, *et al.* 2020). It was found that the use of *Centella asiatica* extracts as components of oil-in-water emulsions and hydrogels improved skin hydration and reduced transepidermal water loss. The tested cosmetic preparations also had an anti-inflammatory effect (Ratz-Łyko, Arct & Pytkowska, 2016).

Current research reported (Fonseca-Santos, Corrêa & Chorilli, 2015) that during the last decades a rising of interest on natural products and biodiversity among the so-called “green consumers” has been observed. Consequently, the companies in cosmetic industry are focused more on formulating and developing the products with organic and natural ingredients such as e.g. plant extracts. Above-mentioned phenomenon also prompts potential consumers to seek natural resources and materials intended for self-preparation of cosmetics. *Centella asiatica* extracts due to the health-promoting properties are one of the proposed options. Therefore, the aim of the study was to assess and compare the antioxidant activity of cosmetic components from *Centella asiatica* available on the Polish market.

## Materials and Methods

### Materials

Three commercially available cosmetic components from *Centella asiatica* for self-production of formulations were purchased from local internet retailers. The studies were conducted on powdered extract of *Centella asiatica* standardized to contain 10% of triterpenes (as 2% w/w water solution – hereinafter referred to as extract I), water-glycerin liquid extract of *Centella asiatica* (hereinafter referred to as extract II) and infusion of *Centella asiatica*. All materials were prepared for the research according to manufacturers recommendations.

To prepare an infusion of *Centella asiatica*, 0.50 g of dried herb was poured with 100 mL boiling distilled water and brewed for 20 min under cover. Subsequently, 1.5 mL of clear infusion was taken and cooled to ambient temperature for further analysis.

### Methods

#### Total Phenolic Content (TPC)

Total phenolic content was investigated with the Folin-Ciocalteu method (Singleton & Rossi, 1965). 0.1 mL of sample was mixed with 0.5 mL of Folin-Ciocalteu reagent, diluted with 6.0 mL of distilled water and allowed to stand for 5 min. Then 1.5 mL of 20% sodium carbonate solution was added. The volume was made up to 10 mL with distilled water and the reaction mixture was left in the dark place for 2 h. Subsequently, absorbance was measured against blank at 765 nm with spectrophotometer UV-VIS (Metertech SP-8001, Taipei, Taiwan). Each sample was analyzed triplicate. A calibration curve of gallic acid was prepared. The results were expressed as milligrams of gallic acid equivalent (GAE) per liter of extract/infusion.

## Ferric Reducing Antioxidant Power (FRAP)

Total antioxidant capacity was evaluated by method described by Benzie & Strain (1996) with further modifications (Malinowska, Gliszczynska-Świągło & Szymusiak, 2014). Before the test the following solutions were prepared:

- 10 mM 2,4,6-tris-(2-pyridyl)-s-triazine (TPTZ) in 40 mM hydrochloric acid solution,
- 20 mM ferric chloride (III) solution,
- 300 mM acetic buffer solution (pH 3.6).

FRAP reagent was prepared by mixing respectively mentioned solutions in a ratio of 1:1:10. Then 30  $\mu\text{L}$  of sample was added into 2970  $\mu\text{L}$  of FRAP reagent and allowed to stand for 4 min. Subsequently, the absorbance with spectrometer UV-VIS (Metertech SP-8001, Taipei, Taiwan) was measured at 593 nm against blank. The FRAP value was calculated as the ratio of the linear regression coefficient of the calibration curve for five dilutions of the sample and the linear regression coefficient of the  $\text{FeSO}_4$  standard curve and expressed as mmol of  $\text{Fe}^{2+}$  per liter of extract/infusion.

## Statistical analysis

All results were expressed as mean  $\pm$  standard deviation for triplicate determinations. The one-way variance analysis (ANOVA) and Tukey's post-test at  $\alpha = 0.05$  (Statistica 12.0 software, StatSoft, Inc. 2013) were conducted to identify differences among means.

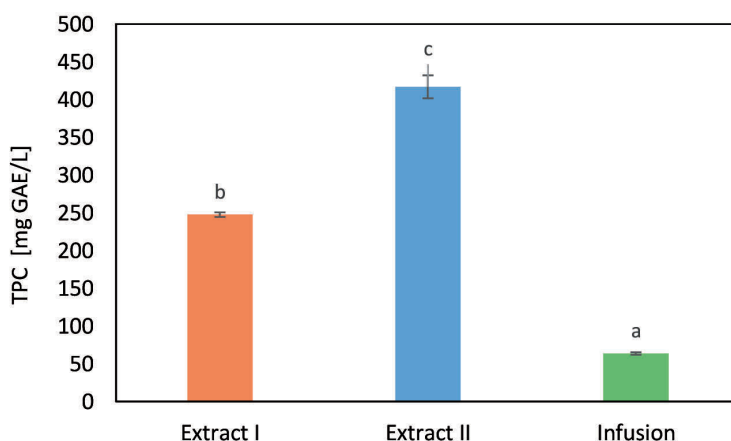
## Results and discussion

Total phenolic content (TPC) values of investigated components from *Centella asiatica* are presented in Figure 1. The obtained results show that TPC values of tested materials varied significantly and ranged from 63.56 mg GAE/L in infusion to 416 mg GAE/L in extract II. Differences in total phenolic content between investigated components from *Centella asiatica* may result from the methods of isolation phenolic antioxidants from plant material. Sepahpour, et al. (2018) revealed that selected herbs showed the lowest TPC values in water extraction compared to different organic solvents. Although the extraction conditions used by the producers of commercially available extracts are unknown, it can be presumed that relatively low TPC value of investigated infusion is related to brewing technique, the proportions of water and herb used, and using of water as extractant.

It is also noteworthy that extract II turned out to be abundant source of phenolic compounds in comparison to different commercially available liquid cosmetic

extracts described in other studies (Malinowska, 2015; Malinowska & Kiewlicz, 2017). It had lower TPC value than apricot extract (510 mg/L), but higher than peony (about 350 mg/L) or grape (about 260 mg/L) extracts.

According to the literature data (Azmin & Nor, 2020; Chew, *et al.* 2011; Ratz-  
łyko & Arct, 2015), *Centella asiatica* contains significant amounts of phenolic compounds such as: flavonoids, phenolic acids, and tannins. The main flavonoids isolated from *Centella asiatica*, includes quercetin, kaempferol (and their glycoside derivatives), catechin, naringenin, rutin, apigenin, luteolin, castilliferol and castillicetin (Mohapatra, *et al.* 2021). Among phenolic acids, chlorogenic acid and its isomers are the most mentioned. All phenolic compounds possess bioactive properties. Therefore, they have been intensively investigated in terms of their potential health-promoting effects. Numerous studies confirmed that phenolic compounds exhibit anti-inflammatory, anti-microbial, anti-allergic, vascular and cytotoxic antitumor activity (Cartea, *et al.* 2011). Nevertheless, their cosmetic and dermatological importance is due to the strong antioxidant activity. Phenolic compounds protect skin against oxidative damages, which may lead to premature aging, as well as exhibit photoprotective activity for the treatment of sensitive or sun-stressed skin by anti-inflammatory activity. The second role of phenolic antioxidants is protection of cosmetic formulations against oxidative deterioration (de Lima Cherubim, *et al.* 2020).



**Figure 1. TPC values of investigated raw materials from *Centella asiatica***

Explanation: a-c: mean values with the different letter were significantly different at  $\alpha = 0.05$ .

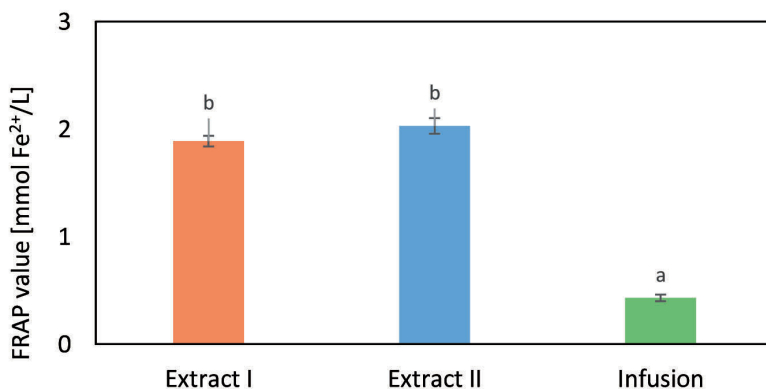
Source: own study.

The Ferric Reducing Antioxidant Power (FRAP) method is used for evaluation of the antioxidant activity through measuring the reduction of TPTZ-Fe<sup>3+</sup> complex



to TPTZ-Fe<sup>2+</sup> complex in the presence of antioxidants. As can be seen in Figure 2, extract I and extract II had several-fold higher FRAP values (respectively, 2.3 mmole Fe<sup>2+</sup>/L and 1.89 mmole Fe<sup>2+</sup>/L) than infusion of *Centella asiatica* (0.43 mmole Fe<sup>2+</sup>/L). Surprisingly, despite the differences in total phenolic content, there were no significant differences observed in FRAP values between both extracts. It may be caused by the presence of non-phenolic antioxidants in extract II. According to manufacturer's declaration, *Centella asiatica* powdered extract was standardized to contain 10% of triterpenes. The literature data (Hashim, *et al.* 2011; Ratz-Łyko & Arct, 2015) show that *Centella asiatica* is abundant source of triterpene glycosides (saponins) and triterpene acids, of which the most biologically active are: asiaticoside, madecassoside and their aglycones – asiatic and madecassic acids. Importantly, Hashim, *et al.* (2015) suggested that asiaticoside may contribute to antioxidant activity of *Centella asiatica* extract. Moreover, Shukla, *et al.* (1999) revealed that asiaticoside may act synergistically with flavonoids, enhancing their antioxidant properties.

Several studies considering the overall antioxidant properties of *Centella asiatica* extracts compared to other sources of natural antioxidants confirm their high antioxidant capacity. Research conducted by Wong, *et al.* (2006) showed that water extract from *Centella asiatica* had FRAP value similar to common rue (*Ruta graveolens* L., Rutaceae) and horse radish tree (*Moringa pteriosperma*, Moringaceae) extracts. Other research (Rattanakom & Yasurin, 2014; Shukla, Rasik & Dhawan, 1999) revealed that antioxidant activity of *Centella asiatica* is comparable to the activity of rosemary extract, vitamin C, and grape seed extract.



**Figure 2. FRAP values of investigated raw materials from *Centella asiatica***

Explanation: a-b: mean values with the different letter were significantly different at  $\alpha = 0.05$ .

Source: own study.

## Conclusions

*Centella asiatica* is a valuable herb commonly used in traditional Chinese system of medicine. In dermatology and cosmetology, preparations containing *Centella asiatica* successfully support the treatment of hypertrophic wounds, burns, psoriasis, and scleroderma (Ratz-Łyko & Arct, 2015). *Centella asiatica* is also considering as a good source of natural antioxidants, which protect skin against oxidative damages and their consequences. Obtained results show that investigated *Centella asiatica* extracts exhibited strong antioxidant activity. Interestingly, the presence of non-phenolic compounds, such as triterpene glycosides, had a significant influence on the antioxidant activity of powdered extract of *Centella asiatica*. Unlike the extracts, infusion from *Centella asiatica* was poor source of phenolic antioxidants and thus exhibited much lower antioxidant activity. It can be concluded that the use of commercially available *Centella asiatica* extracts as cosmetic components may significantly enrich the formulations in natural antioxidants improving their care properties and oxidative stability.

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# 4

## COMPARISON OF SORPTION PROPERTIES OF WHITE (*SINAPIS ALBA*) AND BLACK (*BRASSICA NIGRA*) MUSTARD SEEDS WITH DIFFERENT DEGREE OF COMMINUTION

Anna Flis<sup>1</sup>

### Abstract

Spices have been widely used in human nutrition since time immemorial. Among the popular domestic spices, it is worth paying attention to mustard seeds. The quality of spices, which consists of many factors, is extremely important for the consumer. Among them, hygroscopic properties are significantly important because they determine the safety and storage stability of the product. Simultaneously, it is an important factor in the process of production planning, transport and distribution. The aim of this study was to evaluate the sorption properties of white and black mustard of different degree of comminution, perceived as a factor determining its storage stability. In order to achieve the aim of the study, water sorption isotherms were determined using the static-desiccator method at a temperature of 20°C. The BET model was used to describe the water sorption isotherms. In the course of the research, it was found that both tested mustard types were characterized by similar hygroscopic properties. Their different degree of comminution did not differentiate the sorption properties of the tested material. Black mustard was characterized by a higher water content in the monolayer than white mustard, and based on the size of the sorption specific surface area, it was found that black mustard seeds were a more hygroscopic product.

### Keywords:

sorption isotherm, BET model, white mustard, black mustard, sorption specific surface area

<sup>1</sup> Gdynia Maritime University, Faculty of Management and Quality Sciences, Department of Quality Management; [a.flis@wznj.umg.edu.pl](mailto:a.flis@wznj.umg.edu.pl)

## Introduction

Spices and spice herbs have a special place in human nutrition. From time immemorial, they have been valued for their taste, aromatic and healing properties. The primary purpose of most spices was to preserve food and prevent gastric diseases resulting from its consumption (Kudęłka & Kosowska, 2008). Nowadays, the group of spices is extremely opulent, and consumers appreciate them for their many valuable properties and commonly use them in the process of preparing dishes.

Particular attention should be paid to mustard seeds. The name of mustard seed includes annual, oil-bearing and honey-bearing herbaceous plants from the Brassicaceae family – brassicas, the subfamilies Brassicoideae, from the genera *Sinapis*, *Brassica* and *Eruca*. White mustard (*Sinapis alba*), also known as light or yellow, probably comes from the Mediterranean region and was introduced to north-eastern Europe. Black mustard (*Brassica nigra*) comes from the Mediterranean center, and its second place of origin is the Middle East. Nowadays, both types are found all over Europe, North Africa, Asia Minor and Western India (Sawicka & Kotiuk, 2007).

Black and white mustard seeds, in conjunction with the high content of glycosides, give the dishes a spicy taste and intense aroma, and due to the presence of biocatalysts, they have a positive effect on digestive processes and metabolism. In addition, mustard seed extract supports the treatment of lung cancer in humans (Ahmed, *et al.* 2020). White and black mustard seeds belong to the group of domestic spices and oilseeds. Due to its high fat content (25–30% fat for white mustard, 35–40% for black mustard), mustard is particularly prone to rancidity, so it is recommended to protect it from sun, heat and water (Kudęłka & Kosowska, 2008; Sawicka & Kotiuk, 2007). Furthermore, mustard seeds are a viable source of non-allergen phospholipids, including lecithin with potentially novel emulsifying characteristics (Wade, *et al.* 2021). White and black mustard seeds can be used in the preparation of meat, fish and seafood dishes, salads and marinades. However, the most popular form of using mustard seeds is their use in the production of mustard, which has found wide application in Polish cuisine, being one of the favorite cold sauces of Poles. According to Newerli-Guz (2018), about 30% of Poles, especially in old age, declare that they regularly eat mustard. The Polish Classification of Products and Services PKWiU, item 10.84 indicates that mustard, as a finished product, is also classified as spices (Journal of Laws of 2015, item 1676).

Due to its structure, consistency and long storage time of mustard seeds in consumers' houses, it is worth paying attention to ensuring their uninterrupted, high quality. One of the critical quality assurance parameters for loose and dry products are their sorption properties. Exceeding the critical water content and

water activity by the product leads to irreversible storage changes and prevents its further use in the preparation of food.

The aim of this study was to evaluate the sorption properties of white and black mustard of different degree of comminution, perceived as a factor determining its storage stability.

## Material and methods

The research material consisted of samples of white mustard and black cabbage called black mustard, differing in the degree of comminution: white mustard seeds (WM), white, ground mustard seeds (WGM), black mustard seeds (BM) and black, ground mustard seeds (BGM). Mustard seed samples were purchased in one of the supermarkets in Gdynia, and the amount accepted for research was not less than 2 kg. The research material was stored in non-perforated multi-layer packages.

Taking into account the fact that mustard seeds, included in a broad group of spices, are characterized by variability related to the differences in the physical structure of the seeds and chemical composition, which determine their sorption properties, a comparison of these properties was made by determining the water sorption isotherms. Moreover, the empirically determined sorption isotherms were transformed using the theoretical sorption model of Brunauer, Emmett and Teller (BET) (1938). In this way, the water content and water activity in seeds of selected types of mustard, appropriate for the maintenance of quality and safety, were estimated. This allowed to determine the relative air humidity, which would be in equilibrium with the mustard seeds. Parameters determined in this way can be used to stabilize the quality of seeds and it is recommended to keep them in warehouses during the storage of mustard seeds.

The initial water content in the tested products was determined by thermal drying to constant weight at 373–378 K (100–105°C) under normal pressure (Krełowska-Kułas, 1993).

The water activity was determined in the AquaLab 4TE apparatus (version AS4 2.14.0 2017 by Decagon Devices, Inc.) with an accuracy of  $\pm 0.0003$  at a temperature of 293 K (20°C)  $\pm 2.5$  K. The research material was ground with a WŻ-1 type laboratory grinder.

Water sorption isotherms were determined by the static-desiccator method with the use of saturate solutions of appropriate substances. The scope of the research included the environmental water activity from 0.03 to 0.98 and the test temperature was 293.15 K (20°C). The mustard samples, intended for the determination of sorption isotherms, were placed in the amount of about 1 g

± 0.01 mg in weighing vessels with a diameter of nearly 40 mm in appropriate desiccators until the system equilibrium state. Samples incubated in desiccators with a water activity greater than 0.7 were protected against the development of microflora with thymol.

Based on the knowledge of the initial water content in the product and its change related to the release or absorption from the environment, in the time necessary to achieve the state of dynamic equilibrium, the equilibrium water content was estimated. Water activity was measured in parallel. Based on these data, sorption isotherms was determined. The evaluation of the differentiation of the course of sorption isotherms in the entire range of water activity was analyzed statistically using the t-test, considering the differences at the significance level  $p \leq 0.05$  as statistically significant.

To determine and compare the differences in the sorption properties of the tested mustard seed samples, the parameters of the BET equation were identified in the form:

$$V = \frac{V_m C a_w}{(1 - a_w)[1 + (C - 1)a_w]}$$

where:

$a_w$  – water activity [-],

$V$  – equilibrium water content [g H<sub>2</sub>O/100g d.m.],

$V_m$  – water content in monolayer [g H<sub>2</sub>O/100g d.m.],

$C$  – constant energy (Paderewski, 1999).

Parameters for the selected water sorption model were determined using the Microsoft Office Excel 365 spreadsheet. The choice of the classic BET model was conditioned by the fact that, despite its limitations, this model is still used to calculate the value of the monolayer in various areas of physicochemical research, therefore the obtained results can be compared (Ociecek & Mesinger 2020). In addition, it is endorsed by the International Union of Pure and Applied Chemistry (IUPAC) (Timmermann, 2003).

In order to determine the suitability of the model for the description of the adsorption isotherms obtained, an analysis of the mean square error (RMS) expressed in % was made, which was calculated on the basis of the equation:

$$RMS = \sqrt{\frac{\sum \left( \frac{z w_e - z w_p}{z w_e} \right)^2}{N}} * 100,$$

where:

$z w_e$  – empirical, equilibrium water content [g H<sub>2</sub>O/100g d.m.],



$zw_p$  – predicted, equilibrium water content [g H<sub>2</sub>O/100g d.m.],  
N – number of measuring points (Lewicki, 1998).

Moreover, knowing the surface area of water sitting, i.e. the surface area occupied by a water molecule at a temperature lower than the boiling point of water, the sorption specific surface area of the adsorbent was calculated from the equation:

$$a_{sp} = \omega \frac{V_m}{M} N,$$

where:

$a_{sp}$  – sorption specific surface area [m<sup>2</sup>/g d.m.],

N – Avogadro's number [6,023 · 10<sup>23</sup> molecules/mol],

M – molecular weight of water [18 g/mol],

$\omega$  – water setting surface [1,05 · 10<sup>-19</sup> m<sup>2</sup>/molecule] (Paderewski, 1999).

## Results and discussion

Water contained in food products is one of the main factors influencing the intensity of the processes taking place in a given product. The right amount of water in the product affects its sensory features and determines the susceptibility to microbiological changes. Many ways of preserving food consist in reducing the amount of water in the product, while reducing its activity (Sikorski, 2007). The sorption properties of spices are the result of many factors determining the affinity of the grain surface to vapors and gases. The most frequently mentioned factors conditioned by the sorption phenomenon, which are important in shaping the quality and safety of spices during storage, are water activity and water content. They determine the direction and dynamics of the processes taking place at that time (Ocieczek, 2021).

Table 1 shows the mean initial water content and activity in the tested mustard types. It was found that white, ground mustard had the highest initial water content and activity (7.8455%; 0.6314), white mustard seeds had a slightly lower water activity (7.8455%; 0.6288), and the lowest water content and water activity black mustard seeds (5.4106%; 0.4312) were characterized. It was found that the level of initial water activity for WM and WGM samples was high enough to pose a threat to microbiological safety, calling into question their storage stability. The technological processes typical for mustard production do not include activities that could increase the water content and, consequently, the activity of water. Therefore, it can be assumed that the tested white mustard samples were characterized by low initial quality, which could result from improper transport conditions, improper storage and storage or leaky packaging of the tested white mustard seeds.

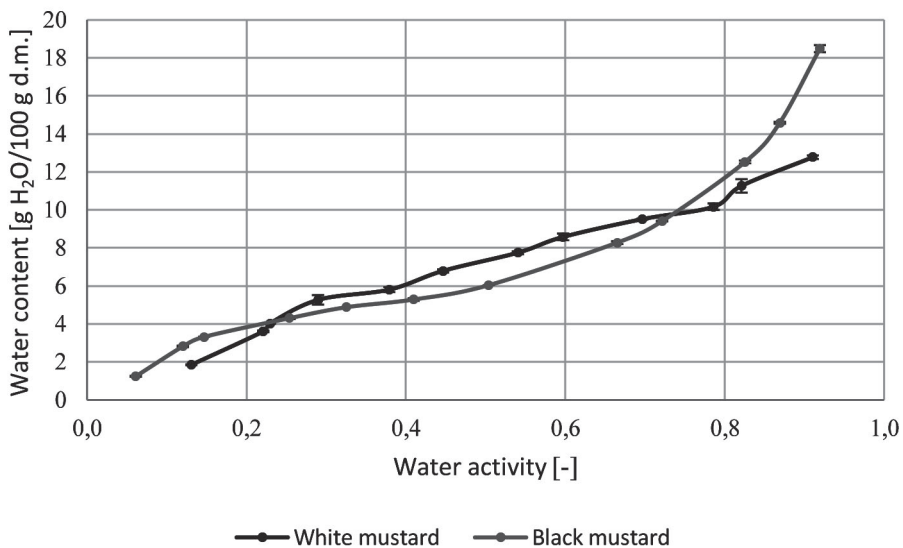
**Table 1. Initial water content and water activity of the tested samples of white and black mustard**

Product	Initial water content [%]	SD [%]	Initial water activity [-]	SD [-]
WM	7.8455	0.2151	0.6288	0.0060
WGM	7.8455	0.2151	0.6314	0.0060
BM	5.4106	0.0502	0.4312	0.0037
BGM	5.4106	0.0502	0.4322	0.0061

Source: own study.

The source of many important information on the state of water in the material are undoubtedly sorption isotherms. This characteristic curve for each product can be presented graphically or in the form of mathematical parameters of the models used to describe them. The BET model was used to describe water vapor sorption isotherms of mustard seeds.

In order to assess the differences in hygroscopic properties between white and black mustard, their water vapor sorption isotherms were determined (Figure 1).



**Figure 1. Adsorption isotherms of water vapor of white and black mustard**

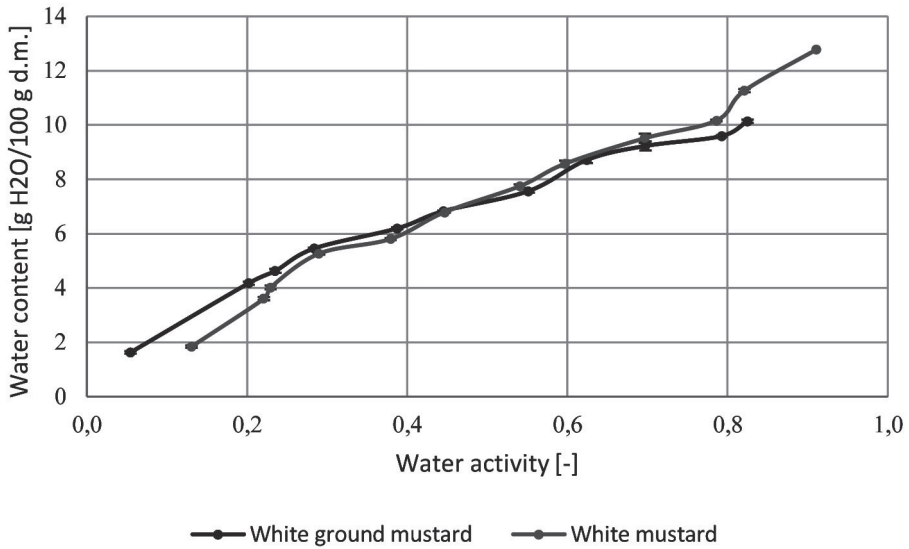
Source: own study.

Preliminary analysis of the distribution of results for the tested mustard samples, based on a comparison of the mutual positions of the determined isotherms, allowed to conclude that their course was similar (Figure 1). The supposition was

confirmed by the results of the t-test, which showed that the lack of differences in the course of the curves is statistically significant at the significance level not exceeding  $p = 0.05$ . The calculated statistics for white and black mustard seeds ( $t = 0.4798$ ) were lower than the critical value read from the statistical tables ( $t_{0.05} = 2.201$ ). Therefore, it can be concluded that both tested mustard types were characterized by similar hygroscopic properties.

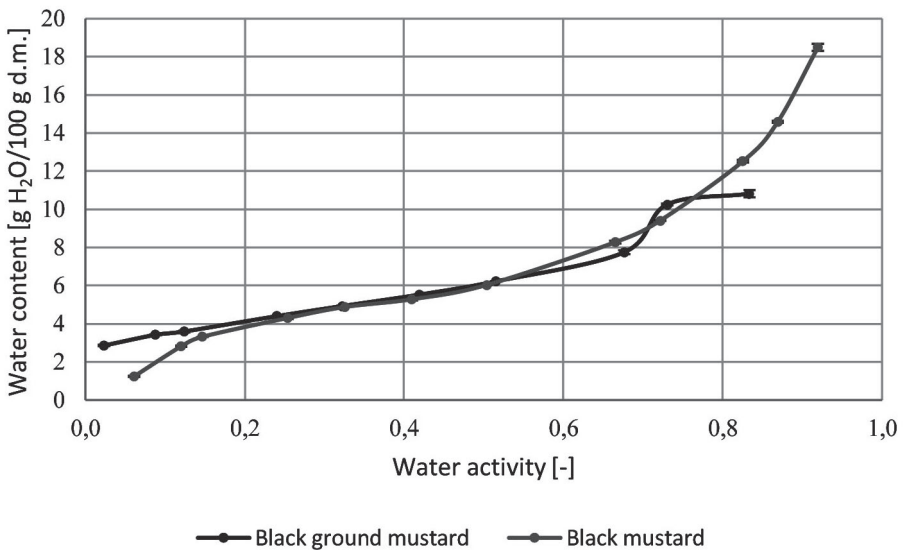
Figures 2–3 show the course of water sorption isotherms for the tested mustard samples of different degree of comminution. The points marked on isotherms are average values and the coefficients of variation ranged from 2.18% to 11.33% for ground white mustard seeds, from 1.18% to 13.37% for whole white mustard seeds, from 1.05% to 10.31% for ground black mustard seeds and from 0.04% to 6.93% for whole black mustard seeds. The obtained water sorption isotherms, irrespective of the type and degree of grinding of mustard seeds, were characterized by a typical sigmoidal shape, characteristic for type II isotherms, in accordance with the classification of Brunauer, *et al.* (1940). This sorption isotherm shape indicates that the water adsorption process took place in three steps. In the first one, the water molecules took over all the free hydrophilic groups. The first inflection point on the sorption curves corresponded to the end of the filling of the monomolecular layer. Water present in mustard seeds in the amount corresponding to this level is most strongly bound to the material, thus it is not an environment necessary for the course of changes of a hydrolytic nature (Rahman, 2009). The water contained in the monolayer is a kind of protection for the seeds against adverse changes depending on external factors. The size of the monolayer and therefore the amount of water that fills it depends on the proportion of hydrophilic polymers such as proteins and polysaccharides in the seed matrix. Mustard seeds are a protein-rich product. Its content in white mustard seeds varies between 27–35%, while in black mustard seeds it is about 27%. Exceeding the amount of water contained in a monolayer, which most often occurs as a result of storing seeds in a damp room or leakage of packages, leads to the initiation of the multilayer absorption phenomenon. Multilayer adsorption is indicated as the second stage of sorption, during which the mechanical properties of the grain change and the dynamics of chemical and biochemical reactions increases significantly. At this level of water content, the seeds begin to breathe vigorously, and this leads to the release and accumulation of metabolic water and energy in the form of heat. The coexistence of these two factors (heat and water), considered critical for the safety and quality of seeds, leads to a sharp reduction in the value of the commodity. The third stage in the sorption process, described as the phenomenon of capillary condensation, is initiated by filling the capillaries on the grain surface with water, which radically increases the dynamics of all biochemical processes typical for a living organism and enables the multiplication of a wide spectrum of microorganisms and the production of toxins by these microorganisms (Rahman, 2009). The commencement of the

capillary condensation process and all the changes deciding about the safety risk and the reduction in the quality of mustard seeds are so advanced that it should be considered that this material is unsuitable for its further storage.



**Figure 2. Adsorption isotherm of water vapor of white granular and ground mustard**

Source: own study.



**Figure 3. Adsorption isotherm of water vapor of black granular and ground mustard**

Source: own study.

Preliminary analysis of the distribution of results for the tested mustard samples, based on a comparison of the mutual positions of the determined isotherms, allowed to conclude that their course was similar (Figures 2–3). The supposition was confirmed by the results of the t-test, which showed that the lack of differences in the course of the curves is statistically significant at the significance level not exceeding  $p = 0.05$ . The calculated statistics for white mustard ( $t = 0.2589$ ) was lower than the critical value read from the statistical tables ( $t_{0.05} = 2.228$ ). An analogous relationship was found for black mustard, where the statistic  $t = 0.2805$  was also lower than the critical value read from the statistical tables ( $t_{0.05} = 2.262$ ). Thus, it was found that the different degree of mustard seed comminution did not differentiate the sorption properties of the tested material. At this point, it should be noted that the observed phenomenon differs from the expected results of the experiment. According to the literature (Ocieczek & Flis, 2020; Ruszkowska & Palich, 2016) the grinding process significantly expands the specific surface area of the adsorbent, which may lead to an increase in the dynamics of surface phenomena related to the influence of water vapor and oxygen, which was not observed in the case of both tested mustard types. This fact can be explained by the high content of fats in the mustard matrix (25–30% white mustard, 35–40% black mustard), which due to their hydrophobic properties limit the ability to bind water molecules by the adsorbent in both examined cases. Comparing the hygroscopic properties of mustard seeds to sunflower seeds, which also represent oilseeds, a similar sorption capacity was found, characteristic for this group of products (Pałacha & Kęsik, 2019).

The BET model was used to describe the phenomenon in the water activity range from 0.05 to 0.44, and its parameters and the degree of model fit expressed by the coefficient of determination ( $R^2$ ) as well as the mean square error (RSM) are presented in Table 2.

**Table 2. BET equation parameters of the tested samples of white and black mustard divided by the degree of fragmentation**

	Parameters of BET model					
	$C_{\text{energ.}}$ [-]	$V_m$ [g H <sub>2</sub> O/100 g d.m.]	Sum of squared deviations (SKO)	RSM [%]	$R^2$	Specific sorption surface ( $a_{sp}$ ) [m <sup>2</sup> /g d.m.]
WM	5.84204	5.5181	5.5799	8.7111	0.9543	193.7773
WGM	10.7416	6.9724	51.2327	34.1625	0.9660	244.8474
BM	9.3279	8.3299	110.9579	135.982	0.9097	292.5183
BGM	3.40058	10.6096	91.0235	61.9413	0.9979	372.5738

Source: own study.

Assuming that the mean square error of less than 10% (Ociecek & Ruskowska, 2018; Pałacha & Sas 2016) is a good agreement of the model fit to sorption data in the selected range of water activity, it was found that the BET model described the experimental data well only for whole white mustard seeds. The highest water content in the monolayer was found for black, ground mustard (10.6096 g H<sub>2</sub>O/100g d.m.), and the lowest for white mustard seeds (5.5181 g H<sub>2</sub>O/100g d.m.). At the same time, it was found that black mustard, regardless of the degree of grinding, was characterized by a higher water content in the monolayer than white mustard. The comparison of SKO and the relatively significant difference in the value of this parameter for different mustard types indicated that the phenomenon was similar to the model for samples of whole white mustard seeds. Table 2 shows the values of the sorption specific surface area calculated on the basis of the BET monolayer capacity. The largest sorption specific surface area, 372.5738 m<sup>2</sup>/g d.m. had whole seeds of white mustard, and the smallest, amounting to 193.7773 m<sup>2</sup>/g d.m. – black ground mustard. The obtained results showed that black mustard had a greater ability to bind water, making this product the most hygroscopic.

The obtained results should constitute a starting point in the process of planning and implementing the production and transport of mustard seeds, as well as storage recommendations of various degrees of usefulness, related to reloading works, the packaging process and, finally, consumer expectations.

## Conclusions

White mustard seeds had a lower storage stability than black mustard seeds. The variety of mustard seeds did not differentiate their hygroscopic properties. The different degree of comminution of mustard seeds did not differentiate the sorption properties of the tested material. Black mustard seeds were a more hygroscopic product.

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# 5

## ANTIMICROBIAL ACTIVITY OF COMMERCIAL NARROW-LEAVED LAVENDER (*LAVANDULA ANGUSTIFOLIA*) EXTRACTS AS AN ALTERNATIVE TO DISINFECTANTS IN FOOD INDUSTRY

Krzysztof Juś<sup>1</sup>, Szymon Frąk<sup>1</sup>, Daniela Gwiazdowska<sup>1</sup>,  
Agata Stolarczyk<sup>1</sup>

### Abstract

Microorganisms are still one of the most frequently identified hazards in food products. To counteract this phenomenon, it is necessary to constantly control microorganisms throughout the food chain, especially at the production stage. An important element in the microbiological safety management of products is the appropriate selection of disinfecting methods for abiotic surfaces in the production environment. An increasing number of reports in the literature indicate the possibility of using natural substances of plant origin, such as essential oils and other extracts which become a promising alternative as sanitizers in the food industry.

The aim of the study was to verify and compare the antimicrobial activity of different type of commercial plant extracts obtained from the narrow-leaved lavender (*Lavandula angustifolia*) against six strains of microorganisms. The obtained results indicate that the tested lavender extracts exhibit strong antimicrobial properties depending both on the type of extract and the type of indicator microorganism. The strongest inhibitory effect was observed for lavender CO<sub>2</sub> extract and for lavender oil (France) towards *E. coli* and *S. saprophyticus*. The obtained results confirm that commercially available plant substances from lavender are effective antimicrobial agents and may also play an important role in microbial infection preventing.

### Keywords:

essential oils, CO<sub>2</sub> extract, antimicrobial activity, food safety, microbial safety management, biological disinfection methods

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Natural Science and Quality Assurance; [krzysztof.jus@ue.poznan.pl](mailto:krzysztof.jus@ue.poznan.pl)



## Introduction

According to RASFF Portal data (2019), an increasing number of reports on microbial contamination in food can be observed in recent years. The most common infections are with the participation of microorganisms such as: *Salmonella enteritidis*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica* and *Campylobacter jejuni*. Infections caused by these microbes can lead to food quality deterioration, health risk for consumers and even death (Zhao, *et al.* 2017). In the prevention of microbiological contamination of food, washing and disinfection processes in food production and processing plants play a key role (Płaza, 2017). Over the years, the most effective disinfectants have been developed based on knowledge and experience. The most popular, commonly available on the market are those based on chlorine compounds, quaternary ammonium salts or inorganic acids. However, the growing problems related to the more and more frequent detection of food contaminants suggest that traditional disinfectant compounds lose their effectiveness due to their abuse and the emergence of microbial resistance mechanisms (Baranowska & Rodziewicz 2008; Donaghy, *et al.* 2019; Fagerlund, *et al.* 2017). Therefore, attempts are needed to develop new solutions in the field of disinfection allowing the development of a non-environmentally friendly solution that does not induce resistance mechanisms and at the same time an effective sanitizer.

## Characteristic and potential of plant extracts

Essential oils and their valuable physicochemical properties have been known for many years. Initially, they gained popularity as fragrances used in the production of perfumes. With the development of knowledge, their broad antibacterial and antiseptic properties began to be used in medicine and even in the food industry (Zdrojewicz, Minczakowska & Klepacki, 2014). One of the well-known plants used in obtaining oils and extracts is *Lavandula angustifolia*. Lavender contains, among others: essential oil, minerals, sugars and phytosterols (Bakkali, *et al.* 2008). The herbal raw material of lavender is its flower, which contains active substances such as: terpenes, polyphenols, sterols, tannins and lactones, coumarins, tannins, as well as essential oil (in the amount of 2 to 3%) (Radosz, *et al.* 2018). Lavender essential oil is a multi-component mixture. It consists of over 300 chemical compounds, including the main ingredients: linalool (9.5–68.9%) and linalyl acetate (12.4–32.2%). In addition, it also includes:  $\alpha$ -terpineol, 1-terpinen-4-ol, lavandulol, lavandulyl acetate, borneol and geraniol (Prusinowska & Śmigielski, 2014).

Plant substances such as oils or extracts, apart from their use as a cosmetic component, are a promising alternative to traditionally used disinfectants (Orhan-Yanikan, *et al.* 2019). Due to the content of bioactive compounds, they are successfully used to eliminate microbiological hazards of both planktonic bacteria and biofilms (Falcó, *et al.* 2019). The great advantage of these substances is their complete safety for both users and the natural environment, and what is particularly important, they do not increase the resistance mechanisms of microorganisms (Medina-Rodríguez, *et al.* 2020). This is a key factor, because the problem of the weakening effectiveness of traditional disinfectants due to the immunization of microorganisms due to their excessive use is becoming more and more serious (Baranowska & Rodziewicz, 2008; Yuan, *et al.* 2020). In recent years, researchers have assessed the effectiveness of plant compounds and their possible use as biocides, such as: grape seed extract, clove extract, oregano essential oil, risin extract, cinamon oil or black tea extract (Abouzeed, *et al.* 2018; Ishaq, *et al.* 2019; Kaskatepe, *et al.* 2016; Patil, *et al.* 2016; Rodrigues, *et al.* 2018; Rossi, *et al.* 2016).

The aim of the study was to verify and compare the antimicrobial activity of different type of commercial lavender essential oils and CO<sub>2</sub> extract obtained against several microorganisms typical for food industry. The antagonistic properties of tested plant extracts were carried out using two different methods – disc diffusion methods as well as microdilution method to evaluated minimal inhibitory concentration and minimal bactericidal/fungicidal concentration.

## Materials and methods

### Plant extracts

The research material consisted of commercial narrow-leaved lavender (*Lavandula angustifolia*) extracts (essential oils and CO<sub>2</sub> extract) purchased in the ECOSPA online store. In the experiment 5 different lavender extracts were used: lavender essential oil (*Lavandula Angustifolia*, linalool, geraniol, limonene, coumarin), Grosso variety lavender essential oil (*Lavandula hybrida* grosso herb oil, limonene, linalool, leraniol, coumarine), ecological lavender essential oil (*Lavandula Angustifolia* [Lavender] oil, linalool, limonene, geraniol), ecological Grosso variety lavender essential oil (*Lavandula hybrida* grosso herb oil, limonene, linalool, geraniol, coumarine) and lavender CO<sub>2</sub> extract (*Lavandula Angustifolia* (Lavender) flower extract, linalool, limonene, coumarin, isoeugenol, geraniol, farnesol, citral). According to producer description, all essential oils were obtained during water steam extraction while the lavender extract was obtained in the supercritical CO<sub>2</sub> extraction method. The narrow-leaved lavender from which the plant extracts were obtained came from France.

## Microorganisms

The antimicrobial activity of the studied narrow-leaved lavender extracts was determined against six microorganisms from the collection of the Department of Natural Science and Quality Assurance of the Poznań University of Economics and Business. All microbial strains were purchased from the American Type Culture Collection (ATCC). In the experiment three Gram – positive bacteria: *Micrococcus luteus* ATCC 10240, *Staphylococcus saprophyticus* ATCC 15305, *Bacillus subtilis* ATCC 11774, Gram – negative bacteria: *Escherichia coli* ATCC 8739, *Pseudomonas putida* ATCC 31483 and fungi *Candida albicans* ATCC 10231 were used. The indicator microorganisms were cultivated on liquid media Trypticasein Soy Broth (BioMaxima, Poland) for *M. luteus*, Nutrient Brtoh (BioMaxima, Poland) for *S. saprophyticus*, *B. subtilis*, *E. coli* and *P. putida* and Sabouraud Dextrose Broth (BioMaxima, Poland) for *C. albicans* in the optimal temperature condition (30°C for *M. luteus* and *P. putida* as well as 37°C for *S. saprophyticus*, *B. subtilis*, *E. coli* and *C. albicans*).

## Disc diffusion method

Antimicrobial activity of tested lavender essential oils and CO<sub>2</sub> extract was evaluated using disc diffusion method according to Paiano *et al.* (2020) with some modification. The 24-hour cultures of indicator microorganisms were diluted in 8.5% sterile saline to obtain bacterial and fungi suspensions with an optical density of 0.5 McFarland (~10<sup>8</sup> CFU/mL). The prepared suspensions of microorganisms in an amount of 1 ml were applied to a sterile Petri dishes (diameter 9 cm) and poured with a liquefied medium Trypticasein Soy Agar (BioMaxima, Poland) for *M. luteus*, Plate Count Agar (BioMaxima, Poland) for *S. saprophyticus*, *B. subtilis*, *E. coli* and *P. putida* and Sabouraud Dextrose Agar with chloramphenicol (BioMaxima, Poland) for *C. albicans*. After the substrate solidified, sterile 6 mm discs (Oxoid, Canada) soaked with tested lavender essential oils and CO<sub>2</sub> lavender extract were applied to its surfaces. The plates were incubated at 30°C (*M. luteus* and *P. putida*) and 37°C (*S. saprophyticus*, *B. subtilis*, *E. coli* and *C. albicans*) for 24 hours in aerobic condition. All experiments were carried out in three independent replicates. Antimicrobial activity was expressed as an average of growth inhibition zone (mm) around the disc soaked with tested plant extracts.

## MIC and MBC/MFC determination

The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of tested lavender extracts were determined using microdilution method for three microorganisms (*M. luteus*, *P. putida* and *C. albicans*) according to Rzemieniecki, *et al.* (2019) and Perczak, *et al.* (2019) with modifications. Firstly, the solutions of tested lavender essential oils and CO<sub>2</sub>

extract (50% concentration) were prepared by mixing the initial material with sterile water and Tween 80 (10%) as an emulsifying agent. Next, twofold dilutions were prepared in 96-well microtiter plates obtaining final concentrations in the range from 0.4 to 25%. Mueller-Hinton broth (BioMaxima, Poland) was used for bacteria and Sabouraud Dextrose Broth for fungi. Subsequently, from 24 h cultures of indicator microorganisms inoculates in Mueller-Hinton broth (for bacteria) and in Sabouraud broth (for fungi) were prepared obtaining optical density 0.5 of McFarland's scale. Next, 100 µl of prepared strains inoculates were introduced into the microdilution plate wells containing 100 µl of tested lavender essential oils and CO<sub>2</sub> extract in dilution series to achieve a final density  $5 \times 10^5$  CFU/ml. The samples were incubated at optimal temperature for microorganisms (described above) for 24 h in aerobic condition. Medium with lavender essential oils and CO<sub>2</sub> extract without bacteria/fungi inoculum was used as a negative control, whereas bacterial or fungal culture without an inhibitory factor was used as the positive control. After incubation, the optical density of samples was determined at 600 nm wavelength using BioTek Epoch 2 microplate reader. All experiments were prepared in three replicates. The MIC value was defined as the concentration of tested lavender extracts which exhibit bacterial/fungal growth inhibition by at least 90%. Minimum Bactericidal/Fungicidal Concentration was evaluated by application of 100 µl of microbial inoculate from wells with no growth observed (optical density equal to the positive control) on an agar medium and incubation at 30 or 37°C for 24–48h (depending on microorganism). The lowest concentration of the tested commercial plant extracts with no microorganisms growth was defined as the MBC/MFC.

## Results

The results of antimicrobial activity of the lavender oils and extract are shown in the Table 1. Commercially available lavender substances exhibited broad-spectrum of antimicrobial effects against indicator microorganisms with inhibition zone ranged between 11.0 mm and 34.1 mm. Similar results were obtained by other investigators for the plant compounds such as clove extract (15.8 mm to 25.8 mm for *E. coli* depending on the solvent), black tea extract (16.0 mm for *S. saprophyticus*), *Nepeta faassenii* essential oil (23.7 mm for *E. coli* and 28.3 for *C. albicans*) (Ishaq, *et al.* 2019; Jianu, *et al.* 2021; Patil, *et al.* 2016).

Results vary widely depending on the used type of essential oil or extract. Lavender CO<sub>2</sub> extract showed the most effective antimicrobial activity against all tested microorganisms with inhibition zone 23.1 mm to 27.4 mm depending on the strain. Lavender oil and lavender oil type Grosso were also very effective, with growth inhibition zone ranging from 23 mm to over 34 mm for *E. coli*, *S. saprophyticus* and *C. albicans*. However, for the two remaining bacteria, *B. subtilis*

and *M. luteus*, the results of the inhibition zone were visibly lower than for the CO<sub>2</sub> extract, ranging from 12.7 mm to 18.3 mm. The weakest results of growth inhibition (11.0 mm–20.3 mm) were achieved for ecological type of both tested essential oils. In general, *S. saprophyticus* and *E. coli* turned out to be the most susceptible microorganism to the commercial lavender extracts, whereas the strains of *M. luteus* and *B. subtilis* showed the weakest sensitivity.

**Table 1. Antimicrobial activity of lavender oils and extract against indicator microorganisms**

Treatment	Growth inhibition zone (mm)				
	<i>E. coli</i>	<i>M. luteus</i>	<i>B. subtilis</i>	<i>S. saprophyticus</i>	<i>C. albicans</i>
Lavender oil (France)	29.26 ± 0.57	13.14 ± 0.33	13.47 ± 0.76	25.22 ± 1.24	25.77 ± 1.79
Lavender oil (Grosso)	34.17 ± 1.52	18.36 ± 0.45	12.78 ± 0.14	23.07 ± 0.81	27.24 ± 1.60
Ecological lavender oil (France)	11.00 ± 0.31	13.41 ± 0.57	12.82 ± 0.43	21.00 ± 0.53	18.30 ± 0.67
Ecological lavender oil (Grosso)	19.00 ± 0.47	11.55 ± 0.57	17.42 ± 0.57	20.30 ± 0.78	18.51 ± 0.51
Lavender CO <sub>2</sub> extract	27.47 ± 0.98	23.11 ± 0.81	24.8 ± 1.11	23.52 ± 0.90	26.85 ± 1.32

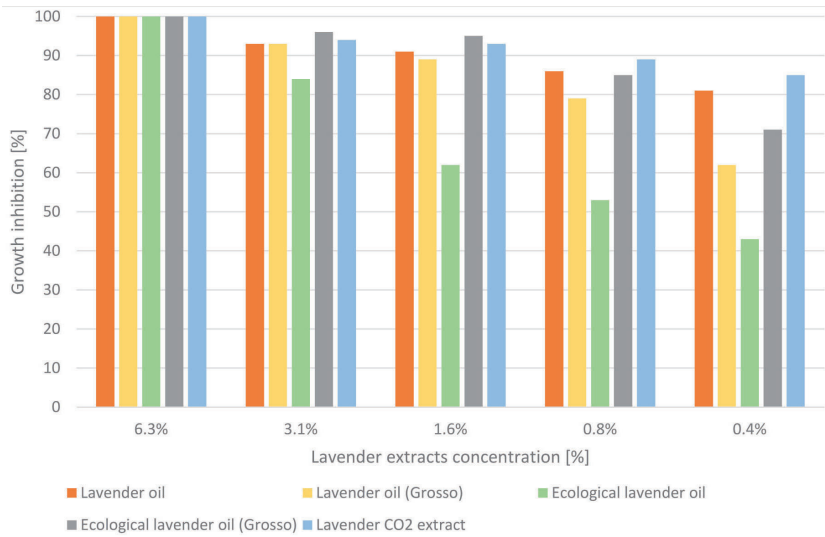
The diameter of the zone of inhibition is presented as mean (n = 3) ± standard deviation.

Source: own study.

### The growth inhibition degree depending on lavender extract concentration

The values of the growth inhibition degrees were determined by examining the antimicrobial potential of tested lavender extracts in the concentration range from 0.4 to 25%. Impact strength of the individual concentration, except for high concentrations (6.3–25%), was depended both on the type of the tested substance as well as on the indicator microorganism. The results from this experiment are presented in Figures 1–3.

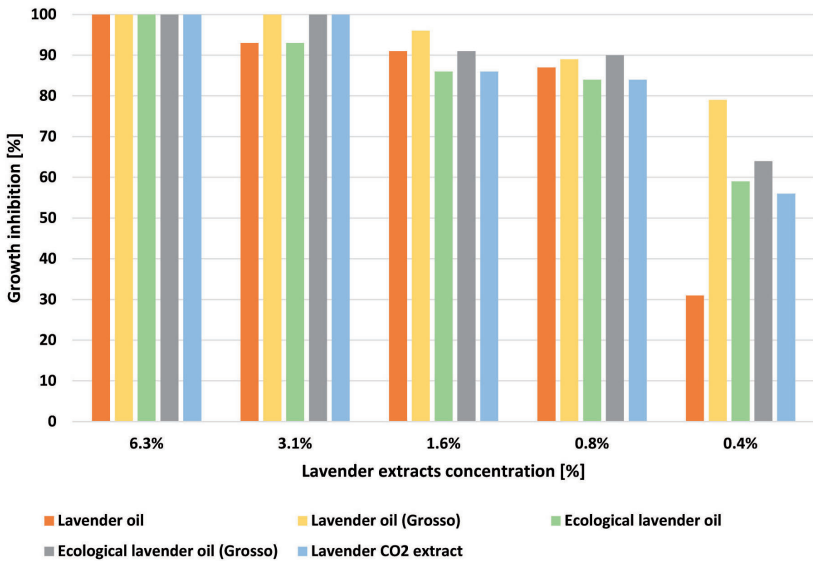
The growth of *M. luteus* was inhibited in range 43 to 100% depending on used concentration of plant substances (Figure 1). At the concentration 6.3% and above the complete growth inhibition was observed for all tested lavender oils and CO<sub>2</sub> extract. The lowest inhibitory effect was obtained by a concentration of 0.4% wherein the lavender oil and CO<sub>2</sub> extract in this concentration inhibited the growth of *M. luteus* in over 80%. The lowest activity was demonstrated by ecological lavender oil, which at a concentration of 1.6, 0.8 and 0.4 inhibited the growth of *M. luteus* only in 62, 53 and 43% respectively. The CO<sub>2</sub> extract turned out to be the most effective and inhibited the growth of bacteria by more than 80% (89 and 85% respectively for the concentration of 0.8 and 0.4%).



**Figure 1. Effect of different concentration of lavender extracts on the growth of *M. luteus***

The percentage of growth inhibition is presented as mean (n = 3); RSD (relative standard deviation) < 2%

Source: own study.



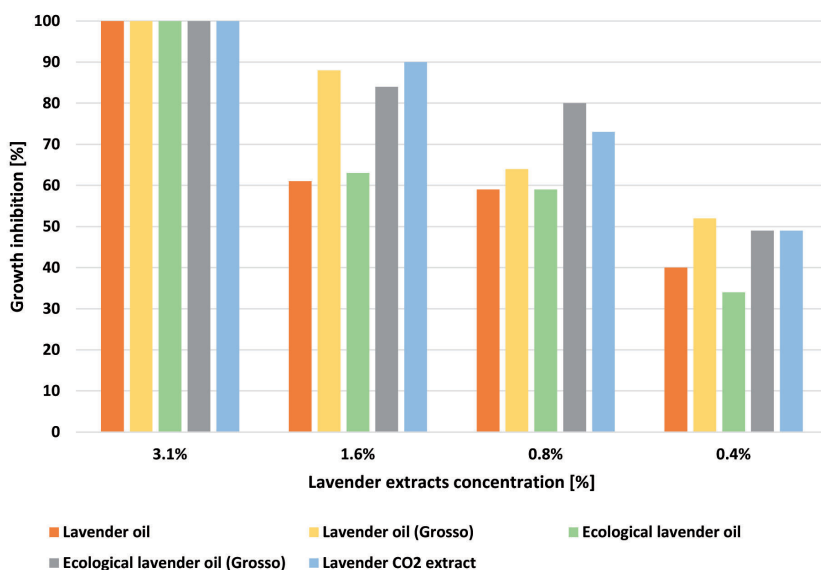
**Figure 2. Effect of different concentration of lavender extracts on the growth of *P. putida***

The percentage of growth inhibition is presented as mean (n = 3); RSD (relative standard deviation) < 2%

Source: own study.

The growth of *P. putida* was completely inhibited by Grosso lavender oil, ecological Grosso oil and CO<sub>2</sub> extract at a concentration 3.1% (Figure 2). The best results were obtained for Grosso type lavender essential oils (conventional and ecological). At the concentrations of 0.8 and 0.4% the growth inhibition was 89 and 79% and 90 and 64% respectively. The weakest inhibitory effect demonstrated lavender oil at the concentration 0.4% with the growth inhibition of bacteria at the level 31%. At the concentration 1.6 and 0.8% all tested lavender extract exhibited above 80% growth inhibition of *P. putida*.

Yeast *C. albicans* showed quite high sensitivity to all tested commercial lavender extracts at the concentration 3.1% where 100% of growth inhibition was noticed (Figure 3). At the concentration 1.6% the strongest antifungal activity was observed for lavender CO<sub>2</sub> extract (90% of growth inhibition) and for Grosso variety lavender oils (88 and 84% inhibition for conventional and ecological oil respectively). The other tested lavender oils were slightly less effective, with ecological lavender oil apparently the weakest at lower concentrations (34% of fungal growth inhibition).



**Figure 3. Effect of different concentration of lavender extracts on the growth of *C. albicans***

The percentage of growth inhibition is presented as mean (n = 3); RSD (relative standard deviation) < 2%

Source: own study.

## Evaluation of MIC and MBC/MFC value of tested plant extracts

The minimum inhibitory concentration investigated for three microorganism strains are presented in Table 2. The MIC values for each oil and extract were different depending on the microorganism. Despite the fact that the lavender-based substances showed a different degree of microorganisms growth inhibition, the MIC value was comparable and amounted around 1.6–3.1%. The exception was observed for Grosso type ecological lavender oil against *P. putida*, where MIC value obtained 0.8%. Among the plant substances tested, only ecological lavender oil was found to have a higher overall MIC value (6.3%) in case *M. luteus*, which additionally confirms the results obtained in the disc diffusion method, in which ecological oils were less effective. Analyzing the results of MBC and MFC it can be stated that these values were generally higher by half or equal to the MIC values. Only in case of lavender oil and ecological Grosso type lavender oil (for *M. luteus* and *P. putida*) as well as CO<sub>2</sub> extract (for *P. putida*) MBC value was much higher than MIC value. In case of *C. albicans* for all tested compounds MFC value was recorded at the concentration 3.1%.

**Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of lavender essential oils and extract against indicator microorganisms**

Treatment	MIC/MBC/MFC value (%)							
	<i>M. luteus</i>		<i>P. putida</i>		<i>C. albicans</i>		common MIC and MBC/MFC value for all tested microorganisms	
	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MBC/MFC
Lavender oil	1.6	6.3	1.6	6.3	3.1	3.1	3.1	6.3
Lavender oil (Grosso)	3.1	6.3	1.6	3.1	3.1	3.1	3.1	6.3
Ecological lavender oil	6.3	6.3	3.1	6.3	3.1	3.1	6.3	6.3
Ecological lavender oil (Grosso)	1.6	6.3	0.8	3.1	3.1	3.1	3.1	6.3
Lavender CO <sub>2</sub> extract	1.6	6.3	3.1	3.1	1.6	3.1	3.1	6.3

Source: own study.



## Conclusion

Ensuring adequate safety in the food chain as well as limiting the negative impact on the environment at the production stage are one of the key elements to achieve the sustainable development goals. Therefore, it becomes necessary to constantly search for new (natural) active substances and to develop effective methods limiting the possibility of microbiological hazards in food. Results of the presented experiment undoubtedly described that commercial lavender oils and extract has strong efficiency to inhibit the growth of chosen indicator microorganisms. The analysis of the results shows that it is difficult to select the most effective plant product due to the relatively similar antimicrobial activity of the tested plant extracts, which depended mainly on the type of indicator microorganism. The exception is the lavender CO<sub>2</sub> extract, which in the well diffusion method most effectively inhibited the growth of all indicator microorganisms. The reason of such results may be the richest composition of active compounds among all tested plant extracts. Also, in the case of serial dilution method on the microplate, the type of indicator microorganism affected the activity of the tested plant extracts, which was similar for all variants in the concentration range from 6.3–0.8%. The obtained results confirm that commercially available plant substances are effective antimicrobial agents and may also play an important role in microbial infection preventing in, for instance, food or cosmetic industry.

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# 6

## MICROBIOLOGICAL QUALITY CHANGES DURING SPONTANEOUS AND CONTROLLED FERMENTATION OF CURLY KALE POMACE

Katarzyna Marchwińska<sup>1</sup>, Daniela Gwiazdowska<sup>1</sup>,  
Katarzyna Czerniewicz<sup>1</sup>

### Abstract

The problem with organic waste, especially originated from agriculture and industrial processing, is increasing each year. A significant group of residues is fruit and vegetable waste including, among others, high amounts of pomace generated during processing. Pomace may imply difficulties with their storage, however the high amount of bioactive compounds makes them a valuable material to transform and reuse. Fermentation is a process leading to many changes in the profile of bioactive compounds, therefore may be used as a waste utilization process to obtain new, valuable products. However, different factors, including method of fermentation – spontaneous or controlled with selected microorganisms, may affect the course and result of the process.

The aim of the presented work was a comparison of the microbiological quality changes of curly kale pomace during spontaneous and starter dependent fermentation with *Lactiplantibacillus plantarum* L04 in two variants of hydration, ratio 1:9 and 1:1 pomace with distilled water. During the process microbiological quality and pH control were determined. The results showed that hydration degree and fermentation method affect the microbiological quality of pomace. The differences between spontaneous and controlled processes were more pronounced in samples with 50% hydration.

**Keywords:** circular economy, curly kale pomace, fermentation, waste management

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Natural Science and Quality Assurance; [katarzyna.marchwinska@ue.poznan.pl](mailto:katarzyna.marchwinska@ue.poznan.pl)

## Introduction

The problem with organic waste including household food waste, agricultural and industrial waste as well as human and animal wastes is increasing each year. Various methods of waste management such as incineration, composting or landfilling (Mussatto, *et al.* 2021) depending on the waste composition are usually used, however they have some disadvantages. For example, agricultural residues as well as co-products and by-products including, among others, bagasse, stem, stalk, fruit and fruit peel, straw or husk are usually used in animal feed or bioenergy production (Valenti, *et al.* 2020). The organic waste could be destroyed by microorganisms and forms leachate contaminating the groundwater or produce methane, which is a more harmful gas compared to carbon dioxide (Abu Yazid, *et al.* 2017; Sánchez, *et al.* 2015). Therefore, the proper waste management is important due to public health and environmental point of view and is an essential step towards a bioeconomy.

Industrial organic wastes are differentiated as they originate from various industries such as dairy industry, sugar industry, fruit and vegetable processing, slaughterhouses or poultry processing (Abu Yazid, *et al.* 2017). A significant group of residues are fruit and vegetable waste (Plazzotta, Manzocco, & Nicoli, 2017) generated during processing, including pomace. Pomace accounts approximately 10 – 35% of raw biomass, implying difficulties with their storage such as limited space in processing plants or easy contamination due to the high content of water in some of them (Gouw, Jung, & Zhao, 2017). According to the literature data pomace contains large amounts of different bioactive substances, often more than in juice, therefore they could be used as a functional ingredient in the food or pharmaceutical industry (Młynarczyk, Walkowiak-Tomczak, & Łysiak, 2018).

The high amounts of pomace are generated during processing fruits such as grapes or apples, although increasing consumer awareness of a healthy lifestyle prompts producers to use also vegetables known as superfoods, such as curly kale, in their production. Curly kale is a leafy green vegetable belonging to the family *Brassicaceae*, whose popularity is growing due to the high content of substances with beneficial effects on health. Kale contains vitamins (e.g. C, K, A, niacin, pyridoxine, riboflavin, folic acid), essentials macro and microelements such as K, Ca, Mg, Fe and Mn as well as phenolic compounds (e.g. caffeic acid, quercetin, kaempferol) and glucosinolates (GLS) like glucoiberin or sinigrin (Šamec, Urlič, & Salopek-Sondi, 2018; Szutowska, *et al.* 2020). Moreover, antioxidant properties of curly kale support protection against reactive oxygen species and free radicals in the organism helping to prevent some chronic diseases (Becerra-Moreno, *et al.* 2014; Šamec, Urlič, & Salopek-Sondi, 2018).

The fermentation of vegetables leads to many changes in the metabolites profile and bioavailability of bioactive compounds (Arqués, *et al.* 2009; Peres, *et al.* 2012)

and may be used to extend the shelf-life of products, to improve their nutritional value, as well as, waste utilization process. Solid state fermentation (SSF), defined as a process in which microorganisms grow on solid material in the absence or near absence of free water (Bhargav, *et al.* 2008), has some advantages in utilization of agriculture and industrial waste. Moreover, SSF is environmentally friendly due to the production of less wastewater and low energy requirements (Singhania, *et al.* 2009). Different factors may affect the course and result of the process including kind of microorganisms, water activity, temperature, aeration. Therefore, the aim of the presented work was a comparison of the microbiological quality of spontaneous and control curly kale fermentation processes taking into account different degrees of substrate hydration.

## Materials and methods

### Curly kale pomace preparation

Fresh green curly kale (*Brassica oleracea* L. var. *acephala* L.) leaves were purchased from local stores in Poznań, Poland. The material was washed with deionized water, drained on a sieve and dried in air for 4 hours at room temperature (~25°C). Curly kale pomace was obtained as a waste product of juice squeezing. The process took place using Hurom HP-PPE12 slow juicer (Hurom HP, South Korea). Freshly obtained pomace was subjected to the sample preparation for the fermentation process.

### Bacterial strain and growth conditions

Fermentation of curly kale pomace was performed spontaneously as well as using the starter culture *Lactiplantibacillus plantarum* L04 (Tang, *et al.* 2021). Fermentation starter strain was isolated from haylage and identified using MALDI-TOF Microflex mass spectrometry (MALDI-TOF MS) and on the basis of the 16S rRNA gene fragment sequencing. Lactic Acid Bacteria (LAB) strain used for the studies is deposited in the collection of the Department of Natural Science and Quality Assurance, in the Poznań University of Economics and Business. *Lb. plantarum* L04 was, each time, propagated on the MRS broth medium (de Man, Rogosa and Sharpe Broth), at 30°C for 48 h.

### Identification of Lactic Acid Bacteria strain

Starter strain *Lb. plantarum* L04 was determined using MALDI-TOF MS (Bruker, Germany) according to the standard producers' protocol (Dec, *et al.* 2014, 2016). LAB strain has been identified on the basis of MALDI-TOF mass spectra stored

in the BioTyper reference library and The National Center for Biotechnology Information (NCBI). For the interpretation of the confidence level, fingerprinting of cell ribosomal proteins (MALDI-TOF MS) results are divided according to the Bruker MALDI-TOF BioTyper criteria. High-confidence of results is provided by the identification index value  $\geq 2$ . The results 1.99–1.70 indicates identification on a low-confidence level, and the value  $< 1.70$  enables the microorganism identification.

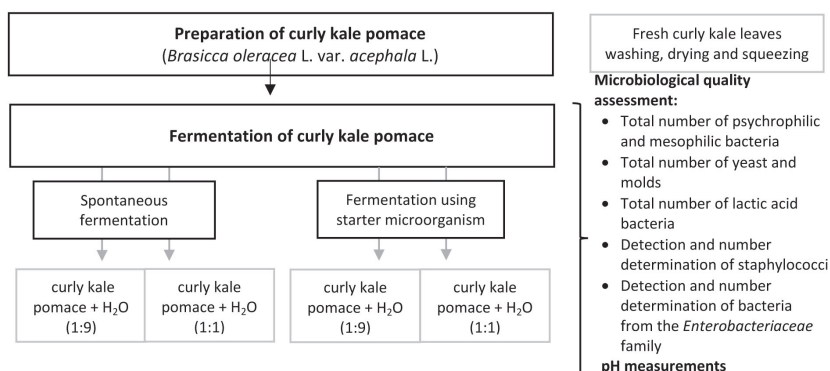
Genomic DNA of *Lb. plantarum* L04 strain was isolated using the Genomic Mini AX Bacteria+ Spin kit (A&A Biotechnology), according to the manufacturer's protocol. Genetic identification was carried out on the basis of the 16S rRNA gene partial sequence analysis. Polymerase chain reaction (PCR) was performed in the thermocycler (Biometra) using an appropriately selected pair of starters: 1492r (5' – 3' ggT TAC CTT gTT ACg ACT T) and S-D-Bact-0008 (5' – 3' AgA gTT TgA TCM Tgg CTC AG) (Leser, *et al.* 2002; Pang, *et al.* 2011) and PCR mixture: PCR Mix Plus HGC (12.5  $\mu$ l), S-D-Bact-0008 (1.0  $\mu$ l), 1492r (1.0  $\mu$ l), matrix DNA (1.0  $\mu$ l), ultrapure sterile distilled water (9.5  $\mu$ l) (according to the PCR Mix Plus, A&A Biotechnology). The total volume of the reaction mixture was 25  $\mu$ l. The PCR was performed according to the protocol: initial denaturation at 95°C (180 s), primers binding at 45°C (30 s), elongation at 78°C (120 s), 30 amplification cycles of: denaturation at 94°C (30 s), primer binding at 45°C (30 s), as well as elongation at 72°C (120 s), and finally one cycle of denaturation at 95°C (30 s), primers binding at 45°C (30 s) and elongation at 72°C (420 s). The analysis of the obtained PCR products was performed by electrophoresis on a 1% agarose gel with Midori Green dye. Further on, the external laboratory Genomed S. A. performed sequencing of the PCR product. Finch TV 1.4.0 and GeneDoc 2.7.000 softwares were used for the edition, combination and generation of 1500 base pairs sequences. Homologous sequence searching of LAB aligned sequences was analyzed using the BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/>).

### Preparation of starter culture

The *Lb. plantarum* L04 strain was freshly cultivated before the studies in MRS broth to make the starter culture inoculum. The broth culture was resuspended in 0.85% NaCl solution in order to obtain a bacterial suspension density of approximately 9 log colony forming units per ml (CFU/ml).

### Fermentation of curly kale pomace

Curly kale pomace was processed by spontaneous lactic acid fermentation and by starter dependent fermentation with *Lb. plantarum* L04 according to Figure 1. Curly kale pomace fermentation was performed in two variants of hydration, ratio 1:1 and 1:9 pomace with distilled water.



**Figure 1. The overall scheme of the conducted curly kale pomace fermentation**

The fermentation of curly kale pomace was prepared by mixing, appropriate for each ratio amount, of pomace with distilled water, and in case of controlled fermentation, starter culture inoculum, in sterile Erlenmeyer flasks. Fermentation took place in 30°C. Samples were collected aseptically before and during fermentation for measurements of pH and enumeration of chosen groups of microorganisms. Fermentation of each sample was performed in parallel (Tang, *et al.* 2021).

## Microbiological quality analyses

Microbiological quality of curly kale pomace solutions before and during the fermentation process was assessed using the standard plate method. The amount of ten grams of curly kale pomace solutions were each placed in sterile

**Table 1. Microbiological quality analyses specifications**

Medium	Application	Incubation conditions
Plate Count Agar	Total number of psychrophilic and mesophilic bacteria	15°C, 30°C 24 h
Sabouraud Dextrose Agar with chloramphenicol	Total number of yeast and filamentous fungi	23°C, 72 h
de Man, Rogosa and Sharpe (MRS) Agar	Total number of LAB	30°C, 24 h
Mannitol salt acc. to Chapman LAB-AGAR	Detection and number determination of staphylococci	37°C, 24 h
Violet Red Bile Glucose (VRBG) Agar	Detection and number determination of bacteria from the <i>Enterobacteriaceae</i> family	37°C, 24 h



stomacher filter bags (Bag Light 400) with 90 mL of 0.85% NaCl sterile solution and homogenized in the stomacher (BagMixer® 400, Interscience, France) for 5 min. Next, 10-fold dilutions were prepared, finally 1 ml of aliquots were transferred into sterile Petri dishes, mixed with medium and incubated. Direction of the experiment, appropriate microbiological media and incubation conditions are presented in Table 1. Pomace solutions samples were collected before and during the fermentation processes after 1, 2 and 4 days.

## pH measurements

Curly kale pomace samples were tested for the changes of pH values before and during fermentation processes. A digital pH meter (ThermoScientific™ ORION™ STAR A111 pH Benchtop Meter) was used for the pH level measurements. The equipment was calibrated before the readings using buffer solutions with pH values of 4.00, 7.00 and 10.00 at 25°C.

## Chemicals and media

In the experiments different microbiological media were used depending on the aim of analysis. Microbial media for LAB cultivation and enumeration: MRS broth and MRS agar were from BIOMAXIMA, Poland. For yeast and filamentous fungi count analyses Sabouraud Dextrose Agar was purchased from Oxoid, Canada. Total psychrophilic and mesophilic count was determined using Plate Count Agar medium from BIOMAXIMA, Poland. Violet Red Bile Glucose Agar used for detection and enumeration of *Enterobacteriaceae* family in tested samples was purchased from Oxoid, Canada. Mannitol salt acc. to Chapman LAB-AGAR from BIOMAXIMA, Poland, was used for the detection and number determination of staphylococci. Sodium chloride used for 0.85% water solutions was from Chempur, Polska. Reference standards of pH values: 4.00, 7.00, and 10.00 for calibration were from Merc, Germany.

## Statistical analysis

Statistical analysis of the obtained results was carried out using SPSS Statistics 25 and Microsoft Excel®. All of the performed analyses were carried out in three parallel repetitions, the results were expressed as means ± standard deviations. One-way analysis of variance (ANOVA) and Tukey's post-hoc test were performed for determination of differences between stages of fermentation at the significance level  $P < 0.05$ .

## Results and Discussion

In the presented work, the fermentation of curly kale pomace was carried out spontaneously and with LAB strain in different sample hydration degrees. As a starter for controlled fermentation experiments *Lb. plantarum* L04 was chosen. The starter strain was isolated from haylage and selected on the basis of its rapid bacterial growth, high cell concentration and high amount of lactic acid production. LAB strain was previously characterized for its functional properties, e.g. antimicrobial activity, tolerance of high concentrations of bile salts and low pH values, enzymatic activity, adhesion ability, and antibiotic susceptibility (unpublished studies). The *Lb. plantarum* L04 was identified by MALDI-TOF mass spectrometry and sequencing of the 16S rRNA gene (Table 2) and deposited in the collection of Department of Natural Science and Quality Assurance, Poznań University of Economics and Business. The LAB strain cell ribosomal proteins fingerprinting results were considered reliable as the obtained log (score) equaled 2.40 with the MALDI Biotyper database *Lb. plantarum* (1590) record. Genotype characteristic was performed on the basis of DNA sequence analysis using Finch TV 1.4.0 and GeneDoc 2.7.000 softwares. Sequence similarity searching was carried out by the BLAST algorithm of the NCBI, showing homology of tested strain to *Lb. plantarum* with the identification value of 99.45% and query coverage of 99%.

The microbiological quality of the curly kale pomace samples before and during fermentation processes was evaluated using the standard plate method, with both the propagating and selective media. Obtained results are presented in Table 3. Obtained results confirmed the presence of LAB, psychrophilic and mesophilic bacteria in all tested curly kale pomace samples, fermented both spontaneously and with starter strain, during the whole process. The number of LAB, psychrophilic and mesophilic bacteria in samples fermented without *Lb. plantarum* addition rapidly increased by about 5.5 log CFU/ml (90% hydration) and 5.0 log CFU/ml (50% hydration) in the first 24 hours of the process. Cell proliferation stopped after the first day of fermentation in case of mesophilic bacteria and slowed down for the LAB and psychrophilic bacteria. The amount of LAB in spontaneously fermented curly kale pomace samples, despite different hydration levels, after 98 h was at a comparable level ( $9.07 \pm 0.06$ – $9.55 \pm 0.06$  log CFU/ml). Various microbiota was detected in spontaneously fermented curly kale pomace, regardless of the water addition in the tested samples. Growth of yeasts wasn't observed on any of the stages of spontaneous fermentation. Occurrence of filamentous fungi was noted only for 50% hydration samples after the second day of the fermentation. The amount has increased from the amount  $<1.50$  log CFU/ml after 2<sup>nd</sup> day of process, up to  $2.39 \pm 0.09$  log CFU/ml after 4<sup>th</sup> day. Furthermore, bacteria of family *Enterobacteriaceae*, and genus *Staphylococcus* were also detected in both tested hydration levels samples fermented spontaneously. For samples containing 90% water addition, *Enterobacteriaceae*

Table 2. Identification of *Lb. plantarum* L04 by MALDI-TOF MS and 16S rRNA gene sequence

MALDI-TOF MS identification	
Reference strain (NCBI strain no.)	Log (score) value
<i>Lb. plantarum</i> (1590)	2.40
BLAST 16S rRNA gene sequencing	
L04 strain DNA sequence	Reference strain (GeneBank ID)
GGGGCAGTGGGGCGGTATACATGCAGTGCAGCAACGAACTCTGGTATTGATTGGTCTTCATCATGATTTACATTTGAGT-GAGTGGCGAACTGGTAGTAACACGTGGAAACCTGCCAGAAAGCGGGGATAACACCTGGAAACAGATGCTAATAC-CGCATAACAACTTGGACCGCATGGTCCGAGTTTGAAGATGGCTTCGGCTACACTTTTGGATGGTCCCGGGCGTATTAGC-TAGATGGTGGGTAAACGGCTACCAATGGCCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACT-GAGAACACGGCCAACTCTACGGGAGGACAGTAGGGGAATCTCCACAATGGACGAAAAGTCTGATGGAGCAAACG-CGGCGTAGTGAAGAAGGGTTTCGGCTGTAAACTCTGTTGTTAAAGAAAGAAATACTGAGAGTAAGTCTGAGTTCAGG-TATTGACGGTATTAAACGAGAAAGCCACGGCTAATACGTGCCAGACCGCGGTAAATAGTAGTGGCAAGCGTTGTCCG-GATTTATTGGCGTAAAGCGAGCCAGCGGGTTTTAAAGTCTGATGTGAAAGCCCTTCGGCTCAACCGAAGAAGTGCATCG-GAAAATGGGAAACTTGAGTGCAGAAAGGACAGTGAACCTCCATGTGAGCGGTGAAATGCGTAGATATGGAAGAACAAC-CAGTGGCGAAGCGGGCTGTCTGGTCTGTAACCTGACGCTGAGGCTGAAAAGTATGGGTAGCAAAACAGGATTAAGATACCCTG-TAGTCCATACCGTAAAGATGAATGTAAGTGTGGAGGGTTCCGCCCTTCAAGTGTGAGTCAAGTAAAGCATT-CGGCTGGGGATACGGCGCAAGGCTGAAACTCAAAGGAATTGACGGGGCCGCAACAGCGGTGGAGCATGTGGTTA-ATTGCAAGTACGCAAGAACCCTACCAGGGTCTGACATATGCAAAATCAAGAGATTAGACGTTCCCTTCGGGGACATG-GATACAGTGGTGCATGTTGTCGCAGCTCGTGTGAGATGTTGGTTAAGTCCCGCAACGAGCGCAACCCCTATTAT-CAGTTGCCAGCATTAAAGTTGGGCACCTCTGGTGAAGTGCAGTCCGGTGAACACCGAAGGAGTGGGATGACGTCAAATCATC-ATGCCCTTATGACCTGGGCTACACACGTCAGTCAATGGATGGTACAACGAGTTCGCAACTCGCGAGAGTAAAGCTAATCTC-TAAAGCCATTCTCAGTTCCGATTGAGGCTGCAACTCGCCCTACATGAAGTCGGAATCGCTAGTAATCGGATCAGCATGC-CGCGGTGAATACGTTCCCGGGCCTGTACACACCCCGCTCACCCATGAGAGTTTGTAAACCCCAAAAGTCGGTGGGGTA-ACCTTTTAGGAACCAAGCGGCTAAGTGACATTT	Lb. plantarum (MT538387.1)
Identity compliance	99.45%
Identification result	
<i>Lactiplantibacillus plantarum</i>	

bacteria were detected at the beginning of the fermentation and after 24 hours of the process. The amount of the bacteria increased from  $<1.50$  log CFU/ml up to  $2.15 \pm 0.08$  log CFU/ml, just to be absent in samples after 48 h of curly kale pomace fermentation. For 90% hydration samples, growth of staphylococci was detected only at the beginning of the fermentation, these possible pathogenic bacteria were not observed during the process as well as in the final fermented pomace samples. The presence of the *Enterobacteriaceae* family bacteria has been detected and determined both before and on all stages of 50% hydration curly kale pomace fermentation, however the amount wasn't higher than  $1.79$  log CFU/ml. *Staphylococcus* spp. bacteria occurred in 50% hydration curly kale pomace samples at the beginning and after 24 hours of spontaneous fermentation, in amount of approximately  $2$  log CFU/ml. Further on after 48 hours of process these cocci shaped bacteria were not observed in any of the tested samples.

Fermentation of curly kale pomace using *Lb. plantarum* L04 resulted in a slight increase of LAB viable cell numbers from  $8.65 \pm 0.08$  to  $9.04 \pm 0.04$  log CFU/ml (90% hydration) and from  $8.72 \pm 0.03$  to  $9.71 \pm 0.05$  (50% hydration) after 24 h. The addition of the LAB starter culture did influence the speed and efficiency of the fermentation processes for samples in 1:1 ratio of pomace and water. LAB number increased the most effectively during the first 2 days of 50% hydration sample fermentation, from  $8.72 \pm 0.03$  up to  $10.77 \pm 0.08$  log CFU/ml. For 90% hydration, the amount of LAB bacteria during the 48 hours of the processes differed by one log CFU/ml. In 0% hydration curly kale pomace samples fermented with starter strain, the growth of mesophilic bacteria did not differ at any stage of the process. For the total count of psychrophilic bacteria, only a minimal increase ( $0.14$  log CFU/ml) has been observed in the first 24 hours, next, growth decrease occurred. Finally, the amount of psychrophilic bacteria at the beginning and at the end of the process was equal ( $8.65$  log CFU/ml). On the other hand for pomace samples characterized by 50% hydration value, fermented with the addition of starter culture, the increase of psychrophilic and mesophilic bacteria cell proliferation was observed to the second day of the process. Same trend was noted for the LAB amount. During the first 24 h of fermentation the amount of these bacteria increased by approximately  $0.8$  log CFU/ml, after 48 hours it increased by another  $1.0$  log CFU/ml and further the stationary phase of growth was observed.

Yeast and bacteria of family *Enterobacteriaceae* weren't observed before and during the starter fermentation of curly kale pomace, for both samples' hydration levels. Filamentous fungi and bacteria of the genus *Staphylococcus* were observed in all of the tested samples inoculated with *Lb. plantarum* L04 only at the beginning of the fermentation processes. Total filamentous fungi amount for both sample hydration of 90% as well as 50% was under  $1.50$  log CFU/ml. Staphylococci bacteria amount before the fermentation processes differed within the hydration values of tested pomace samples. Higher amounts of cocci bacteria were observed for lower

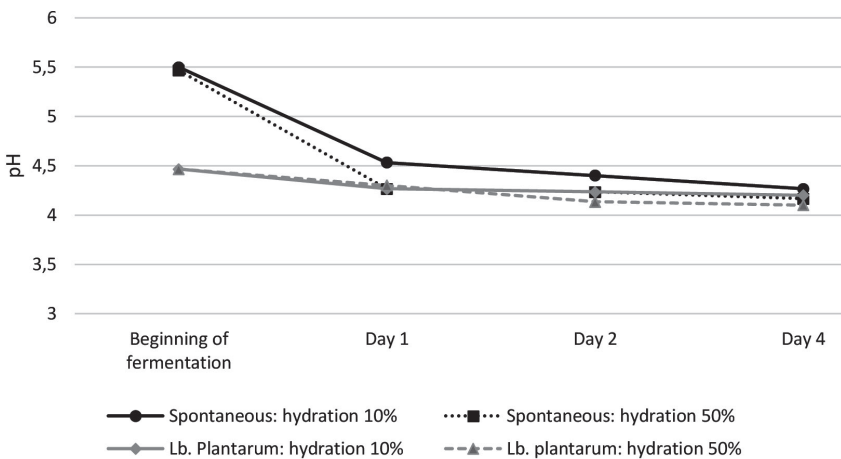
**Table 3. Microbiological quality of curly kale pomace fermented spontaneously and with *Lb. plantarum* L04**

Direction of microbial analysis	Number of microorganisms (log CFU/ml)									
	Spontaneous fermentation					<i>Lactiplantibacillus plantarum</i>				
	Beginning of the fermentation	1 <sup>st</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day	Beginning of the fermentation	1 <sup>st</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day		
	Sample hydration 90%									
Lactic Acid Bacteria	2.72±0.14 <sup>a</sup>	8.42±0.14 <sup>b</sup>	8.49±0.05 <sup>b</sup>	9.07±0.06 <sup>c</sup>	8.65±0.08 <sup>a</sup>	9.04±0.04 <sup>b</sup>	8.68±0.04 <sup>a</sup>	8.72±0.03 <sup>a</sup>		
Total mesophilic count	2.72±0.14 <sup>a</sup>	8.46±0.04 <sup>b</sup>	8.46±0.10 <sup>b</sup>	8.45±0.13 <sup>b</sup>	8.61±0.04 <sup>a</sup>	8.67±0.06 <sup>a</sup>	8.57±0.11 <sup>a</sup>	8.57±0.09 <sup>a</sup>		
Total psychrophilic count	3.01±0.03 <sup>a</sup>	8.38±0.00 <sup>b</sup>	8.70±0.07 <sup>c</sup>	8.81±0.05 <sup>c</sup>	8.65±0.03 <sup>a</sup>	8.79±0.01 <sup>b</sup>	8.69±0.08 <sup>ab</sup>	8.65±0.03 <sup>a</sup>		
Total yeast and molds count	n.d.	n.d.	n.d.	n.d.	<1.50 <sup>1a</sup>	n.d.	n.d.	n.d.		
<i>Enterobacteriaceae</i>	<1.50 <sup>1a</sup>	2.15±0.08 <sup>b</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Staphylococci	<1.50 <sup>1a</sup>	n.d.	n.d.	n.d.	<1.50 <sup>1a</sup>	n.d.	n.d.	n.d.		
	Sample hydration 50%									
Lactic Acid Bacteria	3.18±0.04 <sup>a</sup>	8.36±0.08 <sup>b</sup>	9.09±0.17 <sup>c</sup>	9.55±0.06 <sup>d</sup>	8.72±0.03 <sup>a</sup>	9.71±0.05 <sup>b</sup>	10.77±0.08 <sup>c</sup>	10.77±0.02 <sup>c</sup>		
Total mesophilic count	3.31±0.18 <sup>a</sup>	8.25±0.05 <sup>b</sup>	9.37±0.06 <sup>c</sup>	9.40±0.02 <sup>c</sup>	8.80±0.01 <sup>a</sup>	9.60±0.01 <sup>b</sup>	10.59±0.08 <sup>c</sup>	10.59±0.05 <sup>c</sup>		
Total psychrophilic count	3.23±0.01 <sup>a</sup>	8.15±0.02 <sup>b</sup>	9.35±0.08 <sup>c</sup>	9.45±0.04 <sup>c</sup>	8.73±0.08 <sup>a</sup>	9.49±0.03 <sup>b</sup>	10.52±0.02 <sup>c</sup>	10.60±0.02 <sup>c</sup>		
Total yeast and molds count	n.d.	n.d.	<1.50 <sup>1a</sup>	2.39±0.09 <sup>b</sup>	<1.50 <sup>1a</sup>	n.d.	n.d.	n.d.		
<i>Enterobacteriaceae</i>	1.79±0.49 <sup>a</sup>	<1.50 <sup>1a</sup>	1.78±0.48 <sup>a</sup>	<1.50 <sup>1a</sup>	n.d.	n.d.	n.d.	n.d.		
Staphylococci	2.22±0.01 <sup>a</sup>	2.11±0.33 <sup>a</sup>	n.d.	n.d.	2.36±0.10 <sup>a</sup>	n.d.	n.d.	n.d.		

n.d. – not detected; <sup>1</sup> growth less than 1.50 log CFU/ml, unable to determine precisely; Mean values with the same letter within each sample and the studied group of microorganisms were not significantly different at  $P < 0.05$

hydration samples level (2.36 log CFU/ml), for 90% hydration less than 1.50 log CFU/ml were detected. After the first 24 h of controlled fermentation process, bacteria of *Staphylococcus* genus and filamentous fungi were absent in the samples fermented with starter culture. This can be related to the various metabolites as lactic acid and bacteriocins produced by *Lb. plantarum*, thus the conditions for the growth of other microorganisms were unfavorable and required their adaptation.

Acidic pH of the sample during the fermentation process indicates the increase of the acid-forming bacteria number including LAB. Further on, low pH value by acidifying the environment prevents pathogenic microorganisms' growth in samples (Arquies, *et al.* 2015). The results of pH values changes before and during the fermentation processes, both spontaneous and with LAB starter, are presented on Figure 2. The pH decreased from  $5.5 \pm 0.00$  (90% hydration) and from  $5.47 \pm 0.05$  (50% hydration) to pH values  $4.53 \pm 0.05$  and  $4.27 \pm 0.12$ , respectively, during the first 24 h of spontaneous fermentation. Further on in the next days of fermentation, an acidic pH of approximately 4.2 was maintained in both samples, independent of the hydration value. The conducted studies demonstrated that the controlled fermentation process of curly kale pomace contributed to a slight decrease in pH value from 4.67 at the beginning, regardless of the sample hydration, to  $4.2 \pm 0.00$  (90% hydration) and  $4.1 \pm 0.08$  (50% hydration) at the end of the process.



**Figure 2. pH values of fermented curly kale pomace samples during the process**

Obtained results are comparable with the literature data. The rapid growth of starter culture *Lb. plantarum*, inhibited the growth of undesirable microorganisms such as filamentous fungi, staphylococci or *Enterobacteriaceae* bacteria. Increasing number of LAB cells, both in spontaneous and controlled fermentation, and production of their secondary metabolites such as organic acids, acts against the

growth of potentially pathogenic microorganisms, especially found in the first stage of fermentation. Szutowska, *et al.* (2020) similarly observed during spontaneous fermentation of green curly kale juice, the decreasing number of undesirable bacteria as *Enterobacteriaceae*, enterococci and staphylococci associated with the increasing LAB population. The occurrence of spoilage microorganisms is related with the curly kale leaves contamination during the growth, harvest, and processing. The quality of the raw product as well as the possibility of its fermentation depends also on the qualitative and quantitative composition of the plant autochthonous microbiota (Fröder, *et al.* 2007; Quansah, *et al.* 2018). In the presented study LAB viable cell counts increase was observed in curly kale pomace during both spontaneous and controlled fermentations, with better results for samples with 50% hydration levels. It proves that kale pomace was a suitable substrate for the growth of beneficial LAB microbiota. Similarly, Tang, *et al.* (2021) has successfully performed controlled fermentation of the mulberry pomace with *Lb. plantarum* (ratio 1:3, pomace to water). Authors also observed the increase in the viable cell counts of LAB as well as noted significant drop in pH values in mulberry pomace samples during controlled fermentation. The pH values of fermented green curly kale pomace were significantly reduced during the process, especially with the addition of starter strain *Lb. plantarum*. Similar tendency was observed in the work of Szutowska, *et al.* (2020, 2021) for the curly kale juice fermentation. Consistent with the present study results, Hashemi, *et al.* (2016) reported that pH decrease is related to the lactic acid production by *Lb. plantarum*. Therefore, observed pH value decrease might be the consequence of the considerable growth of *Lb. plantarum* during controlled fermentation and autochthonous LAB in spontaneous process.

## Conclusion

The presented research concerns the possibility of green curly kale pomace fermentation both spontaneous and with beneficial microorganisms such as LAB within samples different hydration values. Curly kale is a good source of various vitamins, macro and microelements and phenolic compounds, therefore its pomace is a desirable waste product for the reuse. However, fermentation of such residues as curly kale pomace requires ensuring appropriate conditions. Among most crucial are: sample hydration, temperature of the process, qualitative and quantitative composition of autochthonous microbiota as well as in case of controlled process high amount of selected starter culture cells. The conducted spontaneous and controlled fermentation of curly kale pomace significantly increased the amount of LAB. It can be concluded that both used hydration values enabled the fermentation of curly kale pomace. However, the differences between spontaneous and controlled processes were more pronounced in samples with 50% hydration. The results showed that the fermentation method



affect the quality of processed pomace. The microbiological quality of curly kale pomace samples at the beginning of the fermentation and after was satisfactory for controlled process. Due to the *Lb. plantarum* development and secondary metabolites production the inhibition of the undesirable microbiota growth was observed. No significant differences were observed in pH values after the fermentation regardless the degree of hydration and whether it was controlled or spontaneous process. Apart from the difficulties associated with the process, reusage of pomace by fermenting, due to containing beneficial microorganisms and bioactive compounds, is becoming more popular as an innovative waste management strategy, which is an essential step towards bioeconomy.

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# 7

## EFFECT OF STORAGE CONDITIONS ON THE MICROBIOLOGICAL QUALITY OF "SPIRULINA" FOOD SUPPLEMENTS

Anita Kukulowicz<sup>1</sup>, Victoria Dąbrowska<sup>1</sup>

### Abstract

In the present health food market, the *Arthrospira platensis*, has been widely used as a dietary supplement under the commercial designation "Spirulina". The aim of the study was to assess the effect of storage place and temperature on microbiological quality of spirulina food supplements. Tests were conducted on spirulina powder, spirulina in tablets and capsules. The tested products were divided into two groups. The first one consisted of spirulina was stored in a cabinet in room temperature, while the second one was exposed to sunlight and different temperature. Spirulina was analysed on the day of opening, after 30 and 90 days of storage. Total counts of aerobic mesophilic bacteria and *Staphylococcus aureus* were measured.

The lowest number of the total counts of aerobic mesophilic bacteria were found in spirulina tablets after opening ( $1.72 \pm 0.34$  log CFU/g), and the highest ( $5.21 \pm 0.11$  log CFU/g) in supplements stored in a cabinet after 90 days. The lowest ( $1.35 \pm 0.49$  log CFU/g) and the highest ( $2.33 \pm 0.04$  log CFU/g) number of *S.aureus* was found in the spirulina in capsules after opening and after 90 days of storage, respectively. The storage time had a significant effect on the number of the test microorganisms, but the form of the supplement and the place of its storage didn't significantly differentiate the average total number of microorganisms.

**Keywords:** spirulina, dietary supplement, *Staphylococcus aureus*, contamination, storage

<sup>1</sup> Gdynia Maritime University, Faculty of Management and Quality Science Department of Quality Management, Poland; [a.kukulowicz@wznj.umg.edu.pl](mailto:a.kukulowicz@wznj.umg.edu.pl)

## Introduction

Dietary supplements are becoming increasingly popular and widely available. Almost 3/4 of Poles (72%) admit taking dietary supplements, while almost half of them (48%) declare that they do so regularly (Czerwiński & Liebers, 2019). PMR Market Experts' data indicates that in 2020 online sales of dietary supplements grew rapidly by 13.5% as compared to 2019, both through online pharmacies, e-shops and other shopping platforms (Handel internetowy..., 2021). In accordance with the definition of the Food and Nutrition Safety Act of 25 August 2006, a dietary supplement is *a foodstuff the purpose of which is to supplement a normal diet, which is a concentrated source of vitamins, minerals or other substances of nutritional or other physiological value, single or complex, marketed in dosage forms, including: capsules, tablets, sugar-coated tablets and other similar forms, sachets with powder, ampoules, dropper bottles and other similar forms with liquids and powders intended to be consumed in small, measured quantities, excluding products with the characteristics of a medicinal product within the meaning of the pharmaceutical law* (Art. 3.3.39, Journal of Laws 2006, no 171, item 1225 as amended). The production of medicinal products is supervised on an ongoing basis by the pharmaceutical inspectorate, and their manufacturers are subjected to regular inspections and have to meet a number of strict requirements in order to ensure their safety. In turn, in the case of dietary supplements, the only requirement is to inform the Chief Sanitary Inspector about the introduction or intention to introduce a given supplement to the market by submitting a specimen label (Co warto wiedzieć..., 2015). On 1 January 2020, the Regulation of the Minister of Health amending the Regulation on notification forms for products introduced to the market for the first time in the Republic of Poland became effective. The Regulation makes the aforesaid form more legible, as it requires to include detailed information about the product intended for marketing and the status of the proceedings, which should contribute to an increase in the level of consumer safety (Journal of Laws 2019, item 2499; Baraniak, *et al.* 2020). Inadequate execution of the procedures related to the introduction of dietary supplements to the market and ineffective supervision over their quality does not allow to ensure a proper level of safety for consumers of such products. The knowledge of Polish people about dietary supplements and the level of awareness of the differences between OTC drugs and supplements is still relatively low. Only 27% of Poles can correctly define what dietary supplements actually are, 41% attribute medicinal properties to dietary supplements, 37% are convinced that supplements are tested for their effectiveness, while 50% believes that they are subject to the same supervision as medicinal products (Czerwiński & Liebers, 2019). Currently, there are no normative documents in Poland that would set the limits for microbiological contamination of dietary supplements. In Polish legislation, dietary supplements are regulated by legal acts on food products

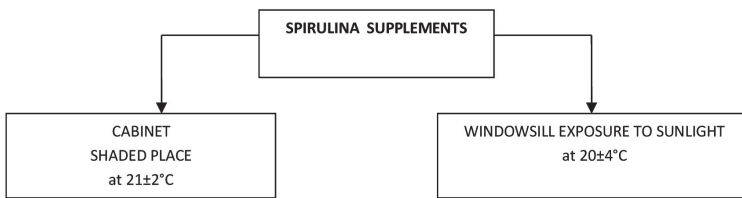
(Ratajczak, *et al.* 2015). Annex I to the Commission Regulation (EC) No. 1441/2007 of 5 December 2007 specifies microbiological criteria for different groups of foodstuffs, however without identifying dietary supplements as a separate group. Any possible contaminants and undeclared substances in supplements have a direct impact on consumers' health. The available literature on the subject reports that in some cases consumption of dietary supplements may be connected with adverse effects on human health. Supplements made from the so-called blue-green algae (spirulina), have raised suspicions regarding their carcinogenicity and toxicity. In some cases, they were found to contain a dangerous neurotoxin: anatoxin-a (Czerwiński & Liebers, 2019).

Spirulina microalgae exemplifies the cyanobacteria biomass that humans and many animals can consume. There are two main types of spirulina (*Arthrospira plantensis* and *Arthrospira maxima*) that are widely used in industry. *Arthrospira* is produced all around the world and has high nutritional value. Spirulina is an excellent dietary supplement containing 65–71% easily digestible protein. Nutritional tests have shown that spirulina algae have a protein efficiency ratio (PER) of 2.2–2.6 (74–87%) and a net protein use (NPU) of 53–61% (85–92%) and a digestibility of 83–84%. Spirulina is 10–15% of polysaccharide, since our cells can absorb them easily. Moreover, spirulina is rich in fat (1.5%–10%), out of which 10–24.5% are omega-6 fatty acids. It also contains eight essential amino acids. Dietary supplements with spirulina are an excellent natural source of vitamins (A, B2, B3, B6, B12, provitamin D and E) and minerals (magnesium, potassium, phosphorus, copper, chromium, manganese, selenium, sodium and zinc) (Großhagauer, Kraemer & Somoza, 2020; Soni, *et al.* 2021). However, some sources in the available literature on the subject report contamination of spirulina with cyanotoxins, heavy metals and pesticides (Großhagauer, Kraemer & Somoza, 2020). *Staphylococcus aureus* is the most osmotolerant foodborne pathogen. Foods of reduced water activity ( $a_w$ ) is common cause of staphylococcal food poisoning worldwide. The ability of *S. aureus* to grow with a reduced  $a_w$  is due to the intracellular accumulation of compatible solutes, including proline, betaine, choline, and taurine (Medved'ová, *et al.* 2019; Shebuski, Vilhelmsson & Miller, 2000). Plant ingredients, due to their origin, are often exposed to microbial contamination from soil, air and water. Consequently, they may also pose a risk of microbial contamination of dietary supplements (Długaszewska, *et al.* 2019).

The aim of the study was to assess the effect of storage place and temperature on microbiological quality of "Spirulina" food supplements.

## Material and methods

The research presented in this paper examined spirulina-based (*Arthrospira platensis*) dietary supplements in capsules (Singularis Superior Spirulina), tablets (Rainforest Foods Spirulina bio) and powder (Singularis Superior Spirulina) purchased from an organic food shop. The products were stored in originally-sealed brown bottles, protecting against UV radiation. Prior to testing, each bottle was inspected for any possible leaks or damage. Microbiological analyses were conducted in winter. The examined products of each form were divided into two parts according to Figure 1. When choosing the place of storage for spirulina supplements, the most frequent consumer behaviour was taken into account.



**Figure 1. Storage of samples for testing**

Source: own study.

Each supplement was subjected to microbiological analysis on the day of purchase, as well as 30 and 90 days after the opening of the product. The labels did not provide information on the permissible period of storage of the supplement after its opening. The total aerobic microbial count (TAMC) on nutrient agar by Merck (incubation at 30°C for 72h) and the number of *Staphylococcus aureus* on Baird Parker RPF base by bioMérieux (incubation at 37°C for 48h) were determined using the deep inoculation method. *S. aureus* species affiliation hasn't been confirmed by any technique (molecular, biochemical). Microbiological analyses were performed using the dilution method and calculations were based on *PN-EN ISO 7218:2008*.

The data was subjected to log transformation. After the verification of the assumptions of conformity with normal distribution (Kruskal-Wallis test) and homogeneity of variances (Cochran's test), a 2-factor analysis of variance with repeated measurements (on the day of opening the package, as well as on the 30th and 90th day after its opening) was performed. The model included main effects and correlations between the place of storage, product form and storage time, which are presented in the graphs. The Newman-Keuls post-hoc test was applied. Basic measures of location and variability (mean and standard deviation) and effect size ( $\eta^2p$ ) were calculated. The analyses were conducted using the Statistica package [StatSoft, Inc. (2011)].

## Results

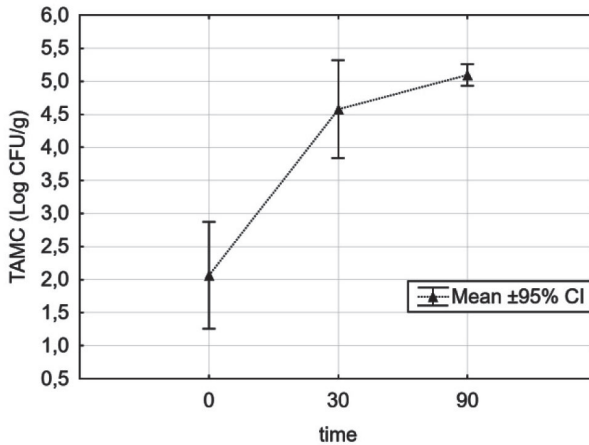
The lowest mean values of total microbial count were recorded on the day of opening for the tablet form ( $1.72 \pm 0.34$  log CFU/g), while the highest ( $5.21 \pm 0.11$  log CFU/g) – for the products stored in the cabinet for 90 days. Spirulina powder contained approximately 0.8 log cycle more mesophilic bacteria than its tablet form (Figure 4) on the day of opening the product. Although the lowest TAMC was observed for the tablets on the day of opening, after 90 days of storage the number increased almost 3 times. Storage time had an impact on the  $\log_{10}$  of the total microbial count (Table 1), with statistically significant differences observed between the mean values on the day of opening and after 30/90 days of storage (Table 1, Figure 2). The largest difference (approximately 0.6 logarithmic cycle) in

**Table 1. ANOVA results for TAMC**

Parameter	F	p	$\eta^2$ partial
place of storage	0.13	0.7546	0.06
product form	0.90	0.5266	0.47
storage time	73.19	<b>0.0007</b>	<b>0.97</b>
storage time * place of storage	1.26	0.3763	0.39
storage time * product form	0.35	0.8351	0.26

F statistics; p value;  $\eta^2$  eta squared partial.

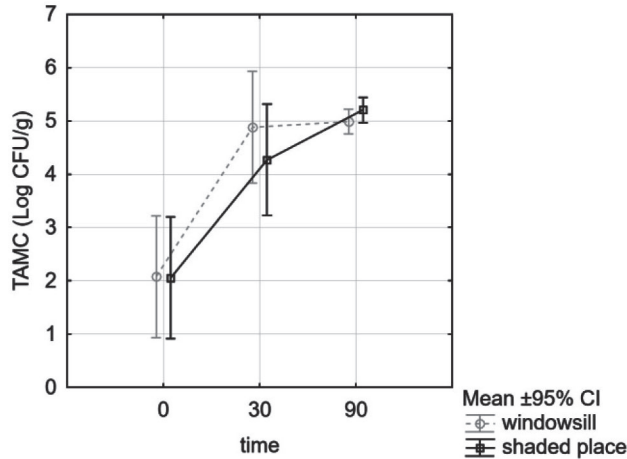
Source: own study.



**Figure 2. Impact of storage time on mean total microbial count**

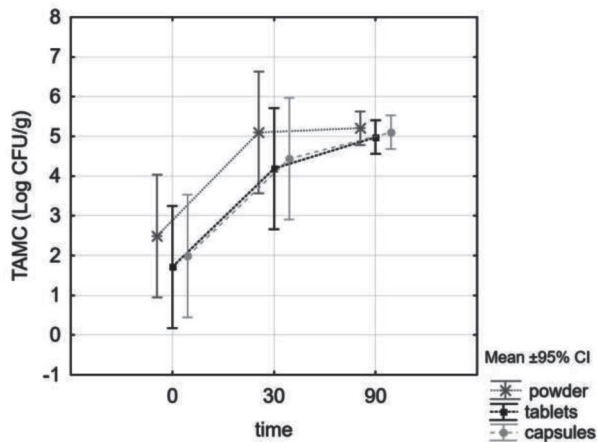
Source: own study.

the number of aerobic mesophilic bacteria between various storage locations was found after 30 days of storage (Figure 3). The supplement form and storage place, as well as correlations form-storage time and location-storage time, caused no significant variations in the mean total microbial count (Table 1, Figure 3 and 4).



**Figure 3. Correlation between the place of storage and storage time after opening**

Source: own study.



**Figure 4. Correlation between the product form and storage time after opening**

Source: own study.

The lowest mean *S.aureus* count was recorded on the day of opening for the capsule form ( $1.35 \pm 0.49$  log CFU/g), while the highest ( $2.33 \pm 0.04$  log CFU/g)

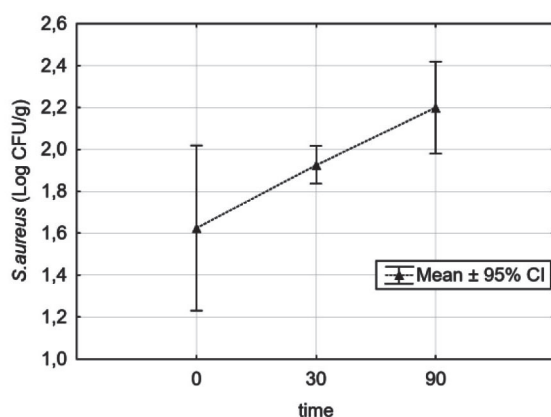
– also for the capsule form stored for 90 days. Spirulina in tablets contained approximately 0.5 log cycle more *S.aureus* than its capsule form (Figure 7) on the day of opening the product. Although the lowest number of *S.aureus* was observed for the capsule form of the supplement on the day of opening, after 90 days of storage their number increased by almost 1 log cycle (to 2.33 log CFU/g). The smallest increase in the number of *S.aureus* was observed for spirulina powder (after 30 and 90 days of storage, by about 0.1 and 0.2 log cycle, respectively) while the most significant increase was recorded for spirulina capsules (after 30 and 90 days of storage, by 0.6 and 0.4 log cycle, respectively) (Figure 7). Storage time had an impact on the number of *S.aureus* (Table 2), with statistically significant differences observed between the mean values on the day of opening and after 90 days of storage (Table 2, Figure 5).

**Table 2. ANOVA results for *S. aureus***

Variables	F	p	$\eta^2$ partial
place of storage	0.2	0.7036	0.09
product form	0.9	0.5141	0.49
storage time	13.7	0.0161	0.87
storage time * place of storage	1.4	0.3547	0.40
storage time * product form	1.9	0.2777	0.65

F statistics; p value;  $\eta^2$  eta squared partial

Source: own study.

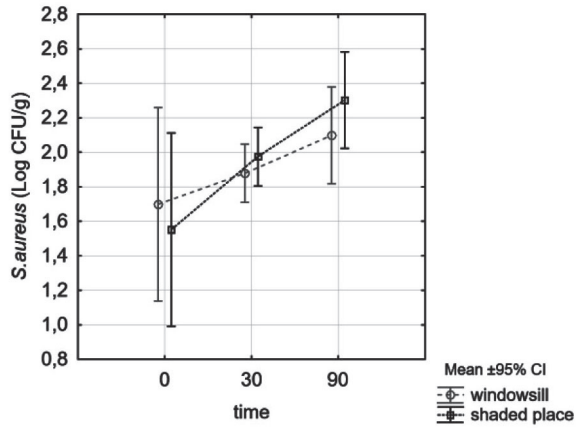


**Figure 5. Impact of storage time on mean *S. aureus* count**

Source: own study.

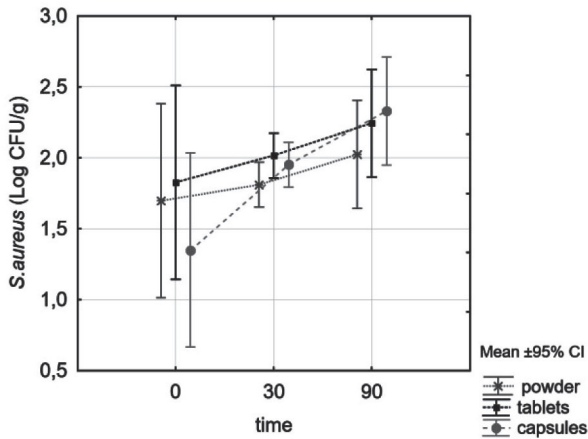


The supplement form and storage place, as well as correlations form-storage time and location-storage time, caused no significant variations in the mean total count of *S.aureus* (Table 2, Figure 6 and 7). In Figure 2 and 5, it can be observed that, compared to aerobic mesophilic bacteria, the number of *S.aureus* did not grow as rapidly over time and the growth was rather linear.



**Figure 6. Correlation between the place of storage and storage time after opening**

Source: own study.



**Figure 7. Correlation between the product form and storage time after opening**

Source: own study.

## Discussion

Dietary supplements, which are legally classified as foodstuffs, are not subject to such strict quality controls as medicinal products. For this reason, manufacturers often neglect the aspect of safety of their supplements. Dietary supplements are purchased by more than 70% of Poles, almost half (48%) of whom do so regularly (Czerwiński & Liebers, 2019). Their consumers include both healthy people and those who are ill or have weakened immunity. The presence of pathogenic microorganisms in such products can be dangerous and potentially lead to infections (Ratajczak, *et al.* 2015; Vardaka, *et al.* 2016) examined spirulina supplements available on the Greek market and found heterotrophic bacteria in these products, which were closely related to animal and human microbiota (e.g., skin, gut). This may indicate that water-attracted animals (e.g., birds, rodents) and humans when handling the product during the different processing steps. The heterotrophic bacteria found included those closely related to taxa, which are known to cause health problems (e.g. *Pseudomonas*, *Flavobacterium*, *Vibrio*, *Aeromonas*, *Clostridium*, *Bacillus*, *Fusobacterium*, *Enterococcus*) (Vardaka, *et al.* 2016). Commission Regulation (EC) No. 1441/2007 of 5 December 2007 does not classify dietary supplements as a separate product group. Edition VII of the Polish Pharmacopoeia (FP) includes the requirement of microbiological purity for medicinal products divided into four categories. Category IV applies to *herbal medicinal products containing exclusively one or more herbal substances (whole, fragmented or powdered) treated with boiling water (group A) or not treated with boiling water before use (group B)*. The spirulina supplements discussed in this paper can be classified as Category IV, group B, non-sterile medicinal products. According to the microbiological criteria listed in the FP, the acceptable total aerobic microbial count (TAMC) for these products is  $10^5$  CFU/g (5.0 log CFU/g) (Marczewska, 2018). All examined spirulina supplements (regardless of their form) met the requirements specified in the FP on the day of opening (Figure 4). After 30 days of storage of the supplements both on the window sill and in the cabinet, the TAMC did not exceed 5.0 log CFU/g (4.88 and 4.27 log CFU/g, respectively). Only spirulina powder stored for 30 days slightly exceeded the permissible values (5.10 log CFU/g). After 90 days of storage, all supplements regardless of their form and storage place contained TAMC at or slightly above the upper limit (4.98 log CFU/g) (Figure 2–4). A similar amount of bacteria in the supplements stored on the window sill and in the cabinet could be caused by the winter period in which the research was conducted. *S. aureus* is not a natural contaminant of plant-derived materials, and its presence usually indicates a lack of good manufacturing practices (GMP) and unhygienic conditions during processing and packaging (Długaszewska, *et al.* 2019). The number of *S.aureus* in the examined supplements was compared with the requirements for oral medicines. According to the limit values listed in the Polish Pharmacopoeia, *S.aureus* should

be absent in this type of products (Marczewska, 2018). The examined spirulina supplements exceeded did not meet this criterion, reaching values from 1.35 to 2.33 log CFU/g. Długaszewska, et. al. (2019) evaluated the microbiological purity of selected dietary supplements containing plant-based ingredients before their marketing, as well as the raw materials of plant origin used in the production of such supplements. The authors found that as much as 92.1% of the samples demonstrated various degrees of bacterial contamination. More than 5% of the samples were contaminated with aerobic bacteria in amounts exceeding 5.0 log CFU/g, while *S.aureus* contaminated dietary supplements and plant ingredients in 16% and 15% of the samples, respectively (Długaszewska, et al. 2019). Plant raw materials may be contaminated by microorganisms present in soil, water and the air. Obtaining high quality raw herbal material is difficult due to the risk of its contamination by pathogenic microorganisms. Contamination is more likely to occur due to: improper hygiene in the production of a given raw material, improper methods of fertilisation, poor quality of water used for cultivation and processing of raw materials, failure to observe proper hygienic conditions in warehouses, drying rooms, as well as during packaging or mixing of raw materials (Baraniak, 2020).

## Conclusions

1. The storage time had a significant effect on the number of the test microorganisms, but the form of the supplement and the place of its storage didn't significantly differentiate the average total number of microorganisms.
2. In order to market "Spirulina" and similar algae products as food supplements suitable for regular use without limitations, manufacturers should be obliged to comply with sanitary and hygiene requirements.
3. Even low levels of microbiological contamination of supplements manufactured from spirulina can pose a considerable health risk to consumer.

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# 8

## THE INFLUENCE OF EFFECTIVE MICROORGANISMS ON THE CONTENT OF SELECTED NUTRIENTS IN VEGETABLES

Agnieszka A. Pilarska<sup>1</sup>, Krzysztof Pilarski<sup>2</sup>,  
Agnieszka Wolna-Maruwka<sup>3</sup>

### Abstract

Regular intake of vitamins, mineral salts and fibre in the form of fresh vegetables is essential for the proper functioning of the human body. Vegetables, as food, affect all metabolic pathways and regulate the water-electrolyte balance of cells. Nowadays, the quality of vegetables that comes from various sources and crops, is the subject of consumer discussion, as well as scientific considerations. Fertilisation of soil with the use of the special microbiological products, in the form of the so-called effective microorganisms, may significantly affect the properties of the soil, and thus the quality of the crops.

As part of the study, vegetables were cultivated in allotment gardens with the use of specific fertilisers. Vegetable samples (control [1], fertilised with mineral preparation [2] and EM preparation [3]) were analysed in terms of their nutritional value, including vitamin A and C, selected micro- and macroelements, as well as the content of dietary fibre. Based on the obtained results, the effect of the application of EM technology on the biochemical and microbiological properties of soil was verified (activity of dehydrogenases, urease, acid phosphatase, development of bacterial and fungal biomass), and thus the nutritional value of the grown vegetables.

### Keywords:

effective microorganisms, fertilisation, chemical composition of vegetables, soil properties, ecology

<sup>1</sup> Poznań University of Life Sciences, Department of Dairy and Process Engineering, Poland; [pilarska@up.poznan.pl](mailto:pilarska@up.poznan.pl)

<sup>2</sup> Poznań University of Life Sciences, Department of Biosystems Engineering, Poland; [pilarski@up.poznan.pl](mailto:pilarski@up.poznan.pl)

<sup>3</sup> Poznań University of Life Sciences, Department of Soil Science and Microbiology, Poland; [maruwka@up.poznan.pl](mailto:maruwka@up.poznan.pl)

## Introduction

Nowadays, there is a growing trend towards ecological solutions in agriculture and a desire to increase the quality of raw materials. There is a close relationship between the nutrient content of vegetables and soil composition. Fertilisation of soil with the use of mineral fertilizers (Pilarska, *et al.* 2012; Pilarska, *et al.* 2013), as well as special microbiological products, in the form of the so-called effective microorganisms (EM), may significantly affect the properties of the soil, and thus the quality of the crops.

Soil is a complex and dynamic structure, in which there are continuous processes of decomposition and synthesis of organic and mineral matter. The function of soil is to produce biomass, participate in humification of dead organic compounds and accumulate humus. The particles that form farmland contribute to the flow of energy, water and biogenic elements. Soil is the habitat for all living organisms – it ensures appropriate oxygen, water and thermal conditions (Hillel, 2012). Assessment of the level of enzyme activity is a good indicator of the processes that take place in a given environment, including soil, and the quality of organic materials that could be used as food for plants. The level of activity of enzymes that are involved in the process of biodegradation of cellulose and lignin and the content of such elements as nitrogen, phosphorus or selected light metals are most often analysed (Kieliszewska-Rokicka, 2000). One of the group of enzymes, the levels of activity of which are most often analysed are dehydrogenases. Their presence in soil indicates the presence of active microorganisms. The activity of dehydrogenases depends on the number and species of microorganisms, physicochemical properties of soil, catalytic efficiency and the number of compounds involved in enzymatic reactions (Bielińska, 2009). Phosphatase and urease also play an important role in the mineralisation of organic matter in soil. Phosphatase, by catalysing phosphate residues separated from organic compounds (sugars, proteins, fats) and other phosphoric acid esters, enables plants and other organisms to use inorganic phosphorus in the form of  $\text{H}_2\text{PO}_4^-$  and  $\text{H}_2\text{PO}_4^{2-}$  anions (Gibczyńska & Lewandowska, 2005). Urease, in turn, is an enzyme thanks to which organic nitrogen in animal and plant remains can be reassimilated by other microorganisms or plants and used to rebuild proteins and other molecules that are necessary for the functioning of organisms (Wyczółkowski & Dąbek-Szreniawska, 2005).

The use of preparations that contain effective microorganisms is a way to enrich and restore soil, by means of natural biochemical transformations. The high level of activity of lactic acid bacteria (*Lactobacillus casei*, *Streptococcus lactis*) helps to improve the sterility of soil conditions as they prevent the proliferation of fungi (*Fusarium sp.*), bacteria (*Escherichia coli*, *Salmonella sp.*) and enterococci. Photosynthetic bacteria (*Rhodospseudomonas palustris*, *Rhodobacter spae*)

produce organic compounds in the process of partial photosynthesis, including free amino acids that are utilised by yeast (*Saccharomyces albus*, *Candida utilis*). Yeast produces active substances that stimulate the activity of lactic acid bacteria and actinobacteria (*Streptomyces albus*, *S. griseus*) of natural antibiotic effect. Fungi (*Aspergillus oryzae*, *Mucor hiemalis*) significantly improve the quality of soil by eliminating odour and accelerating decomposition of organic matter. The selection of microorganisms in EM is not random; microorganisms often act symbiotically, complementing each other's actions (Olle & Williams, 2013).

Therefore, EM technology supports natural agriculture. Mixed cultures of beneficial microorganisms are to create a more favourable environment for growing plants and their health. EM promotes effective germination, flowering, fruiting and ripening of plants; as a result of application of EM, the physical, chemical, and biological parameters of soil are improved (Boliłtowa & Gleń, 2008). In principle, EMs are widely used in fruit and vegetable production, animal production, environmental protection or composting processes (Kotarba & Paśmionka, 2015; Xu, Wang & Mridha, 2000). Based on the literature, fertilisation of soil with EM, in combination with irrigation, increases the content of various elements (e.g. boron, copper, iron, potassium, nitrogen) in potato tubers and increases yield (Boliłtowa, 2005). It can also be noticed that the size and weight of seeds increase, for example, cereals and legumes (Hu & Qi, 2013; Trawczyński, 2012). Moreover, systematic fertilisation of soil with EM helps to correct nutritional and physiological disorders of plants and reduces the adverse effects of continuous cropping by not allowing the soil to become sterile (Kucharski & Jastrzębska, 2005).

In view of the above premises, the goal of the research was to cultivate vegetables, with the use of specific fertilisers – in the open air (allotment cultivation). Samples of vegetables (carrot, beetroot, leek, parsley and celery): control (1), fertilised with mineral preparation (2) and with EM preparation (3) were analysed in terms of the content of vitamin A and C, selected micro- and macroelements and dietary fibre. Based on the obtained results, the effect of application of EM technology on specific biochemical and microbiological parameters of soil, including the activity of dehydrogenases, urease and acid phosphatase, as well as on the development of bacterial and fungal biomass, was assessed.

## Material and methods

### Cultivation of vegetables

In the research, vegetables cultivated by the author of the study were used, such as: carrot, beetroot, leek, parsley and celery. The vegetables were cultivated on

unfertilised soil (control sample), soil fertilised with organic mineral fertiliser and preparation that contained effective microorganisms. The cultivation of vegetables began in Spring, at the beginning of April 2020, and harvesting of the crop was done at the beginning of October (carrot, beetroot and parsley at the end – the vegetables stayed in the soil until mid-October). Two types of fertilisers were used in the research: *BIO fertiliser for vegetables, fruits and herbs – for organic farming – Florovit*, the composition as follows: nitrogen (2%), phosphorus (0.3%), potassium (5.5%), calcium (0.6%), organic matter. EM technology was also used, *EmFarma* fertiliser, the composition as follows: cultures of beneficial microorganisms (including lactic acid bacteria, photosynthetic bacteria), revitalised water, herbal composition, fruit juice concentrates, natural oils, composition of natural micronutrients, metabolites of beneficial microorganisms (enzymes, vitamins).

## **Analysis of the composition of vegetables**

### ***Determination of vitamin C content***

The analysis was performed with the Tillmans titration method based on the reducing properties of L-ascorbic acid in relation to 2,6-dichlorophenolindophenol, DCIP (Tillmans reagent as an indicator). Determination of vitamin C in the first stage consisted of its extraction with oxalic acid from the sample, and then oxidation of ascorbic acid to dehydroascorbic acid, in an acidic environment, with the standard blue DCIP dye (Gronowska-Senger, 2018).

### ***Determination of vitamin A content***

Reverse phase high-performance liquid chromatography (HPLC) method, using a UV-VIS (ultraviolet-visible) detector. In the first stage, saponification of the fat fraction was carried out using an alcoholic solution of potassium hydroxide with a concentration of 60%, in which vitamin A is dissolved. The saponification time was 40 min and the temperature was 70°C. After the vitamin was released from the sample, it was extracted with chloroform. The vitamin extract was then concentrated on a vacuum evaporator. The distillation residue was dissolved in a hexane (Sobolewska, Rożnowski & Fortuna, 2000). For the determination of vitamins A, HPLC liquid chromatograph, L-3000 series, Rigol Technologies, Inc., Beijing, China.

### ***Determination of total phosphorus***

The method of UV-VIS spectrophotometry with the addition of ammonium vanadomolybdate as a coloring reagent, after ashing the sample. Spectrometry is an analytical technique that uses the phenomenon of absorption of light waves in a medium of different density and structure. After the beam has passed through the test substance, the obtained UV-VIS spectrum, compared with the standard



curve, allows to determine both the presence and concentration of a given substance in the sample. The analysis was performed using the Metertech UV-VIS SP-8001 spectrophotometer, Medson, Poland.

### **Total dietary fiber determination**

The enzyme-gravimetric method, which is based on the separation of fiber from other food ingredients (fat, proteins, starch and sugars) by the use of enzymatic washing and digestion, and then by weight determination of its quantity. In the first stage, samples were prepared (ground) and Schott crucibles. Incubation with enzymes, determination of total dietary fiber (TDF) and weight measurement of sediment were performed according to the procedure presented by Popińska-Gil, Gędek & Kruczyńska, 2018. Determination of light metals using technique of atomic emission spectrometry with induced plasma, ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) was performed (JY 2000 2 ICP-OES Spectrometer, Hitachi, Tokyo, Japan).

### **Biochemical and microbiological analyses of soil**

Collected samples of soil (at the beginning of the experiment [1], during cultivation [2] and at the end of the experiment [3]) were subjected to biochemical analysis to assess the enzymatic activity of dehydrogenases, phosphatase and urease. Also, the samples of soil were tested in terms of the presence of bacterial and fungal biomass.

Activity of dehydrogenases was assessed using 1% TTC (triphenyltetrazolium chloride) as substrate, after 24 h of incubation at 30°C and application of wavelength of 485 nm with the use of Novaspec Plus spectrophotometer by Amersham Biosciences (Uppsala, Sweden), the obtained value was expressed as  $\mu\text{mol TPF}\cdot\text{g}^{-1}\cdot\text{d.m.}\cdot 24\text{h}^{-1}$ , TPF – triphenylformazan, d.m. – dry matter (Camiña, *et al.* 1998; Wolna-Maruwka, *et al.* 2019). Activity of urease was assessed with the use of urea as substrate, after 18 h of incubation at 37°C, at 410 nm wavelength, the obtained value was expressed as  $\mu\text{g N-NH}_4\cdot\text{g}^{-1}\cdot\text{d.m.}\cdot 18\text{h}^{-1}$  (Pilarska, *et al.* 2015; Zantua & Bremner, 1975). Activity of acid phosphatase was assessed with the use of sodium p-nitrophenyl phosphate as substrate, after one hour of incubation at 37°C, at 400 nm wavelength, the obtained value was expressed as  $\mu\text{mol PNP}\cdot\text{g}^{-1}\cdot\text{d.m.}\cdot\text{h}^{-1}$  (Alef, Nannipieri & Trasar-Cepeda, 1995).

Bacterial and fungal biomass was assessed with the application of a direct method based on bacterial cell count or measurement of fungal hyphae in microscope slides, with the use Breed method (a laboratory technique used for counting microorganisms). Bacterial biomass was determined after 24 h of incubation of the preparations in phenol erythrosine by counting the cells with the use of an eyepiece micrometer grid, taking into account the number of cells, average volume

of cells and specific mass. Fungal biomass was determined by measurement of fungal hyphae (length and width) stained with aniline blue (24h), with the use of an ocular micrometer. The biomass of the analysed microorganisms is expressed as  $\text{mg}\cdot\text{g}^{-1}\cdot\text{s.m.}$  of soil (Weyman-Kaczmarkowa, Pędzwiłk, 1996; 2000).

## Results and discussion

### Microbiological and biochemical analyses of soil

Table 1 presents the results of microbiological and biochemical analyses of soil.

**Table 1. Microbiological and biochemical parameters of soil**

Parameter	Unit	I term	II term		III term			
		C <sub>1</sub>	C <sub>2</sub>	S <sub>F2</sub>	S <sub>EM2</sub>	C <sub>3</sub>	S <sub>F3</sub>	S <sub>EM3</sub>
Bacterial biomass	mg/g d.m.	4.58 <sup>g</sup>	4.76 <sup>f</sup>	5.56 <sup>c</sup>	8.98 <sup>a</sup>	4.99 <sup>e</sup>	5.34 <sup>d</sup>	7.29 <sup>b</sup>
Fungi biomass	mg/g d.m.	1.22 <sup>b</sup>	1.13 <sup>c</sup>	0.76 <sup>f</sup>	0.42 <sup>e</sup>	1.44 <sup>a</sup>	0.72 <sup>f</sup>	0.53 <sup>d</sup>
Activity of urease	$\mu\text{g N-NH}_4\cdot\text{g}^{-1}$ $\text{d.m.}\cdot 18\text{h}^{-1}$	1.42 <sup>b</sup>	1.48 <sup>e</sup>	1.53 <sup>a</sup>	1.81 <sup>d</sup>	1.48 <sup>e</sup>	1.39 <sup>c</sup>	1.79 <sup>d</sup>
Activity of phosphatase	$\mu\text{mol PNP}\cdot\text{g}^{-1}$ $\text{d.m.}\cdot\text{h}^{-1}$	0.29 <sup>f</sup>	0.33 <sup>ef</sup>	0.47 <sup>c</sup>	0.88 <sup>b</sup>	0.35 <sup>e</sup>	0.42 <sup>d</sup>	0.92 <sup>a</sup>
Activity of hydrogenases	$\mu\text{mol TPF}\cdot\text{g}^{-1}$ $\text{d.m.}\cdot 24\text{h}^{-1}$	0.05 <sup>b</sup>	0.11 <sup>e</sup>	0.16 <sup>d</sup>	0.21 <sup>a</sup>	0.09 <sup>e</sup>	0.14 <sup>d</sup>	0.17 <sup>c</sup>

Explanations: a-f – averages marked with the same letters are not statistically significantly different ( $p < 0.05$ )

I term – preparing soil for sowing vegetables (end of March and beginning of April)

II term – vegetables growth, starting fertilisation (April – September)

III term – harvesting vegetables (September – October)

Soil sample symbols:

C – control (unfertilised soil sample)

S<sub>F</sub> – sample of soil fed with mineral fertiliser,

S<sub>EM</sub> – sample of soli fertilised with an effective microorganism preparation.

According to the results obtained, the cultivation of plants with the application of mineral fertiliser and EM product increased the proliferation of bacteria and the level of enzymatic activity of soil. Regardless of the period of the experiment, the effect was particularly noticeable in the sample enriched with EM preparation, which, according to the manufacturer, included lactic bacteria (*Lactobacillus casei*,

*Streptococcus lactis*), photosynthetic bacteria (*Rhodospseudomonas palustris*, *Rhodobacter spae*), yeast (*Saccharomyces albus*, *Candida utilis*), actinobacteria (*Streptomyces albus*, *S. griseus*) and fungi (*Aspergillus oryzae*, *Mucor hiemalis*).

In turn, the highest value of fungal biomass (Table 1) was obtained in samples of unfertilised soil. That parameter was reduced when mineral fertiliser was used. EM fertiliser had the greatest reducing effect on the development of fungi. Fertilisation affects properties of soil, including pH, which increases (from 5.8 to 6.4 – mineral fertiliser and 7.6 – EM fertiliser). Slightly acidic and alkaline soil promotes cultivation of vegetables and prevents growth of fungi and pathogens. In the case of fertilisation with EM preparation, that effect is clearly more favourable. Effective microorganisms successfully compete with fungi and with fungus-like organisms by reducing their adverse impact on the properties of soil.

Activity of urease and phosphatase, as a result of fertilisation with mineral fertiliser, did not increase significantly, contrary – as mentioned above – to fertilisation with EM preparation, where the increase in enzymatic activity is noticeable. In essence, the above results may indicate low efficiency of fertilisation with mineral fertiliser – poor mineralisation of organic phosphorus in soil and a small dose of supplied nitrogen but also poor content of humus in examined soil (Gałązka & Kocoń, 2015).

It is worth noting that a stronger proliferation of bacteria and a higher level of metabolic activity of the examined soil enzymes were recorded in the second period of the experiment (April-September), which was most probably related to the abundant production of root metabolites by plants that were rich in sugars, amino acids and vitamins, which is characteristic of the phase of intensive vegetative growth and flowering of plants.

## Chemical composition analyses of vegetables

The following Tables 2, 3, 4 present the results of chemical composition analyses of the grown vegetables.

**Table 2. Composition of vegetables cultivated without fertiliser**

Vegetable type	Vegetables chemical parameters							
	vit.C (mg)	vit.A (µg)	P (mg)	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	diet. fiber (mg)
Carrot	3.1	1601	30	76	251	34	13	3.2
Parsley	42	3	71	47	389	40	26	4.2
Leek	19.8	139	51	6	237	44	8	2.5
Celery	8.1	4.4	73	76	301	38	18	1.8
Beetroots	10	2.2	16	48	338	36	14	2.0

Explanation: vit. C – vitamin C, vit. A – vitamin A, P – phosphorus, Na – sodium, K – potassium, Ca – calcium, Mg – magnesium, diet. fiber – dietary fiber.

**Table 3. Composition of vegetables cultivated with mineral preparation**

Vegetable type	Vegetables chemical parameters							
	vit.C (mg)	vit.A (µg)	P (mg)	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	diet. fiber (mg)
Carrot	3.2	1616	30	79	276	34	14	3.3
Parsley	44	3	73	47	391	41	28	4.5
Leek	20.3	146	53	8	245	46	10	2.6
Celery	8.3	5.1	76	82	321	42	18	1.9
Beetroots	11	3	15	51	342	38	15	2.1

Explanation: as under Table 2.

**Table 4. Composition of vegetables cultivated with EM preparation**

Vegetable type	Vegetables chemical parameters							
	vit.C (mg)	vit.A (µg)	P (mg)	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	diet. fiber (mg)
Carrot	3.4	1668	31	81	278	34	15	3.5
Parsley	48	5	70	46	389	40	24	4.5
Leek	20.6	151	54	9	242	45	12	2.8
Celery	8.3	5.2	76	80	328	44	20	2.0
Beetroots	12	3	18	55	360	39	18	2.3

Explanation: as under Table 2.

Fertilisation with EM increases the decomposition of organic matter in soil, which – as the results in Tables 2, 3 and 4 show – can be a reason of increase of fibre content in vegetables. By analysing the changes in the values of another parameters, it can be also concluded that the effective absorption of organic compounds in the EM-fertilised soil, may have a positive impact on the increase in content of most types of vitamins in vegetables. In the studies conducted, this increase was observed for vitamin C (ascorbin acid) and vitamin A (retinol, also called beta-carotene). The high concentration of vitamin A in carrot and C in parsley (Table 2) also increased due to fertilisation (Tables 3 and 4).

However, the results of the study show that fertilisation with EM does not increase the content of micro- and macroelements.

The results obtained from the study showed the beneficial effect of using effective microorganisms on the values of soil parameters. Higher enzymatic activity was noted, which indicates the presence of organic compounds that improves the quality of soil, which are transformed during the humification process into

humus. Moreover, the EM preparation increased the bacterial mass in the soil, which competitively contributed to lowering the proportion of fungi, which are often a source of disease. The increase in amount of positive microorganisms in soil is also confirmed by authors of other works (Daly & Stewart, 1999).

Thus, an increase of retinol, ascorbic acid, and dietary fibre content in vegetables was noted as result of EM application. The effect of EM and mineral fertiliser on the increase of micro- and macrolelements in vegetables was not confirmed in the study. However, enriching composition of vegetables in nutrients following the application of EM technology in cultivation has been observed by other researchers (Fawzy, *et al.* 2012; Wierzbicka & Trawczyński, 2011; Xu, Wang & Mridha, 2000).

The effectiveness of the use of EM fertilisers in stimulating plant growth and development has been supported by many publications describing faster seed germination and protection against some pathogenic fungi (Czopek, Staniak & Marcinek, 2018; Pietkiewicz, *et al.* 2004). The results from study of Muthaura, *et al.* 2010 demonstrated that growth and yield of pigweeds may be improved by inoculating the plants with effective microorganisms, and as a result reduce the use of fertilizers in production of this vegetable hence promoting sustainable agriculture. As the same author concluded, more studies would be needed to determine the effects of effective microorganisms' inoculation on other amaranthus species. The experiments carried out in next years by Olle, Williams, 2015 were to assess the influence of effective microorganisms on the growth and nitrate content of cucumber, pumpkin and squash transplants. It was observed that cucumber, pumpkin and squash transplants grown with EM were significantly shorter and had thicker stems than those grown without EM. While the nitrate content of transplants was lower in transplants grown with EM than in those grown without. Therefore, it improves the growth and reduces the nitrate content of the vegetables used in the discussed experiment. Interesting results were obtained during a long-term study conducted at China Agricultural University's Qu-Zhou (Hu & Qi, 2013). The field experiment included three treatments: effective microorganisms (EM) compost treatment, traditional compost treatment; and unfertilized control. The results revealed that long-term application of EM compost gave the highest values for the measured parameters and the lowest values in the control plot. The application of EM in combination with compost significantly increased wheat straw biomass, grain yields, straw and grain nutrition compared with traditional compost and control treatment.

To sum up, effective microorganisms increase the beneficial microbial population in the soil for sustainable crop production, which results in the above-described positive research achievements. EM improve the structure of the soil, increase its fertility and radically improve biological diversity, suppress soil-borne pathogens, fix the nitrogen in soil and enhance nutrient uptake, accelerates

the decomposition of organic waste, residues and composting, increases beneficial minerals in organic compound, enhances the activities of indigenous microorganism and boosts the strength of plants and yield of crops (Joshi, *et al.* 2019).

As it is also confirmed by available sources of information (media, interviews, etc.), the greatest approval of EM technology is shown by farm and business owners involved in organic production. Currently, organic farming is experiencing intense development (Kowalska, 2015). Therefore, it is necessary to acquire substantive knowledge and new technologies in order to increase the quality of yields. The priority is to implement and apply methods that will effectively minimize food safety risk and environmental nuisances and threats.

## Conclusions

The results shown that both the application of mineral fertilizer and the EM product increased proliferation of bacterium and level of soil enzymatic activity. This effect was particularly noted in the option enriched by Effective Microorganisms mixture. Intensive bacterium proliferation and increased activity of the enzymes studied were obtained in the second term of the research (April – September) which, as stated, is related to characteristic secretion of root metabolite. In contrast to the value of bacterial biomass, the distribution of results for fungal biomass were different, where the highest values were achieved for unfertilised soil samples. The values of this parameter were reduced by mineral fertilisation, especially EM, which can be attributed to changes in soil reaction. However, no increase of micro- and macroelements during fertilisation was found in the results of the tests performed. The content of phosphorus, sodium, potassium, calcium, and magnesium in the control, fertilised by mineral and EM preparation, were slightly different from each other.

Fertilisation with EM product potentially increases the decomposition of organic matter, which has a positive impact on the increase of fiber content in vegetables cultivated with the use of EM noted during the experiment. The increase of vitamin C (ascorbic acid) and vitamin A (retinol) in vegetables cultivated on EM-fertilised soil was also observed, which was probably due to efficient absorption of organic compound.

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# 9

## FOOD QUALITY MADE FROM BEETROOTS

Katarzyna Mikołajczyk-Bator<sup>1</sup>

### Abstract

Red beet is a valuable source of biologically active compounds. A group of compounds with bitter taste contained in red beet are triterpene saponins. Sensory analyses of the fresh roots and saponin mixture extracts were conducted. The bitterness notes were manifested the strongest in cv. Nochowski and Chrobry. In turn, roots of red beet cv. Bonel had a strongly intensive sweet taste. The analyzed cultivars were different in terms of their total saponin content. The intensities of bitter taste and bitter aftertaste were well correlated ( $R^2 = 0.9378$ ,  $R^2 = 0.9079$ , respectively) with the total saponin content. The saponin mixture extracts, isolated from different red beetroot cultivars, were classified in terms of the bitter taste intensity and compared to caffeine solutions at 0.25 g/L as the bitterness standard. The results of the study indicated that the taste of the red beet depended on the content of mixture saponins in a red beet cultivars and part of the roots. Furthermore, the obtained results indicate an important role of triterpene saponins in shaping the taste of beetroot products.

**Keywords:** *Beta vulgaris*, saponin bitterness, bitter taste, sensory analysis

### Introduction

Changes in the food consumption patterns in the last decade have mostly been the result of newly shaped consumers' preferences but also activities promoting a healthy lifestyle. High quality of food products is the response to customers'

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Natural Science and Quality Assurance, Poland; [katarzyna.mikolajczyk-bator@ue.poznan.pl](mailto:katarzyna.mikolajczyk-bator@ue.poznan.pl)

needs who currently pay particular attention to pro-health and sensory properties of purchased food products. Meeting the consumers' expectation which is purchasing high quality food products is followed by the challenge which producers of plant materials (Kubera, 2002). In Poland a red beet is grown on the significant area of more than 10 000 ha annually, and its harvest amounts to 330 000 tons annually. Large area of beetroot growing and harvesting is related to the high consumption of this material in our country 3–4.5 kg/person/year. A red beet is a material recognized for production specialization of Polish agriculture. Poland is a leader in the EU as far as the production of a red beet is concerned.

Red beet (*Beta vulgaris* L.), is a valuable source of bioactive compounds that exhibit beneficial properties, e.g., antioxidant, anti-inflammatory and anticancer effects (Giglio, *et al.* 2018; Kapadia, *et al.* 2013; Kardum & Glibetic, 2018; Kujala, *et al.* 2002; Ravichandran, *et al.* 2012, 2013; Roemmelt, *et al.* 2014; Sawicki & Wiczowski, 2018; Sheila, *et al.* 2017; Slimen, Najar & Abderrabba, 2017; Wiczowski, *et al.* 2018). The impact on health is nowadays the attribute of food, which apart from sensory characteristics and price, has the biggest influence on purchasing behaviour of consumers. Sensory characteristics is a crucial factor, while choosing food and its consumption by the consumer. The aim of the research was to determine the influence of saponins on sensory quality of the red beets.

The carried out in this study sensory analysis of a mixture saponin extracts, isolated from beetroots has a significant influence from the point of view of commodity science, evaluation of particular types of red beets and their use, in the production of given food products. The knowledge, concerning proper application for processing different types of red beets present on the market, differentiated as regards the content quality of natural substances, is key in the development of processing industry.

## Materials and methods

### Plant materials

The experiments were carried out on the roots of three red beet cultivars, Chrobry, Bonel and Nochowski, obtained from the *Spójnia* Plant Breeding, Seed Production and Horticultural Station in Nochowo, Poland. The fresh red beetroots were washed, and then peeled to obtain the skin and flesh.

### Crude extracts from different parts of the red beetroots

The fresh red beetroots were lyophilized. Then, the saponin mixtures were isolated from the skin and flesh of the cv. Chrobry, Bonel & Nochowski beetroots

and purified using solid phase extraction (SPE) cartridges, as described previously (Mikołajczyk-Bator & Kikut-Ligaj, 2016). The saponin fractions collected from the SPE were evaporated under reduced pressure (in a concentrator RapidVap Vacuum, Labconco, USA), and the residues were re-dissolved in water (see the section: Sensory analyses of the saponin mixtures) and was used for the sensory analyses.

## Sensory analyses of the red beetroots and saponin mixture extracts

### *Selected evaluators*

The tester group included ten female and three male panelists (aged 21–40) with proven sensory sensitivity. To verify the sensory sensitivity of the panelist candidates, the triangle method was applied. Concentrations of individual substances (caffeine, lactic acid, sodium chloride and sucrose in aqueous solutions) were prepared according to the method by Corinna & Hofmann, 2014. Based on conducting the triple test, 24 people were selected as a sensory panel. Afterwards, the testers were trained to determine a taste recognition threshold and taste intensities. For bitterness evaluations, the following concentrations of aqueous caffeine were used: 0.15, 0.17, 0.19, 0.21, 0.23, 0.25, 0.27, and 0.29 [g/L]. The testers were tasked with ordering the different caffeine concentrations based on the intensity of the bitter taste. In this test, 14 people were selected.

### *Sensory analyses of the saponin mixtures*

Different concentrations of the saponin mixture solutions were chosen to a determine, the bitter concentration of the saponin mixtures isolated from the flesh and skin of the 3 red beet cultivars. The extracted saponin mixtures were dissolved in bottled water (Dobrovianka: pH 6.7 and low mineralization of 420 mg/L) at three different concentrations, ranging from 0.050 to 0.150 g/L for the flesh and from 0.035 to 0.132 g/L for the skin of the three analyzed cultivars (table 2). The saponin mixture extracts from the flesh were dissolved in bottled water at a no. 3 concentration of 0.131, 0.150, and 0.140 g/L for cv. Bonel, Chrobry & Nochowski, respectively; from the skin, 0.132, 0.128, and 0.105 g/L, respectively. Subsequently, the sensory analyses were conducted, and the taste attribute type (bitter taste, bitter aftertaste, sweet taste, earthy taste and astringent taste) yielded by the saponin extract was identified by the sensory panel in a so-called control test (0.4% water solution with a saponin mixture concentration ranging from 0.14 to 0.85 g/L, depending on the root part of the analyzed cultivars). The testers were tasked with ordering the three saponin mixture concentrations, based on the intensity of their bitter taste on a linear scale from 0 (not detectable) to 9 (very intense). Then, in the sensory evaluation of the relative intensity of the bitterness of the saponin mixtures, a multiple paired comparison test was

applied (comparing the samples of no. 1–3 extracts to the bitterness standard). In this method, caffeine was used as a reference standard, at seven different concentrations above the stimulus threshold (0.17, 0.19, 0.21, 0.23, 0.25, 0.27, and 0.29 g/L). All the analyses were conducted as three independent replicates, at sensory laboratory. The sensory panel indicated the 0.25 g/L concentration of caffeine as a bitter taste.

### **Sensory examination – a 10-point scale for beetroots**

A questionnaire prepared using the flavor profiling method was used. The following taste attributes were distinguished: bitter, bitter aftertaste, sweet, astringent and earthy. The intensity of the taste notes was measured using a linear scale from 0 (not detectable) to 9 (very intense). All the analyses were performed as four independent replicates.

### **Statistical analysis**

The obtained data are presented as mean values with the standard deviation ( $\pm$  SD). The calculations were performed using a Microsoft Excel spreadsheet and Statistica software version 13.3. The results were statistically analyzed using analysis of variance (two-way ANOVA, where the main factors were the skin and the flesh of red beetroots. The *F*-test was used as a significance of analyzed effects. A value  $p \leq 0.05$  was considered as a statistically significant.). The Pearson correlation coefficient (*R*) was calculated with a 95% as a level confidence.

## **Results and discussion**

### **Sensory analyses of red beetroots**

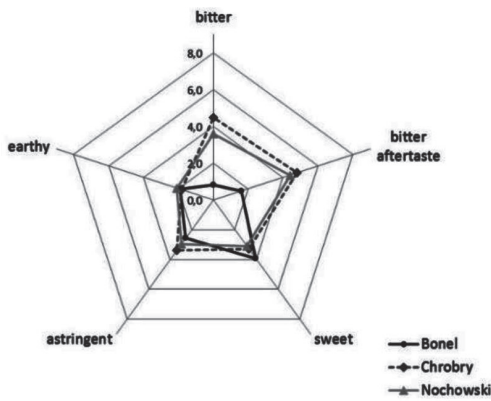
For all the examined cultivars, the intensities of the bitter taste and bitter aftertaste were stronger in the skin than in the flesh of the analyzed roots (Figure 1). The bitterness notes were the highest for the skins of cv. Nochowski & Chrobry. In contrast, the roots of the cv. Bonel red beet had a intense, strong, sweet taste and very low scores for the intensity of the bitter taste. The results of these studies indicated that the bitter taste and the bitter aftertaste, depend on the cultivars of the beetroots. The mean score for the bitter taste in the flesh of cv. Nochowski & Chrobry ranged from 3.6 to 4.5 and in the skin, respectively. The mean scores for the perception of the bitter aftertaste in the cv. Nochowski & Chrobry were 4.6 for the flesh and 6.6 for the skin. A simultaneous, lower sweet taste intensity was observed in the strongly bitter flesh of the Nochowski & Chrobry cultivars (Figure 1). Low scores for the bitter taste intensity, 0.8 for the flesh and 1.5 for the skin, were achieved for the Bonel cultivar. A strong sweet taste intensity was

recorded for this cultivar. The intensity of the astringent taste was evaluated and averaged in all the analyzed cultivars with scores ranging from 2.6 to 5.1.

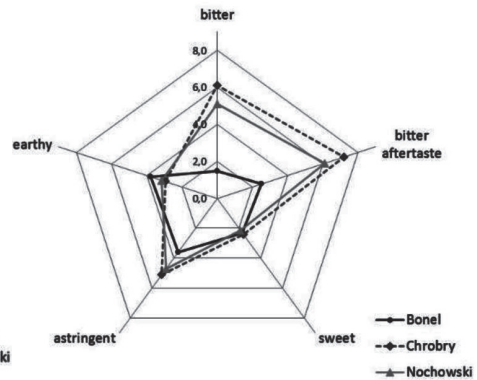
Results of this work show that (two-way ANOVA,  $\alpha = 0.05$ ), the individual cultivars of beetroot differed significantly in the intensities of the bitterness, and there were perceptible differences in the bitter taste and aftertaste depending on the flesh or skin of the beetroot. In addition, there were perceptible differences in the earthy taste depending on the part of the beetroot. The earthy taste was most perceptible in the skin of the analyzed cultivars. In contrast, the analyzed red beet cultivars did not differ significantly in the intensities of the sweet and astringent tastes.

The total saponins content in different beetroot cultivars were described in previous investigations (Mikołajczyk-Bator, 2018). The statistical analysis (two-way ANOVA,  $\alpha = 0.05$ ) revealed that the red beet cultivars differed significantly in their triterpene saponin contents, and perceptible differences in their triterpene saponin contents were found, depending on the part of the beet root (Table 1). The total triterpene saponin contents were significantly different among the analyzed red beet cultivars.

A)



B)



**Figure 1. Sensory profiling of the flesh A – and skin B – of the evaluated red beet cultivars**

The highest total content of these compounds was found in cv. Chrobry (1524.9  $\mu\text{g/g}$  d.w. in the flesh, 1963.7  $\mu\text{g/g}$  d.w. in the skin), and the lowest was found in cv. Bonel (131.4  $\mu\text{g/g}$  d.w. in the flesh, 684.5  $\mu\text{g/g}$  d.w. in the skin). The total saponin contents in the skin of red beetroots were 81, 22 and 18% higher than that in the flesh of cv. Bonel, Chrobry & Nochowski, respectively (Table 1).

The intensity of the bitter taste correlated well ( $R^2 = 0.9378$ ) with the total triterpene saponin contents in the flesh and skin of the roots (Figure 2a). A similar

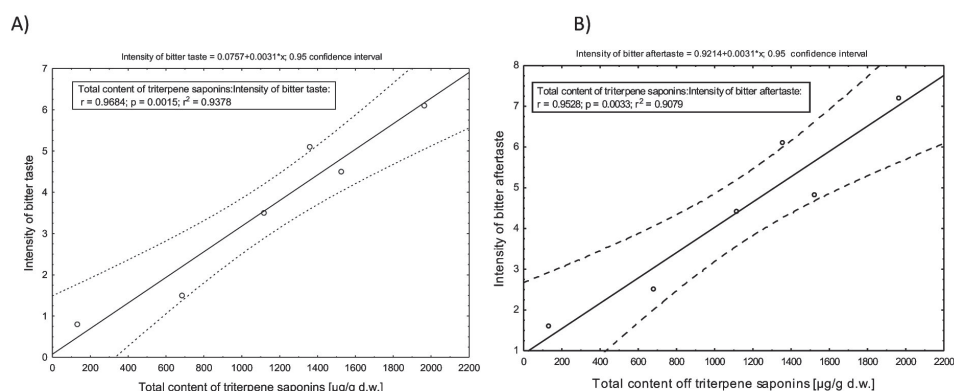
relationship was demonstrated for the bitter aftertaste ( $R^2 = 0.9079$ , Figure 2b). Our previously studies (Mikołajczyk-Bator & Kikut-Ligaj, 2016) indicate, that the red beet cultivars can be divided into two groups: in Group I (the ‘Nochowski’, ‘Chrobry’, and ‘Noe 21’ cultivars), there are the beet root cultivars having a strong level of bitter taste and in Group II (the ‘Wodan’, ‘Opolski’, and ‘Rywal’ cultivars), there are those showing a minimal intensity level of bitter taste. Other studies (Mikołajczyk-Bator, 2018) indicates, that bitter taste of product made from beetroots depend on content of triterpene saponins. It has been shown, that there is a strong correlation between the content of red beet saponins and the bitter taste.

**Table 1. Total triterpene saponin content in different *Beta vulgaris* cultivars**

Red beet cultivar	The total triterpene saponin content ( $\mu\text{g/g}$ d.w.)	
	flesh	skin
Bonel	131.4 <sup>a,A</sup> $\pm$ 24.0	684.5 <sup>a,B</sup> $\pm$ 70.6
Nochowski	1116.8 <sup>b,A</sup> $\pm$ 145.1	1359.0 <sup>b,B</sup> $\pm$ 191.7
Chrobry	1524.9 <sup>c,A</sup> $\pm$ 86.4	1963.7 <sup>c,B</sup> $\pm$ 358.1

Explanation: <sup>a, b, c</sup> The total triterpene saponin content differs significantly between red beet cultivars (significant differences between the meanings in the lines); <sup>A, B</sup> The total triterpene saponin content differs significantly between the skin and flesh of the analyzed red beet cultivars (significant differences between the averages in the columns); The data are presented as the mean  $\pm$  standard deviation.

Source: based on: (Mikołajczyk-Bator, 2018).



**Figure 2. Relationship between the total content of the triterpene saponins and the intensity of the bitter taste (a) and bitter aftertaste (b) of the flesh and skin from the analyzed red beet cultivars**

## Sensory analyses of saponin extracts from *B. vulgaris*

Analyses involving sensory identification of compounds, that caused the high intensity bitter taste and aftertaste in the beetroots were also conducted. The panelists were tested water solutions of SPE-purified samples at three different concentrations (Table 2). The evaluated samples contained mixtures of triterpene saponins obtained from the flesh and skin of the roots of cv. Chrobry, Bonel & Nochowski.

As a results of these analyses, demonstrated that individual samples of saponin mixture extracts had different intensities for bitter taste at concentrations no. 2 and no. 3. The saponin mixtures had a slightly bitter taste at the second concentration, whereas the bitter taste of the third concentration was classified, as comparable to the bitterness of a caffeine solution at 0.25 g/L. The no. 3 concentrations were different for the different cultivars for the skin and flesh, because the intensity of the bitter taste was different depending on the root part of the analyzed red beet cultivars (Table 2).

Based on the statistical analysis, concentration no. 3 (from 0.105 to 0.150 g/L) of the saponin mixture extracts, isolated from the analyzed cultivars did not differ significantly in the total content of the triterpene saponins. The third concentration had a bitter taste in the sensory analyses for all the cultivars. Other concentrations of the saponin mixture extracts had different results. For example, concentration no. 2 had a slight bitter taste, but concentration no. 1 of the analyzed saponin mixture extracts did not have a bitter taste (Table 2).

**Table 2. Different concentrations of the saponin mixture crude extracts used in sensory analyses**

No. concentration	Sensory analysis	Bonel		Chrobry		Nochowski		Standard
		flesh	skin	flesh	skin	flesh	skin	
		Total content of triterpene saponins [g/L] <sup>1</sup>						caffeine solution [g/L] <sup>2</sup>
1	no bitter	0.110	0.105	0.050	0.043	0.056	0.035	–
2	Slightly bitter	0.120	0.118	0.100	0.085	0.112	0.070	–
3	bitter	0.131	0.132	0.150	0.128	0.140	0.105	0.25

<sup>1</sup> Water solution of the saponin mixture crude extract or caffeine prepared for the sensory analysis.

<sup>2</sup> Solutions standard for the bitter taste.

Other research (Mikołajczyk-Bator & Kikut-Ligaj, 2016) confirmed, that saponin extracts isolated from red beets cultivar Nochowski had a strong bitter taste.



## Conclusions

The results of our study indicated, that the bitterness of the raw red beetroots clearly correlated to the total triterpene saponin contents in cv. Chrobry, Bonel and Nochowski, whereas the sweet, earthy and astringent tastes did not correlate to these compounds. The obtained results indicate, an important first step in elucidating the role of triterpene saponins in the sensory-perceived bitterness of red beetroots and in shaping the taste of beetroot products.

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## COMPARATIVE ASSESSMENT OF THE QUALITY ALOE VERA AND ALOE JUICE

Beata Borkowska<sup>1</sup>, Paulina Bialkowska<sup>1</sup>

### Abstract

The aim of the study was to compare the quality of juice obtained from aloe leaves and aloe juices purchased at retail. An attempt was made to verify the differences in certain physicochemical parameters between individual products.

The research material consisted of two aloe juices from selected producers (A, B) and a juice prepared from aloe leaves. The research material was subjected to the following physicochemical analyzes: determination of total acidity by titration method, vitamin C content by Tillmans method, total extract by refractometric method, chloride content by Mohr method, crude fiber by Scharrer-Kurschner method, color parameters by colorimetric method, antioxidant activity and polyphenol content by spectrophotometric method. A test of single variance analysis was carried out on the basis of the test results obtained.

The conducted research shows that the prepared aloe juice obtained a higher antioxidant activity compared to juices purchased at retail sale.

As a result of the research, it was found that the content of raw fiber in aloe vera juice was ten times higher than in the aloe juice produced by the manufacturer A and B. The individual parameters during the color determination using the colorimetric method showed that the juice obtained from the aloe leaves prepared alone was characterized by the most intense coloration. When determining the antioxidant activity and vitamin C content, significant differences were noticed in the results obtained in the producer's juices (A and B) compared to the juice produced by us.

**Keywords:** aloe vera, aloe juice, quality, comparative analysis

<sup>1</sup> Gdynia Maritime University, Department of Quality Management, Faculty of Management and Quality Science, Poland; [b.borkowska@wznj.umg.edu.pl](mailto:b.borkowska@wznj.umg.edu.pl)

## Introduction

Aloe vera is a green plant belonging to the family of Aloe vera (from Latin *Aloaceae*) with broad, fleshy, stout, pointed leaves which are usually covered with a waxy sheath. The appearance is likened to the leaves of a sea onion. The stem is located in the middle of the ground rosette and is topped with a yellow, red or orange flower that is rich in nectar. The smell of aloe vera is intense and the resulting juice is quite bitter in taste (Zamiara, 2010). There are about 400 species of aloe vera such as shrubs, trees, perennials and lianas. Of the species listed, 20 genera have been found to be medicinal. Two species are used in the cosmetic industry and in dermatology: *Aloe barbadensis* Mill, also known as Aloe vera L. and tree aloe *Aloe arborescens* Mill (Cieślak & Turcza, 2015).

The dry matter of aloe vera contains many valuable components, some of which are characterized by high biological activity. The main component is dietary fiber, its content in the dry matter of aloe vera is over 73%. This plant is also rich in fatty acids: linoleic, arachidonic, caprylic, stearic, palmitic, linolenic, which amount to 2.9%. Aloe vera contains large amounts of water. In raw pulp its amount is almost 98.5% and its pH value is 4.5. Additionally, aloe vera contains vitamins, enzymes, endogenous and exogenous amino acids, carbohydrates and other biologically active substances. In addition, the plant contains minerals that contribute to the proper functioning of enzymes and act as antioxidants including copper, chromium, selenium, calcium, magnesium, manganese, potassium, zinc and sodium (Matejczyk, Golonko & Chilmon, 2017).

The plant is native to the dry, desert and savanna areas of South Africa, East Africa, Madagascar, Mascarene and Socotra. Today, aloe vera is found in the Mediterranean and Caribbean, India, Indonesia, North America and Australia. Despite its exotic origin, aloe vera has long been used in Polish folk medicine to treat lung diseases, wounds and burns. In Poland, mainly fresh aloe vera leaf preserves grown in greenhouses are used (Czerwionka, 2016).

Nowadays, aloe vera is a plant that has been broadly applied in various industries. The plant is grown at home and used as a food or cosmetic product. However, most often consumers choose to purchase aloe vera products. When choosing products with aloe vera leaf juice, it's advisable to pay attention to the nutritional value on the package. The effect of aloe vera on health is determined, among other things, by its percentage content in a given product. Many manufacturers claim that their brand juices are 100% natural aloe vera juice, which is often not true. Another important issue is the type of production used in the processing of aloe vera, the type of transport and storage conditions of aloe vera. There are many factors that can greatly impair the quality of aloe vera juice.

The aim of the study was a comparative assessment of the quality of juice obtained from aloe vera leaves and aloe vera juices purchased in retail sale of selected producers. The following research hypotheses were formulated:

1. Aloe vera juices purchased in retail have lower antioxidant activity, vitamin C content and dietary fibre.
2. The technological process of aloe vera processing changes the color of the aloe vera juice.

## Material and methods

### Characteristics of research material

The research material consisted of 2 aloe vera juices of selected producers purchased in retail sale and aloe vera leaf juice prepared by the author herself. The aloe leaves were cleaned of dust by washing them with distilled water and the thorns along with the outer woody skin were removed with a knife. The gelatinous substances and the fillet were homogenized with a blender until smooth. The aloe vera juice was squeezed through sterile gauze. The common aloe vera used in the study was a six-year-old plant. For a clearer interpretation of the test results, the test material has been marked with symbols: aloe vera juices purchased in retail (A and B) and aloe vera leaf juice obtained by the author (C). The aloe vera juices of the selected manufacturers had the following composition:

- aloe vera juice from producer A: juice squeezed from aloe vera leaves 99.90%, acidity regulator (citric acid),
- aloe vera juice from manufacturer B: aloe vera juice reconstituted from 99.99% concentrated juice.

In the purchased aloe vera juices shown above, only the juice manufacturer (B) included storage information on the package at the end of the ingredients description. Another additional information was a note not to consume in case of hypersensitivity to any of the ingredients and that aloe vera juice is not recommended for pregnant women or nursing mothers.

Due to the increasing prevalence of food intolerances and allergies among consumers, manufacturers are obliged to include a declaration of the presence of commonly occurring allergenic ingredients in their products and must also include information on contraindications to consumption. According to Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, the manufacturer is obliged to include the nutrition declaration on the label of the product. Only the manufacturer's aloe vera juice (B) contained such nutritional claim.

## Research methodology

Aloe vera juices of selected manufacturers (A and B) and the juice prepared from aloe vera leaves (C) were subjected to laboratory tests in order to determine selected physicochemical parameters. Comparative assessment of the quality of the tested aloe juices comprised the following physicochemical analyses: determination of color parameters with colorimetric method (Wilczyńska, 2012), vitamin C content with Tillman's method (PN-A-04019:1998), total extract with refractometric method (PN-A-75101-02:1990/Az1:2002), chloride content with Mohr's method (PN-A-75101-10:1990), acidity content with titration method (PN-A-75101-04:1990/Az1:2002), crude fibre content with Scharrer-Kürschner's method (Przybyłowski, Śmiechowska, 1996), antioxidative activity and polyphenols content with spectrophotometric method (Wilczyńska, 2012).

The results of the research have been subjected to statistical analysis with the application of the test of variance analysis for many averages for the evaluation of selected physicochemical parameters of aloe vera juice in order to check whether the examined factor, i.e. the process of aloe vera production, exerted significant influence on the shape of average values of the examined physicochemical parameters. The calculated values of F statistic for the examined quality indicators were compared with the critical value read from the Snedecor's F distribution table for the assumed significance level  $\alpha = 0.05$  (Stanisz, 1998).

## Results and discussion

The results of selected physicochemical analyses in aloe vera juices are presented in Table 1.

During the preparation of the samples of juices with aloe vera for testing, a considerable difference in intensity of color was visible between the juice made by the author herself (C) and the juices purchased from producers (A) and (B). The hue and intensity of color is mostly influenced by environmental factors, therefore it is important that the products are stored in a cool place without access to light and in a dark bottle. During the colorimetric method the most intensive green color was found in freshly squeezed juice from aloe vera leaves (C), which was determined by the lowest, negative value of coordinate  $a^*$  (-7,02). The highest negative  $a^*$  value was found in the aloe vera juice of producer A (-0.23) which color was the lightest green. The parameter  $b^*$  represents the proportion of blue or yellow color. The juice from aloe vera leaves showed the most intense yellow color  $b^* = 8.44$ . The highest coordinate value of  $L^* = 25.27$  was obtained by the aloe vera leaf juice (C) because its color was the darkest, and the lowest coordinate value of  $L^* = 22.92$  was obtained by aloe vera juice by producer (B).

Acidity of aloe vera juice was another quality parameter determined. The highest total acidity was found in the juice of aloe vera leaves of producer C – 0.3897 g/100 g. The juice of producer A had the lowest acidity and it was 0.1340 g/100 g.

The highest vitamin C content in the study was found in the aloe vera juice of producer A (5,411.1 mg/100 g), however this result is not reliable because the producer added ascorbic acid as an antioxidant to the aloe vera juice. The vitamin C result is adulterated and is not indicative of the actual vitamin content of the aloe vera juice (A). The actual highest amount of vitamin C was in the juice that was made from aloe vera leaves (C), as it is pure juice with no antioxidants added. The vitamin C content was 4.0793 mg/100 g. The lowest content of vitamin C was found in aloe juice of producer B – 3.4992 mg/100 g.

The results of the research conducted by Cieślak and Turcza prove that vitamin C content in aloe vera leaves was 4 mg/100 g. The content of vitamin C in the tested aloe vera juices made by the author ranged from 3. 4992 to 5. 4101 mg/100 g. The content of ascorbic acid was at a similar level as in the study by Cieślak & Turcza (Cieślak & Turcza, 2015).

**Table 1. Results of physicochemical analyses of tested aloe vera juices**

Designation name	Aloe juice A	Aloe juice B	Aloe juice C
acidity [mg/100 g]	0.1340	0.3126	0.3897
chlorides [%]	0.0127	0.0175	0.0175
crude fibre [g]	0.89	0.64	7.53
antioxidant activity[AA%]	6	14.7	22.45
vitamin C [mg/100g]	5.4101	3.4992	4.0793
total extract [%]	0.5	0.5	0.75
polyphenols [mg GAE/100 g]	–	–	8.95
color parameters			
<i>L</i> *	23.8	22.92	25.27
<i>a</i> *	-0.23	-0.46	-7.02
<i>b</i> *	0.71	1.89	8.44

Source: own study.

The total extract assay was performed to determine the sum of all components that transferred to the aloe vera extract as a result of the extraction process and are non-volatile up to the boiling point of the solvent.

The highest percentage of the total extract content was found in the aloe vera leaf juice (C) prepared by the author herself (0.75%), lower values of the total extract were determined in the aloe vera juices of the producers A and B, which remained at the same level of 0.5%.

Aloe vera leaf juice (C) and producer's (B) juice contained equal amounts of chloride at the level of 0.0175%. Juice of producer A had a lower chloride content of 0.0127%.

After the determination of crude fibre with the Scharrer-Kürschner's method it was found that the juice from aloe vera leaves (C) had the highest content of crude fibre and it amounted to 7.5 g. Aloe vera juices purchased in retail outlets had about 10 times lower crude fibre content. The juice of producer A contained 0.89 g of crude fibre, whereas aloe vera juice of producer B contained 0.64 g of crude fibre. In spite of the highest crude fibre values obtained from own production of aloe vera juice, the obtained result is not satisfactory. Compared with the results of a study on crude fibre content in aloe vera leaves conducted in Great Britain ( $73.35 \pm 0.30\%$ ) (Liu Chun-hui, *et al.* 2007), it is 9 times lower. The crude fibre content of aloe vera leaves depends on the age of the plant, the older the aloe the better the healing properties.

Aloe (C) leaf juice showed the highest antioxidant activity (22.45%) it was almost 4 times higher than the lowest value found in the aloe vera juice of producer A (6%). Aloe vera juice of the manufacturer B had antioxidant activity of 14.7%.

Three aloe vera juices were tested for determination of polyphenols content with spectrophotometric method. The content of polyphenols could be determined only in aloe vera leaf juice (C) and amounted to 8.95 [mg GAE/100 g]. Aloe vera juices purchased from retail manufacturers A and B had insufficient color intensity that prevented absorbance reading. In the study by Heś *et al.* total polyphenols content in the tested aloe extract was determined at the level of 17.85 mg GAE per 1 g d.m. (Heś, *et al.* 2016). This value is twice lower compared to the data reported by Ray, *et al.* (Ray, Dutta Gupta & Ghosh, 2013), which is also confirmed by our own results of the aloe vera juices tested.

The results of the statistical analysis of the tested quality indicators of aloe vera juices have been presented in Table 2.

**Table 2. Results of univariate analysis of variance of the studied physicochemical indices in aloe vera juices**

Designation name	F calculated	Test F Snedecora
acidity	0.0016	7.708647
chlorides	0.0807	7.708647
crude fibre	1.0996	7.708647
antioxidant activity	9.0584	7.708647
vitamin C	0.0634	7.708647
total extract	0.125	7.708647

Source: Own research.



As a result of statistical analysis, no significant differentiation of the examined physicochemical parameters in aloe vera juice depending on the producer (method of obtaining the juice) has been demonstrated, except for antioxidative activity, which showed significant differentiation in the examined aloe vera juices.

## Conclusion

The Author's own research has shown significant differences, albeit not statistically significant in contents of physicochemical indicators between particular aloe juices. The juice obtained independently from aloe vera leaves (C) obtained the highest contents in each of tested quality parameters.

In own study, the dietary fiber content between the different products was significant. The juice obtained from aloe vera leaves obtained 10 times higher crude fibre content in comparison with the juices of producers A and B.

During determinations of antioxidative activity with spectrophotometric method and vitamin C content it was found that prepared aloe vera juice (C) demonstrated higher antioxidative activity in comparison to juices (A) and (B) purchased in retail stores.

In conclusion, the aloe vera juices of manufacturers A and B had a low content of quality components tested, and the health benefits of aloe vera in this case will be negligible. The research results indicate that the tested juices contained a lower percentage of aloe vera juice than the producers declared on their products. This may be proved by a much lighter color of the juice in comparison with aloe vera juice prepared by the Author as well as a manufacturer's choice of a more economic form of aloe juice production, which results in a reduction of its quality and higher losses of nutrients.

When determining the polyphenol content in the study, it was only possible to test the aloe vera juice obtained from the leaves because the color of the product was darker. The other purchased juices had too weak a color for the absorbance reading.

The results of own research have shown that the highest contents in all physicochemical analyses were obtained in aloe vera leaf juice prepared by the author herself.

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# 11

## THE INFLUENCE OF JAPANESE QUINCE JUICE ADDITION ON SENSORY PROFILE AND COLOR PARAMETERS OF CLOUDY APPLE JUICE

Inga Klimczak<sup>1</sup>

### Abstract

Recently, new plant materials have been searched for due to the competitive fruit juice market and growing consumer demands. Due to the valuable chemical composition and attractive aroma, Japanese quince fruits (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach) can be interesting and valuable addition to fruit juices. When assessing the quality of juices, sensory testing has become a useful tool for evaluating these products.

The aim of the study was to evaluate the influence of addition of Japanese quince juice (JQJ) on sensory attributes and color parameters (CIE L\*a\*b\*) of cloudy apple juice (AJ). Moreover, in all analyzed juices, total phenolic content using Folin-Ciocalteu method was determined.

The addition of Japanese quince juice to apple juice had a significant effect on the color parameters of apple juice. As the concentration of JQJ increased, the sensory quality of juices was evaluated through descriptive tests with the aid of trained panel. Only the addition 15% of the Japanese quince juice had a positive effect on the score of overall sensory evaluation. Fortification of apple juice with 25 and 35% JQJ was negatively evaluated by the sensory panel and was associated with increased astringent and sourness rates in apple juice.

**Keywords:** Japanese quince juice, apple juice, sensory quality, color

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Food Quality and Safety, Poland; [inga.klimczak@ue.poznan.pl](mailto:inga.klimczak@ue.poznan.pl)

## Introduction

The genus *Chaenomeles* is represented by four East Asian species: *C. japonica* (Thunb) Lindl., *C. speciosa* (Sweet) Nakai, *C. cathayensis* (Hemsl.) Schenider and *C. thibetica* Yü. The plant is a type of shrub belonging to family Rosaceae and subfamily Pomoideae. These species commonly known as Japanese quinces, because firstly plants were introduced into Europe from Japan and their fruit with numerous seeds resembles the fruit of the common quince genus *Pseudocdonia*. Japanese quince fruit is a valuable dietary product. The fruit is an important source of polyphenols, vitamin C, organic acids, minerals and dietary fiber (Du, *et al.* 2013; Hellin, *et al.* 2020; Nahorska, 2014; Tarko, Duda-Chodak & Pogoń, 2010; Watychowicz, *et al.* 2017), This plant having many medicinal properties. Recent *in vivo* and *in vitro* studies suggest that *Chaenomeles* fruit can help in the healing process of diabetes, tumor, allergies and liver diseases. Its anti-inflammatory, analgesic, antispasmodic, antioxidant, immunoregulatory, antibacterial, and antinociceptive properties have been confirmed. These bioactivities have been mainly ascribed to the high content of phenolic compounds and vitamin C (Antoniewska, Rutkowska & Adamska, 2017; Watychowicz, *et al.* 2017; Zhang, *et al.* 2014).

A raw Japanese quince fruit has a sour, astringent flavor which disqualifies these fruits from direct consumption. The unacceptable taste of quince is due to three times more acids than sugars. It is considered that fruit intended for direct consumption should contain at least 10 times more sugars than acids (Lesińska, 1986; Tarko, *et al.* 2010). However due to the valuable chemical composition and attractive aroma, quince fruits can be an appropriate material for processing in the food industry. Several products were developed based on fruit juice, purée and extracts, including products such as quince ice cream, lemonade, jam, curd, yoghurt, cake and chips (Antoniewska, *et al.* 2017; Hellín, *et al.* 2003; Kowalska, *et al.* 2020; Nawirska-Olszańska, 2010).

Today's consumers are more educated than ever before. They want to know what ingredients are used in their products and where they come from. They are looking for food that tastes, looks attractive and provides potential nutritional benefits (Bech-Larsen & Scholderer, 2010). The recently observed increase in the consumption of chilled fruit juices as well as NFC juices (not from concentrate) also proves the interest of consumers in natural and high-quality products. In Poland among NFC juices, apple juice is produced in the largest amount (Nosecka, 2020). It has been shown that cloudy apple juices contain much more fiber and polyphenolic compounds than clear varieties and have a significantly greater antioxidant potential than clear juices (Markowski, *et al.* 2015; Oszmiański, 2007).

Due to the considerable competition on fruit juices market and consumers' needs and tastes change over time, firms often redesign and reformulate food products

once they are already on the market. Apple and quince juices are well known for their distinctive taste and aroma as well as health benefits. Mixing of these fruit juices can increase their quality, give a unique flavor, color and improve nutritional composition of new product. When assessing the quality of juices, sensory testing has become a useful tool for evaluating these products.

The aim of the study was to evaluate the influence of addition of Japanese quince juice (JQJ) on sensory attributes and color parameters (CIE  $L^*a^*b^*$ ) of fresh, cloudy apple juice (AJ). Moreover, in all analyzed juices, total phenolic content using Folin-Ciocalteu method was determined.

## Materials and methods

### Apple juice

Freshly prepared (unpasteurized), cloudy apple juice (AJ) was obtained from the local juice manufacture. Apples used to obtain juice derived from the so-called old varieties: Cytrynówka, Żelaźniak, Grey Reneta.

### Japanese quince juice preparation

Japanese quince fruit (JQJ) was cut in half and juice was obtained by pressing in a high-speed juicer. Sample of JQJ was frozen and then subjected to freeze-drying process with the use of lyophilizer Ralph 1-2 LD (Martin Christ Gefriertrocknungsanlagen GmbH). Next, the juice was prepared by diluting with distilled water at 8.8°Brix a lyophilisate of Japanese quince.

### Mixture of juices preparation

Japanese quince juice and apple juice were combined in different proportions, so that the addition of Japanese quince juice in apple juice was 15, 25 and 35% (v/v) and samples were coded as follows: AJ – apple juice 100%; AJ+15%PJ – apple juice with the 15% addition of Japanese quince juice; AJ+25%PJ – apple juice with the 25% addition of Japanese quince juice; AJ+35%PJ – apple juice with the 35% addition of Japanese quince juice.

The experiment was performed in duplicate.

### Determination of color parameters

The instrumental color parameters of the juices were evaluated in triplicate using Minolta Chroma Meter CR-300 colorimeter (Konica Minolta, Tokyo, Japan).

The instrument was calibrated with black and white tiles before use. All color parameters were calculated for D65 illuminant and 10° angle observer. CIE Lab parameters:  $L^*$  (lightness; 0 = black, 100 = white),  $a^*$  ( $-a^*$ , greenness;  $+a^*$ , redness) and  $b^*$  ( $-b^*$ , blueness;  $+b^*$ , yellowness) were obtained. The total color difference ( $\Delta E^*$ ) between the values for sample with Japanese quince juice and samples without Japanese quince juice was calculated using the following equations:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

### Total polyphenols (TP)

Total polyphenols were determined by the Folin–Ciocalteu method (Singleton & Rossi, 1965) using gallic acid as the standard. Briefly, to 0.1 mL of diluted juice, 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of 20% sodium carbonate were added, mixed and brought to 10 mL volume with distilled water. Prepared samples were incubated for 2 hrs at room temperature. The absorbance was measured at 760 nm and results expressed in mg gallic acid equivalents per 1l of juice.

### Sensory evaluation

The sensory quality of juices was evaluated through descriptive method with the aid of trained panel, consisting of eight employees of the Institute of Quality Sciences of the Poznań University of Economics and Business. All the judges had high degree of sensory sensitivity and long-standing experience of sensory methodology, including the scaling and profiling of juices (ISO 6565:1985; ISO 13299:1998; ISO 8586:2012). The juices were individually coded with three-digit numbers and served to the assessing team members in 50 mL plastic transparent cups. Mineral water and unsalted crackers were available during studies.

The intensities of sensory attributes were rated on semi-structured scales with a length of 10 cm (corresponding to 10 conventional units, 10 cu), anchored on the left side as “untraceable” and on the right as “very intense”. Apart from unitary characteristics, the assessment was also concerned with the overall quality of the juice samples. The boundary designations for overall quality were: “bad quality” – “very good quality”.

### Statistical analysis

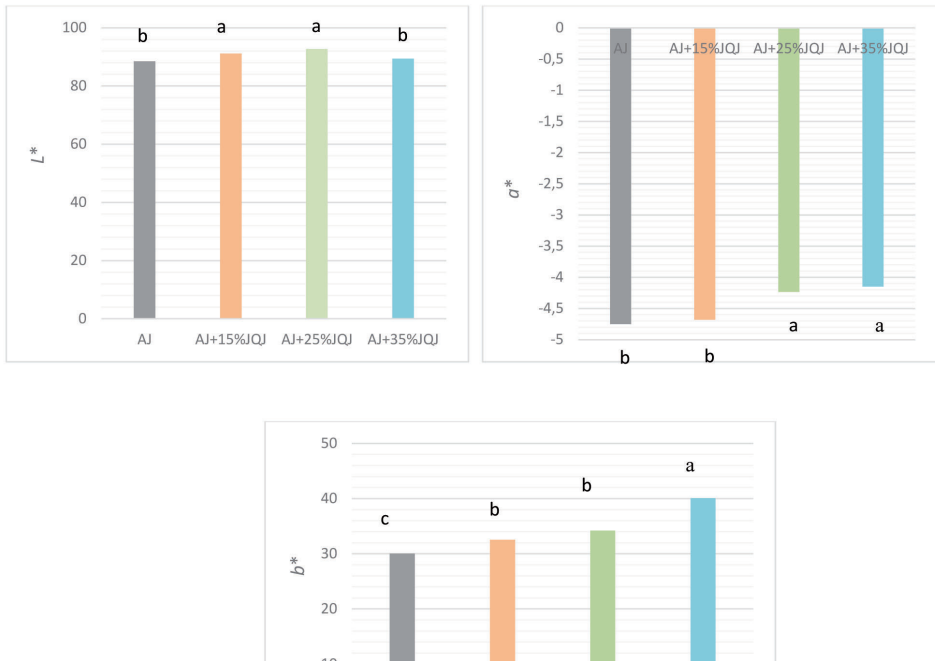
Statistical analysis were performed using Statistica 13.0 (StatSof, Inc., 2000) program. In order to determined the influence of the addition of Japanese quince juice on the analyzed parameters one-way analysis of variance (ANOVA). Differences between means were evaluated by Tukey’s test, at the level of significance  $\alpha = 0.05$ .

## Results and discussion

### The effect of Japanese quince juice addition on $L^*$ , $a^*$ and $b^*$ values of cloudy apple juice

The color of the product is usually the most important factor assessed visually, it indicates what aroma and taste sensations the consumer can expect. Lack of compatibility between these characteristics usually causes lack of acceptance of the product (Garber, Hyatt & Nafees, 2015).

The initial  $L^*$ ,  $a^*$ , and  $b^*$  values of the cloudy apple juice (AJ) were  $88.55 \pm 1.53$ ,  $-4.75 \pm 0.08$  and  $30.07 \pm 0.60$ , respectively (Figure 1). The addition of Japanese quince juice (JQJ) to apple juice changed its color. The analysis of variance showed a significant influence of the Japanese quince juice share in the mixed juice on the



**Figure 1.** The effect of Japanese quince juice addition on color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) of apple juice

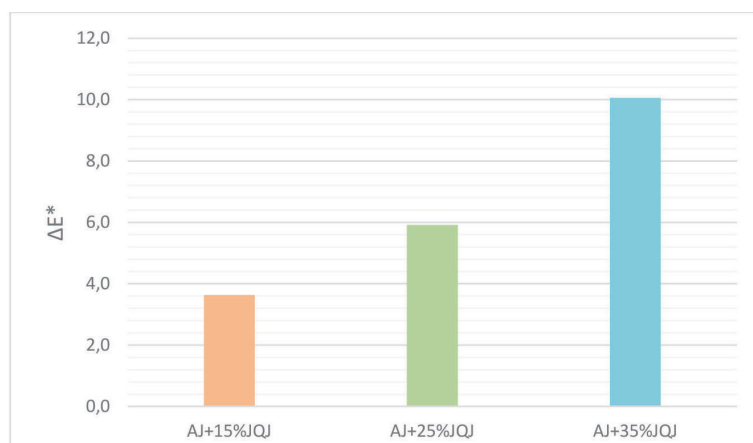
Explanation: AJ – apple juice 100%; AJ+15%PJ – apple juice with the 15% addition of Japanese quince juice; AJ+25%PJ – apple juice with the 25% addition of Japanese quince juice; AJ+35%PJ – apple juice with the 35% addition of Japanese quince juice.

a-c – diagrams marked with the same letters do not differ significantly in statistical terms according to Tukey's multiple range test ( $\alpha = 0.05$ ).

color parameters of apple juice. Addition of 25% and 35% of JQJ to apple juice resulted in brightening of these samples. There was no significant difference in the  $L^*$  value of apple juice without and with 50% JQJ. Increasing proportion of Japanese quince juice resulted in significantly higher  $b^*$  values as compared to juice without Japanese quince juice addition. Also, a slight increase in  $a^*$  value for apple juice with 25 and 35% JQJ was observed.

Wojdyło (2011) also noted an increase in the  $b^*$  parameter of apple purees enriched with quince puree (20%). Similar, Nawirska-Olszańska, *et al.* (2010) observed increase brightness and yellow color in pumpkin jam with addition of quince (30 and 50%) to compare apple juice.

The total color difference ( $\Delta E^*$ ) between two samples determines color perception by human observer.  $\Delta E^*$  value can be interpreted as follows (Cserhalmi, *et al.* 2006): 0–0.05 (difference in color is unnoticeable), 0.5–1.5 (difference is slightly noticeable), 1.5–3.0 (noticeable), 3.0–6.0 (well-visible) and > 6.0 (great difference, observer recognizes two different colors). As it was shown in Figure 2, the values of total color difference ( $\Delta E^*$ ) increased upon addition of increasing proportion of Japanese quince juice. Addition of 15, 25 and 35% of JQJ resulted in  $\Delta E^*$  values about 4, 6 and 10.



**Figure 2.** The effect of Japanese quince juice addition on total color difference ( $\Delta E^*$ ) value of apple juice

Explanation:– as in Figure 1.

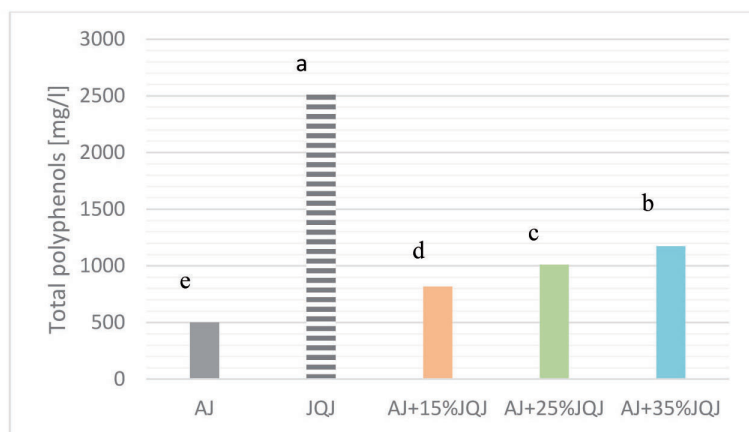
### The effect of Japanese quince juice addition on the content of polyphenolic compounds of cloudy apple juice

Japanese quince juice was characterized by the highest content of polyphenols ( $2510 \pm 75$  mg/l), five times higher than in apple juice (Figure 3). The results are



consistent with the data of other authors (Klimczak, 2017; Lemańska-Pawlak, *et al.* 2018; Oszmiański, *et al.* 2007; Ros, *et al.* 2004).

In designed mixed juices higher concentration of polyphenols was found as compared to the apple juice. In AJ with addition of 15, 25 and 35% of JQJ total polyphenols content was found to be  $816\pm36$ ,  $1012\pm45$  and  $1174\pm52$  mg/l, respectively (Figure 3).



**Figure 3. The effect of Japanese quince juice addition on content of total polyphenols of apple juice**

Explanation: as in Figure 1; JQJ – Japanese quince juice.

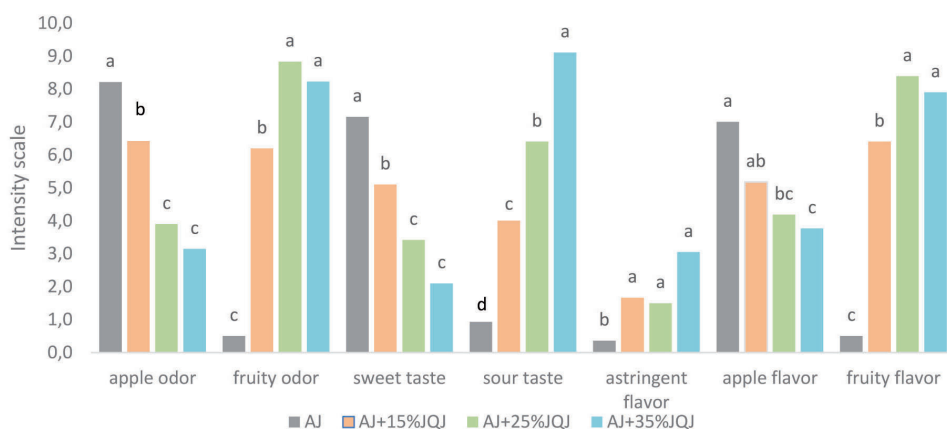
a–e – diagrams marked with the different letters differ significantly in statistical terms according to Tukey's multiple range test ( $\alpha = 0.05$ ).

Phenolic compounds are secondary metabolites contributing for the color and sensory characteristics of fruit and juices (Teleszko & Wojdyło 2014). The main phenolic compounds in apple juice are flavan-3-ols (catechin, epicatechin and proanthocyanidins), dihydrochalcone and hydroxycinnamic acids (Gliszczynska-Świgło & Tyrakowska, 2003; Klimczak, 2017). A major part of phenolic compounds of Japanese quince are flavonoids, among which procyanidins constitute 95% of total content of the total amount of polyphenols (Du & Wu, 2013; Nahorska, 2014; Watychowicz, *et al.* 2017). Proanthocyanidins are compounds with proven effects on sensory characteristics of food. It has been shown that above all, they are compounds responsible for astringency, and they can also shape bitterness and sourness of plant raw materials (Rauf, *et al.* 2019).

## The effect of Japanese quince juice addition on sensory quality of cloudy apple juice

When assessing the quality of juices, sensory tests have become a useful tool to evaluate these products (Klimczak, 2017; Klimczak & Małeczka, 2011). Descriptive sensory analysis is considered to be one of the most comprehensive, flexible and useful sensory methods to obtain detailed information on the perceived sensory properties of product. In the food industries, company can use descriptive methods to develop the sensory profile of new food products, to modify formulations, and in the quality control of products (Murray, Delahunty & Baxter, 2001; Świąder, Marczevska, 2021).

Apple juice and three juices prepared by mixing apple juice with Japanese quince juice, were evaluated sensorially and scores attributed by the panelists are shown in Figure 4 and 5. During the initial session, 2 odor (*apple, fruity*), 3 flavor (*astringent, apple, fruity*) and 2 taste (*sweet, sour*) characteristics were adopted and defined for the apple juice and Japanese juice samples. Beyond unitary characteristics, the *overall quality* was also assessed. Apple juice was characterized by a high intensity of apple odor and flavor (note 8.2 and 7.0), and sweet taste (note 7.2). Sour taste and astringent flavor were slightly noticeable. Research of Jaros, et al. (2009) has shown that sensory attributes such as sweet and sour taste are the most important in the consumer assessment of apple juices. In general, apple juice taste is mainly related to the sugar content (sweetness) and organic acid content (sourness); aroma is associated with odor-active volatile compounds (Iaccarino, et al. 2019).



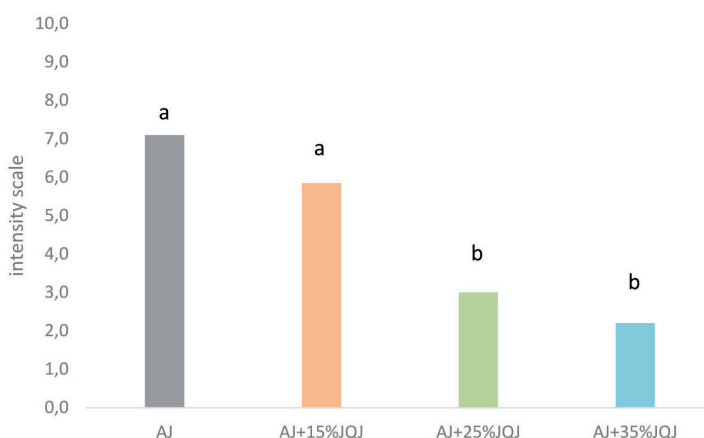
**Figure 4. Sensory quality profile of apple juice with and without Japanese quince juice**

Explanation: AJ, AJ+15%JQJ, AJ+25%JQJ, AJ+35%JQJ – as in Figure 1.

a–d – diagrams marked with the different letters within one and the same characteristic differ significantly in statistical terms according to Tukey's multiple range test ( $\alpha = 0.05$ ).

The addition of Japanese quince juice to apple juice significantly influenced its sensory quality. The mixed juices (AJ+15%JQJ, AJ+25%JQJ, AJ+35%JQJ) were characterized by a higher intensity of sour taste and astringent flavor (Figure 4). Increasing the proportion of JQJ in mixed juice (up to 35%) significantly decreased the intensity of apple odor, apple flavor and sweetness. In mixed juices, compared to apple juice, a significant increase in fruity odor and flavor associated with Japanese quince was found.

Figure 5 presents the effect of addition of Japanese quince juice on overall sensory quality of apple juice. Only the addition of 15% of Japanese quince juice had a positive effect on the score of sensory quality of apple juice. There were no significant differences ( $p > 0.05$ ) in the overall quality between apple juice with the addition of JQJ in the amount of 15% and the control apple juice. Apple juices with the addition of JQJ in the amount of 25 and 35% were negatively assessed by the sensory panel (note 3.0 and 2.0, respectively). Therefore, 15% addition of Japanese quince juice to apple juice seems to be the optimal.



**Figure 5. The effect of Japanese quince juice addition on overall sensory quality of apple juice**

Explanation: AJ, AJ+15%JQJ, AJ+25%JQJ, AJ+35%JQJ – as in Figure 1.

a–b – diagrams marked with the same letters do not differ significantly in statistical terms according to Tukey’s multiple range test ( $\alpha = 0.05$ ).

Pawlak-Lemańska, *et al.* (2018) observed a similar trend of changes in the sensory profile of apple juice enriched with Japanese quince juice. As the concentration of Japanese quince juice increased (8.0–20%), so did the intensity of astringent and sour flavours, whereas the intensities of sweet and apple flavours and apple aroma decreased. Tarko, *et al.* (2015) tested the effect of different fruit extract on total sensory evaluation of beverages (apple, orange and grapefruit). It was shown

that the addition of Japanese quince extract (2 and 5%) was (in the case of apple beverage) was preferred than the other extracts (Cornelian cherry, lingonberry).

## Conclusions

The results obtained can be used in the development of new apple juice products with good sensory quality by mixing with Japanese quince juice. The optimal sensory quality of apple juice was found by addition of 15% JQJ.

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# 12

## ANTIOXIDANT ACTIVITY OF HOME-MADE ISOTONIC DRINKS PREPARED FROM FRUIT JUICES

Ewa Stasiuk<sup>1</sup>

### Abstract

In this work, isotonic drinks based on citrus fruit juices with NaCl and KCl were prepared and their antioxidant activity was determined. Freshly squeezed juices of lime, lemon, grapefruit, orange and tangerine were used in the study. Two variants of beverages were made: the first with NaCl addition, the second with NaCl and KCl addition. Their osmolality ranged from 271 to 302 mOsm/kg. These beverages conformed to the isotonicity requirements (300 mOsm/kg $\pm$ 10%).

The antioxidant activity of isotonic drinks was defined by the DPPH method as the scavenging ability against free radicals (expressed as a percentage). Measurements were taken on the day the drinks were made, the day after, and two day after the drinks were made. Beverages prepared from tangerine juice had the lowest average scavenging activity against radicals (59,2% and 62,2%). The highest antioxidant activity had beverages prepared from orange juice (95,5% and 96,3%). The other beverages also showed high antioxidant activity (82,3%–94,6%).

Studies have confirmed that home-made isotonic drinks based on citrus juices have strong antioxidant effects. It was also found that the high free radical scavenging capacity of the home-made isotonic drinks persisted for two days.

**Keywords:** DPPH, isotonic drinks, antioxidant activity

<sup>1</sup> Gdynia Maritime University, Department of Quality Management Faculty of Management and Quality Science; [e.stasiuk@wznj.umg.edu.pl](mailto:e.stasiuk@wznj.umg.edu.pl)

## Introduction

Functional drinks are becoming increasingly popular among consumers. Two largest categories among them are energy drinks and isotonic drinks. The primary function of energy drinks is to stimulate the human body to mental effort, while for isotonic drinks the primary role is to provide water and electrolytes to the human body. Energy drinks are consumed to improve mental concentration, for example while studying or during long car rides. Isotonic drinks, on the other hand, are consumed by athletes, fitness people, cyclists, etc. According to a report prepared by Nielsen, the sales value and volume of the energy, isotonic and vitamin drinks category grew 17.2% and 13.8%, respectively, in the country in September 2017-September 2018 compared to the same period in the previous one. This report also notes that consumers are moving towards products that are perceived to be healthier (Gwóźdź & Gębczyński, 2015; Kociubińska, 2019). As a result, consumers are increasingly trying to make their own isotonic drinks at home. Very often these drinks are made using fruit juices. For people engaging in physical exercise, isotonic drinks are a source of energy (glucose requirement), minerals (sodium and potassium ions) and water (body thermoregulation factor) (Czaja, Stachowicz & Lebedzińska, 2018).

There is no set legal standard for isotonic drinks in the European Union. However, already in 2001 the SCF (Scientific Committee on Food of the European Union) and later in 2011 the EFSA (European Food Safety Authority) consider isotonic drinks to be isotonic drinks:

- containing 80–350 kcal/1000ml,
- in which at least 75% of the energy comes from metabolized carbohydrates with a high glycemic index,
- containing 20–50 mmol sodium/1000 ml (as Na<sup>+</sup>) or 460–1150 mg sodium/1000 ml,
- whose osmolality is within the range of 300 mOsm/kg water±10% (270–330 mOsm/kg water) (SCF, 2001; EFSA, 2011).

Citrus fruit are present on the Polish market all year round. It is very convenient for consumers that they have access to these fruits in the market regardless of the season. Therefore, you can make isotonic drinks with citrus juice all year round. Citrus fruits themselves are, among other things, a source of vitamin C or bioactive compounds such as flavonoids and phenolic acids (Człapka-Matysiak, *et al.* 2011; Sembratowicz & Rusinek-Prystupa, 2015). Consumption of citrus fruit (including juices) in Poland is diversified and depends on consumers' education, their income and social group or place of residence. Educated people and those from large urban centers consume the most citrus fruits. On the other hand, the poorest and those from smaller towns consume the least citrus (Dorosz & Dudek, 2020; Murawska, 2018). Statistical Yearbook 2021 reports that retail sales of food



and non-alcoholic beverages increased from 198,388 million zlotys to 212,870 million zlotys in 2020 compared to 2019 (Mały Rocznik Statystyczny Polski, 2021). Interestingly, the first studies about the impact of the pandemic on the agri-food market have already appeared. Ambroziak reports that citrus fruit imports to Poland in January-June 2020 compared to the previous year increased by 15.6% (Ambroziak, 2020).

Antioxidant properties are related to the presence of compounds that counteract free radicals responsible for oxidation processes in the human body. These processes are responsible for aging, cancer, inflammation and atherosclerosis (Majewska & Czeczot, 2009). They are detrimental to human health (Nowak, *et al.* 2014). Compounds that act as antioxidants include flavonoids, vitamin C, carotenoids and melatonin. Citrus fruits have such antioxidant compounds in their composition (Klimek-Szczykutowicz, Szopa & Ekiert, 2017; Sembratowicz & Rusinek-Prystupa, 2015). Therefore, the possibility of using citrus fruit juices to prepare isotonic drinks seems very promising. There are many sites on the Internet dedicated to the preparation of isotonic drinks based on juices or compotes. Not all beverages prepared according to these recipes meet the osmolality criterion (Świtalski, Stasiuk, & Rybowska, 2020). Therefore, an attempt was made to prepare isotonic drinks using citrus fruit juice and to determine their free radical scavenging capacity.

The aim of this article is to compare the antioxidant activity of home-made isotonic drinks prepared from citrus fruit juice.

## Material and methods

The research material consisted of isotonic drinks made from citrus fruit juice, water and NaCl or NaCl and KCl. For the purpose of the research the following citrus fruits were purchased in shops in Gdynia: limes, lemons, grapefruits and tangerines. Juices was squeezed from purchase fruit to make isotonic drinks. The juices was mixed with boiled and cooled tap water. Two variants of salt addition were used: NaCl and NaCl with KCl. In order to determine the exact recipe of isotonic drinks, the extract content in squeezed juices was measured using a hand refractometer RR1 produced by Polskie Zakłady Optyczne. Measuring range of the device is 0-35%, accuracy of reading  $\pm 0,25\%$ . The results of refractometer measures are listed in Table 1.

The extract content is mostly influenced by the sugar content. In the studied citrus fruit juices the least amount of sugar contained in lemon juice – 5,92% on average, then lime juice – 8,83%, grapefruit juice – 9,17%, orange juice – 11,50% and the most in tangerine juice – 13,08%.

Osmolality of beverages was measured by cryoscopic method using OS3000 osmometer from Marcel. Measuring range of the device is 0–2000 mOsm/kg H<sub>2</sub>O, osmolality accuracy ± 2 mOsm/kg H<sub>2</sub>O. 100 µl of the drink was taken and placed in special vials and then the osmolality of the drink was read based on the freezing point.

**Table 1. Extract content of juices**

Juice	Extract content [%]			
	1	2	3	average
lime – L	9.00	8.75	8.75	8.83
lemon – Ln	6.00	5.75	6.00	5.92
grapefruit – G	9.25	9.25	9.00	9.17
orange – O	11.50	11.50	11.50	11.50
tangerine – T	13.25	13.00	13.00	13.08

Source: own study.

The antioxidant activity of isotonic drinks was defined by DPPH method as the scavenging against free radicals (expressed as a percentage). DPPH solution (2,2-Diphenyl-1-picrylhydrazyl from Aldrich Chemistry) was prepared by dissolving 0.0078 g of DPPH in methanol in a 100 ml volumetric flask. For the test, 1 ml of isotonic drink was taken after centrifugation using microcentrifuge, then 4 ml of DPPH solution was added. The whole thing was stirred and set aside for 45 minutes. Similarly, a blank (control) was performed with 1 ml of water instead of beverage. Spectrophotometric measurement was performed at  $\lambda = 517$  nm using a UV/VIS-UV2 spectrophotometer from ATI Unicam (Cambridge, UK). Zeroing was done with methanol. The absorbance of the blank and isotonic beverage samples were measured. The results of antioxidant activity (AA) were calculated as the percentage of free radical inhibition according to the following formula:  $AA\% = [(A_B - A_A) / A_B] \times 100$ , where  $A_A$  – the absorbance of the tested isotonic drink sample,  $A_B$  – the absorbance of the control sample. Absorbance measurements were performed three times on three consecutive days. The choice of three consecutive days was to test the variation in free radical scavenging capacity over 48 hours.

## Results and discussion

The hand-made isotonic drinks had to meet the osmolality criterion of  $300 \pm 10\%$  mOsm/kg. Due to the different sugar content of citrus fruit juices, the composition of each isotonic drink was determined and the results are shown in Table 2. The established beverage compositions had two variants of salt addition: the first with

0.13 g NaCl per 100 ml of beverage, the second with 0.13 g NaCl and 0.041 g KCl per 100 ml of beverage. As can be seen the established drink recipes contained between 30 ml (tangerine juice) and 55 ml (lemon juice) of citrus juice per 100 ml of isotonic drink. This formulation ensured that the drinks were isotonic. A similar relation was found in refractometric measurements, where the lowest extract content was in lemon juice – 5.92% and the highest in tangerine juice – 13.08%.

In turn, the isotonicity of hand-made beverages prepared according to established recipes ranged from 272 to 302 mOsm/kg. The isotonic beverage prepared with lemon juice and NaCl addition had the lowest osmolality – 272 mOsm/kg, whereas the beverage prepared with lime juice and NaCl and KCl addition had the highest osmolality – 302 mOsm/kg.

It can be seen that there are differences in osmolality between the two salt addition variants. In beverages with the addition of the two salts NaCl and KCl, the osmolality is higher by 5 to 20 mOsm/kg compared to beverages with only NaCl (Table 3).

**Table 2. Composition and osmolality of isotonic drinks**

Isotonic drink	Composition of isotonic drink		Osmolality of isotonic drinks [mOsm/kg]			
	juice [ml]	water [ml]	1	2	3	average
lime – L	40	60	296	297	299	297
lemon – Ln	55	45	272	272	271	272
grapefruit – G	40	60	282	282	282	282
orange – O	35	65	284	282	281	282
tangerine – T	30	70	279	279	277	278
lime – L1	40	60	302	302	301	302
lemon – Ln1	55	45	289	291	297	292
grapefruit – G1	40	60	293	278	296	289
orange – O1	35	65	289	293	292	291
tangerine – T1	30	70	297	298	296	296

Explanation: L, Ln, G, O, T – beverages with NaCl addition, L1, Ln1, G1, O1, T1 – beverages with NaCl and KCl addition.

Source: own study.

Establishing recipes for home-made isotonic beverages is very important because meeting the isotonicity conditions is a condition for the authenticity of these beverages (Stasiuk & Przybyłowski, 2017). In turn, the determination of these recipes is determined by the sugar content of the juices and the addition of salt.

**Table 3. Differences in osmolality between two salt addition variants**

Juice	average osmolality [mOsm/kg]		
	salt addition		differences
	NaCl	NaCl+KCl	
lime – L	297	302	5
lemon – Ln	272	292	20
grapefruit – G	282	289	7
orange – O	282	291	9
tangerine – T	278	297	19

Source: own study.

The aim of this article is to compare the antioxidant activity of home-made isotonic drinks prepared from citrus fruit juice. The antioxidant activity was determined by DPPH method. For this purpose, the absorbance was also measured three times on three consecutive days. A blank sample was included in the measurements. The results are shown in Table 4. From these results, the antioxidant activity expressed as % free radical scavenging capacity was calculated and the results are shown in Table 5. On the other hand, Table 6 shows the differences in antioxidant activity between isotonic drinks of two variants of salt addition (NaCl and NaCl + KCl).

The highest free radical scavenging capacity was found in isotonic drinks with orange juice of both salt addition variants – 95.5 % (NaCl) and 96.3 % (NaCl + KCl), respectively. The isotonic drinks with tangerine juice and both variants of salt addition had the lowest free radical scavenging capacity – 59.2 % (NaCl) and 62.2% (NaCl + KCl), respectively.

The difference between the highest and the lowest value of antioxidative activity for isotonic beverages with NaCl was 36,3%, and for beverages with NaCl and KCl – 34,1%. This indicates large differences in this activity between beverages made from different citrus juices. However, the differences between orange juice drinks and lime juice drinks are smaller at 13.2% for the NaCl addition variant and 7.8% for the NaCl + KCl addition variant.

The differences in antioxidant activity between the two salt addition variants are small, ranging from 0.8% to 8.4% for the isotonic drink with orange juice and the isotonic drink with lemon juice, respectively.

Table 4. Absorbance measurements of isotonic drinks

Isotonic drink <sup>1</sup>	Absorbance measurements											
	26 V 2021				27 V 2021				28 V 2021			
	1	2	3	average	1	2	3	average	1	2	3	average
<b>L</b>	0.342	0.333	0.331	0.335	0.318	0.317	0.316	0.317	0.195	0.194	0.193	0.194
<b>Ln</b>	0.125	0.123	0.121	0.123	0.124	0.124	0.125	0.124	0.462	0.461	0.461	0.461
<b>G</b>	0.180	0.179	0.178	0.179	0.115	0.114	0.114	0.114	0.485	0.488	0.490	0.488
<b>O</b>	0.074	0.073	0.072	0.073	0.067	0.067	0.068	0.067	0.080	0.081	0.081	0.081
<b>T</b>	0.970	0.965	0.963	0.966	0.243	0.242	0.240	0.242	0.784	0.782	0.781	0.782
<b>L1</b>	0.201	0.199	0.198	0.199	0.184	0.185	0.185	0.185	0.172	0.171	0.172	0.172
<b>Ln1</b>	0.070	0.069	0.070	0.070	0.116	0.116	0.116	0.116	0.076	0.075	0.075	0.075
<b>G1</b>	0.264	0.262	0.254	0.260	0.159	0.160	0.159	0.159	0.112	0.112	0.113	0.112
<b>O1</b>	0.028	0.028	0.028	0.028	0.086	0.086	0.086	0.086	0.068	0.069	0.068	0.068
<b>T1</b>	0.508	0.505	0.503	0.505	0.593	0.592	0.592	0.592	0.763	0.762	0.761	0.762
<b>control</b>	1.525	1.515	1.519	1.520	1.550	1.563	1.564	1.559	1.804	1.804	1.813	1.807

Source: own study.

**Table 5. Antioxidant activity**

Isotonic drink	Antioxidant activity [%]			
	26 V 2021	27 V 2021	28 V 2021	average from 3 days
lime – L	77.96	79.67	89.26	82.3
lemon – Ln	91.91	92.05	74.49	86.2
grapefruit – G	88.22	92.69	72.99	84.6
orange – O	95.20	95.70	95.52	95.5
tangerine – T	36.45	84.48	56.72	59.2
lime – L1	86.91	88.13	90.48	88.5
lemon – Ln1	95.39	92.56	95.85	94.6
grapefruit – G1	82.89	89.80	93.80	88.8
orange – O1	98.16	94.48	96.23	96.3
tangerine – T1	66.78	62.03	57.83	62.2

Explanation: as under Table 2.

Source: own study.

**Table 6. Differences in antioxidant activity between two salt addition variants**

Juice	average antioxidant activity [%]		
	salt addition		differences
	NaCl	NaCl+KCl	
lime – L	82.3	88.5	6.2
lemon – Ln	86.2	94.6	8.4
grapefruit – G	84.6	88.8	4.2
orange – O	95.5	96.3	0.8
tangerine – T	<u>59.2</u>	<u>62.2</u>	3.0

Source: own study.

The study showed that the highest antioxidant activity, as determined by DPPH, was found in both variants of salt addition prepared with orange juice, followed by lemon juice, grapefruit juice, lime juice, and the lowest in tangerine juice. It can be concluded that all isotonic drinks made with citrus fruit juices showed very good (orange, lemon, grapefruit, lime juice) or good (tangerine juice) free radical scavenging capacity. This proves that it is possible to produce an isotonic drink at home, which will also have a health-promoting effect on the human body.

The literature mostly contains data about antioxidant activity of fruit juices, there is no data about isotonic drinks based on juices. Żukiewicz-Koc & Kalbarczyk, examining fruit juices, stated that aronia juices have the highest antioxidant activity because of the content of anthocyanins and polyphenols. According to

the authors, citrus fruit juices have a weaker radical scavenging ability (Żukiewicz-Koc & Kalbarczyk, 2007). On the other hand, Człapka-Matysiak and others in ready-made juices and drinks show good properties against free radical reactions in orange and grapefruit juices. Higher values are found in dark juices, i.e. from chokeberry, blackcurrant or grapes (Człapka-Matysiak, *et al.* 2011). The study also showed a higher amount of antioxidant compounds in organically grown fruit than conventionally grown fruit (Sembratowicz & Rusinek-Prystupa, 2015). Most studies on the antioxidant activity of juices or drinks are conducted on ready-made products on the market. These juices and drinks are standardized and usually filtered. In the case of homemade isotonic drinks, as was the case in this work, the juices were pressed by hand, hence comparisons against purchased products are not always valid. This explains the obtained very good and good free radical scavenging results for isotonic drinks made with citrus fruit juices.

Isotonic drinks with the use of citrus fruit juice made manually at home are intended for people associated with sports or amateur practitioners, as well as for people who care about their fitness. The only thing to note is that people who are allergic to citrus should not drink it (Karczewska, Ukleja-Sokołowska, & Bartuzi, 2018).

## Conclusions

Consumers are increasingly preparing their own isotonic drinks. Citrus fruit juice may be an ingredient in such beverages. The study showed that isotonic drinks prepared with fruit juices have adequate osmolality and very good (orange, lemon, grapefruit, lime) or good (tangerine) antioxidant properties.

The results obtained confirm the benefits of using home-made isotonic drinks prepared with citrus fruit juices. These benefits are due to its high antiradical activity, which helps maintain good health and prevent lifestyle diseases.

It seems that the use of isotonic drinks with citrus fruit juices can replace the use of standard isotonic drinks. This is also confirmed by the market, as more and more isotonic drinks with enriched ingredients can be found on store shelves.

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## FAST SORTING OF APPLE JUICE ACCORDING TO THE TOTAL ANTIOXIDANT CAPACITY USING SYNCHRONOUS FLUORESCENCE SPECTROSCOPY AND CHEMOMETRICS

Katarzyna Włodarska<sup>1</sup>, Przemysław Nowak<sup>2</sup>, Ewa Sikorska<sup>1</sup>

### Abstract

The application of front-face synchronous fluorescence coupled with chemometrics for discrimination between the apple juices with low and high total antioxidant capacity (TAC) was studied. The synchronous fluorescence spectra (SFS) of juices were recorded for emission-excitation wavelength offsets ( $\Delta\lambda$ ) from 10 to 160 nm, with 10 nm step. The TAC was evaluated using Trolox equivalent antioxidant capacity (TEAC) assay. Partial least square discriminant analysis (PLS-DA) was used for development of multivariate models for sorting the juices into the respective categories. The performance of PLS-DA models showed dependence on  $\Delta\lambda$  selected for synchronous fluorescence measurements and on pre-processing applied to the spectra. The lowest misclassification errors rates of 0.0625 for both cross-validation and external validation were obtained for the model based on unit vector normalized SFS measured at  $\Delta\lambda = 120$  nm.

Multivariate discriminant models based on the fast and direct fluorescence measurements enabled sorting apple juices according to their antioxidant properties. The results may contribute to the development of rapid methods for the screening of antioxidant properties of apple juices, providing an alternative to the conventional time- and labour-consuming assays.

**Keywords:** apple juice, synchronous fluorescence, partial least squares discriminant analysis, variable selection.

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Technology and Instrumental Analysis, Poland; [katarzyna.wlodarska@ue.poznan.pl](mailto:katarzyna.wlodarska@ue.poznan.pl); [ewa.sikorska@ue.poznan.pl](mailto:ewa.sikorska@ue.poznan.pl).

<sup>2</sup> Adam Mickiewicz University in Poznań, Faculty of Chemistry, Department of Spectroscopy and Magnetism, Poland, [nowapr@ue.poznan.pl](mailto:nowapr@ue.poznan.pl).

## Introduction

Apple juice is one of the most popular among fruit juices and it is an important source of phenolic compounds. Several studies demonstrated that these components mainly determine the antioxidant activity of apples and apple products, with only low contribution of vitamin C, which is present in apples in small amounts (Kalinowska, *et al.* 2005). The highest contribution to the total antioxidant activity among the major apple phenolics exhibited quercetin, followed by epicatechin and procyanidin B2 (Łata, Przeradzka & Bińkowska, 2005). The antioxidant properties are related to the health beneficial effects of apples and apple juices for humans, such as a reduction in the risk of developing several diseases (Candrawinata, *et al.* 2013; Liaudanskas, *et al.* 2015). Thus, control of total antioxidant capacity (TAC) of apple juices may be of primary interest from both manufacturers' and consumers' perspectives.

The TAC of food products is determined using several assays, such as: the Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP), ferric-reducing ability of plasma (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical cation (DPPH<sup>•</sup>) assay (Craft, *et al.* 2012). All these methods rely on conventional wet chemistry approach, and measure the ability of a food to scavenge specific free radicals or reduce other chemicals. They use chemical reagents, and are time- and labour-consuming. These disadvantages make them frequently unsuitable for rapid analysis, especially for in-line food quality assessment.

There is a great interest in development of high-throughput methods for routine analysis of foods. Such methods could be used instead of the conventional assays for rapid analysis of high number of samples. Therefore, they should be direct and fast, eliminating or reducing the usage of reagents and production of laboratory waste. Many publications and applications have demonstrated that coupling of spectroscopic techniques with chemometrics was well suited for these purposes. Besides of wide application of vibrational spectroscopy, mainly in the near infrared region (NIR), an increasing number of publications has demonstrated the potential of fluorescence in food analysis.

Excitation-emission spectroscopy and synchronous fluorescence spectroscopy are the fluorescence techniques most widely used in food studies (Kumar, Tarai & Mishra, 2017). Synchronous fluorescence spectroscopy relies on recording fluorescence intensity as a function of the simultaneously changed emission and excitation wavelengths, with a constant offset between them ( $\Delta\lambda = \lambda_{em} - \lambda_{ex}$ ). The main advantage of this technique as compared to the conventional emission and excitation spectral measurements and excitation-emission spectroscopy is the possibility to record multiple fluorophores present in a sample in a single synchronous spectrum. Other useful features of synchronous measurements

are the improved selectivity and sensitivity, significant reduction in the spectral overlapping, and suppressed light-scattering interferences (Andrade-Eiroa, *et al.* 2010a, 2010b). The main characteristics of the SFS are determined by the  $\Delta\lambda$  value. The SFS spectra recorded over the range of  $\Delta\lambda$  values are known as the total synchronous fluorescence (TSFS) spectra (Patra & Mishra, 2002).

In several studies fluorescence was used to predict antioxidant contents or antioxidant properties in a variety of plant-derived foods. For example, fluorescence spectroscopy coupled with multivariate regression was used to screen for the antioxidant contents and the antioxidant capacity such diverse foods as coffee, peppermint extract (Orzeł & Daszykowski, 2014), tomato paste (Orzeł, *et al.* 2015), plant oils (Fruhwith, *et al.* 2003; Rabiej & Szydłowska-Czerniak, 2020) and peach extract (Trivittayasil, *et al.* 2017).

Fluorescence of apple juices was correlated to the presence of phenolic compounds, new products formed during thermal processing, and other components (Cohen, *et al.* 1998; Poryvkina, Tsvetkova & Sobolev, 2014; Seiden, *et al.* 1996; Zhu, *et al.* 2009). In our previous studies we have demonstrated the relationship between the natural fluorescence of apple juices and their total phenolic contents (TPC), total flavonoid contents, and total antioxidant capacities (TAC) (Włodarska, *et al.* 2016, 2017b).

However, the PLS regression models obtained for the prediction of TAC were characterized by moderate predictive ability, only suitable for semi-quantitative prediction. Therefore, presently we focus on the usage of synchronous fluorescence for the discrimination between commercial apple juices with low and high TAC. Such approach may be useful for quality control in food industry, as the manufacturer may be interested in screening analysis based on sorting products into different categories, rather than in the exact parameter values (Cayuela & García, 2017). Previously, synchronous fluorescence spectra coupled with discriminant analysis was successfully used to distinguish apple juices produced directly and reconstituted from concentrate (Włodarska, Khmelinskii & Sikorska, 2018). The aim of the present study was to investigate the possibility of using synchronous fluorescence spectra to sort apple juices according to their antioxidant properties.

## Materials and methods

### Apple juices

Forty eight commercial apple juices produced in Poland and available on the Polish market were studied. The selected samples included juices reconstituted from concentrate, both clear and pulp-enriched cloudy varieties as well as direct juices

preserved by pasteurization. The juices originated from 16 different producers, and the samples from three different production batches were included for each of the producers. No further systematic information about the raw material characteristics and details of processing procedures was available.

### The total antioxidant capacity (TAC)

The reference TAC values of the juices were determined using the Trolox equivalent antioxidant capacity (TEAC) assay according to (Re, *et al.* 1999), as described previously (Włodarska, *et al.* 2016). Briefly, the method is based on the measurements of absorption decay of the ABTS<sup>•+</sup> radical cation at 734 nm, with the increase of the juice or the standard concentration. Trolox was used as a standard in this assay. The ABTS<sup>•+</sup> cation radical was generated by tracking the interaction of 7.7 mg of ABTS that was dissolved in 1.8 mL of deionized water and 0.2 mL of 0.0069 g/mL potassium persulphate. The cation was incubated in the dark at room temperature for 16 h. The ABTS<sup>•+</sup> cation radical working solution was diluted with methanol to an absorbance of 0.80 at 734 nm. The absorbance was recorded 6 min after mixing 0.008 mL of juice with 0.792 mL of the ABTS<sup>•+</sup> working solution. The TEAC value was calculated from the ratio of the linear regression coefficients of the calibration curve of the juice and the Trolox calibration curve. The TEAC value was expressed in mmol of Trolox per litre of juice (mM). These measurements were performed on Spectronic Genesys 2 spectrophotometer (Milton Roy, USA). The samples of clear juices were measured directly, while the samples of cloudy juices were centrifugated (15,000 × g for 5 min) before measurements. All determinations were performed in triplicate.

### Fluorescence measurements

The synchronous fluorescence spectra (SFS) were available from a previous study (Włodarska, Khmelinskii & Sikorska, 2018). Spectra were recorded in the 240–700 nm excitation range with the emission-excitation offsets ( $\Delta\lambda$ ) in the 10–160 nm range, with a 10 nm step, using a Fluorolog 3–11 spectrofluorometer (Spex-Jobin Yvon, USA). The slit widths of the excitation and emission monochromators were set at 3 nm. The acquisition interval and the integration time were maintained at 1 nm and 0.1 s, respectively. A reference photodiode detector at the excitation monochromator stage was used for the compensation of the xenon lamp intensity fluctuations. The spectra were corrected for the wavelength-dependent response of the excitation and emission channels. The fluorescence of the undiluted juices was recorded in a 10 mm fused-silica cuvette using front-face geometry. The samples of clear juices were measured directly, while the samples of cloudy juices were centrifugated (15,000 × g for 5 min) before measurements.

## Data analysis

The single SFS recorded at particular  $\Delta\lambda$  values were rearranged for the multivariate analysis into matrices sized  $48 \times 461$  elements (number of samples  $\times$  number of excitation wavelengths). Additionally, the entire set of SFS spectra measured at  $\Delta\lambda$  in the 10–160 nm range was arranged into a two-dimensional matrix (unfolded total synchronous fluorescence spectra, uTSFS) sized  $48 \times 7376$  elements (number of samples  $\times$  number of  $\Delta\lambda$  offsets *multiplied by* number of excitation wavelengths). The analysis was performed on both raw and pre-processed spectra. The data pre-processing involved calculation of the first- and second-order derivatives, unit vector normalization, and a combination of derivative calculation and normalization. The derivatives were calculated using the Savitzky-Golay algorithm. The data were mean-centered prior to all of the analyses.

Principal component analysis (PCA) was used for explorative analysis of the spectra. The analysis was performed on the entire uTSFS and on individual SFS for every one of the forty eight samples.

Partial least squares discriminant analysis (PLS-DA), which is an extension of PLS regression for supervised classification, was used for discriminant analysis (Ballabio & Consonni, 2013). Prior to the analysis, all of the studied juice samples were divided into the training set (32 samples) and the test set (16 samples). All of the samples were sorted according to the TAC value, every third sample starting from the second one was included into the test set. The remaining samples constituted the training set. The training set was used for the development and optimization of the calibration models. The test set was used for testing the predictive ability of the final model.

The **X** matrix in PLS-DA included either the entire uTSFS or an individual SFS. The response matrix (**Y**) was a dummy matrix, containing class membership information for each of the samples; in detail, the respective variable was set to 1 for the juices with low TAC (LTAC) and to 0 for the high TAC (HTAC) juices. The discriminant models were validated using the cross-validation and external validation procedures. The optimal number of latent variables was selected as the minimum in the plot of the classification error rate as a function of the number of components. The classification performance was evaluated on the basis of the classification error rate (Ballabio & Consonni, 2013). The classification error rate is defined as the proportion of samples that were classified incorrectly, and varies in the range from zero to unity. To estimate the importance of variables used in a PLS-DA model, the Variable Importance in Projection (VIP) was used (Chong & Jun, 2005).

The data analysis was performed using Solo v. 5.0.1 software (Eigenvector Research Inc., USA).

## Results and discussion

### Total antioxidant capacity of the apple juices

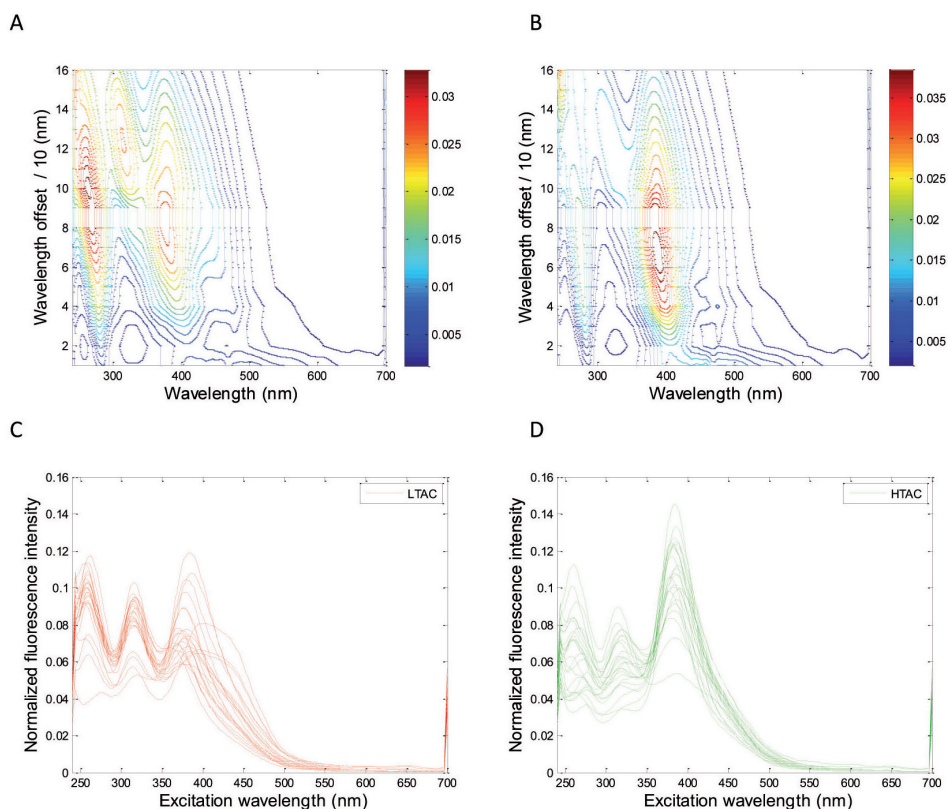
The TAC determined using standard spectrophotometric methods showed significant variations within the studied sample set. The TAC values were in the ranges from 1.06 to 18.77 mM, with the mean value of 4.85 mM and the standard deviation of 3.85 mM. These results demonstrate the variability of commercial juices, which are produced using different methods and from raw material of diverse composition (Biedrzycka & Amarowicz, 2008). The wide range of TAC values has been also reported in literature. For example, the TAC determined using TEAC assay were reported in the range of 1.10–1.95 mM for commercial clear juices reconstituted from concentrate (Gliszczyńska-Świgło & Tyrakowska, 2003), and values in the range of 4.8–11.5 mM were reported for the cloudy juices and the pulp-enriched juices (Will, *et al.* 2008). The TAC values for a diverse set of commercial apple juices were found in the range from 0.8 to 17.0 mM Trolox (Włodarska, *et al.* 2017).

The entire sample set was divided into two classes according to the TAC values. All samples were sorted according to the increasing TAC, and median value of 3.34 mM was used to split the sample set into two categories of juices, those with low (LTAC) and high (HTAC) antioxidant capacity. The mean TAC value for LTAC class was  $2.23 \pm 0.59$  mM (range: 1.06–3.29 mM). The HTAC class was characterized by the mean TAC value of  $7.47 \pm 3.95$  mM (range: 3.39–18.77 mM).

### Explorative analysis of synchronous fluorescence spectra of apple juices

The set of SFS was measured for each sample in the range of  $\Delta\lambda$  offsets from 10 to 160 nm with 10 nm step. The entire set of these spectra formed the TSFS for each of the samples. Figure 1 shows the mean TSFS for juices belonging to the two classes studied, presented as contour maps. The individual SFS in such presentation correspond to the cross-section at the respective constant  $\Delta\lambda$  value. Additionally, Figure 1 presents the individual SFS measured at  $\Delta\lambda = 120$  nm for the two classes of juices, as an example.

The characteristics of TSFS of the juices were reported and discussed in details previously (Włodarska, *et al.* 2017a, 2017b). Briefly, the TSFS of all of the studied juices exhibited three main spectral bands, located in the following  $\Delta\lambda/\lambda_{\text{exc}}$  ranges: 40–100/260–280 nm, 110–140/305–320 nm and 60–100/360–420 nm. The exact positions of the maxima and the relative intensities of these bands vary between different juices. The emission of apple juices is attributed to aromatic amino acids, phenolic compounds and products of non-enzymatic browning.



**Figure 1. The normalized fluorescence spectra of the apple juices studied. Contour maps of TSFS: A) mean TSFS for the LTAC class, B) mean TSFS for the HTAC class. SFS of all juices measured at  $\Delta\lambda = 120$  nm: C) for the LTAC class, D) for the HTAC class**

Source: own work.

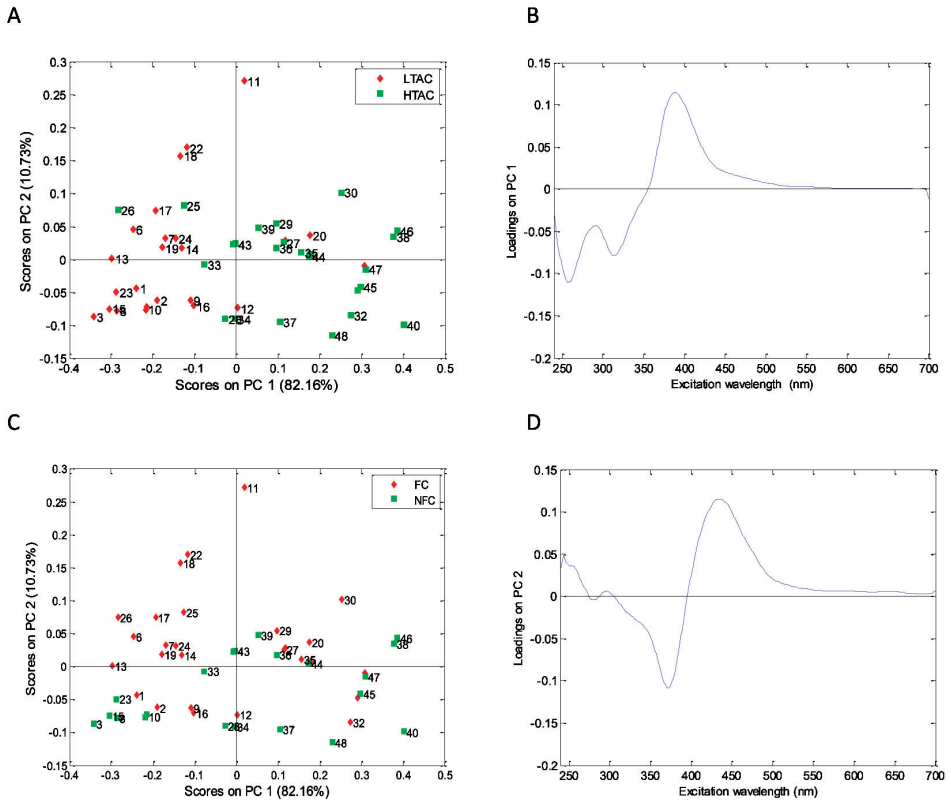
Differences in the fluorescence spectral patterns of the mean TSFS, as well as between the individual SFS of juices that belong to the LTAC and HTAC categories were revealed, Figure 1. It should also be mentioned that significant spectral variability is observed within each of the two classes studied.

For the explorative study, PCA was performed on the uTSFS and separately on individual SFS of each of the studied juices. As an example, Figure 2 shows the results of PCA performed on the normalized SFS measured at  $\lambda\Delta = 120$  nm.

Figure 2A shows the distribution of apple juices in the coordinate system determined by the PC1 and PC2 principal components, with different symbols used for the samples of the LTAC and HTAC classes. The PC1, which represents the main direction of the spectral variability (82.16% of the total spectral variation), already discriminates the juices according to the TAC values to some extent. Generally,



the juices belonging to the LTAC class have lower scores for PC1 than the majority of HTAC juices. Figure 2A shows that HTAC and LTAC juices have different spectral properties, and the majority of the spectral variability is correlated to the TAC value. The inspection of the loading plots for PC1, Figure 2B, revealed that PC1 was positively correlated with the emission band that peaks at about 390 nm and negatively correlated with the bands below 350 nm. Thus, these spectral ranges for the SFS at  $\Delta\lambda = 120$  nm differentiated the two categories of juices studied.



**Figure 2. Principal component analysis of the SFS measured at  $\Delta\lambda = 120$  nm, pre-processed using unit vector normalization, for all of the apple juices studied. Left panel: scores plots of PC1 vs PC2: A) with HTAC and LTAC classes marked; C) with FC and NFC classes marked. Right panel: loadings plots: B) for PC1; D) for PC2**

Source: own study.

The samples used in this study included the juices reconstituted from concentrate (FC) and direct (not from concentrate, NFC) juices. Figure 2C shows exactly the same results of PCA analysis as Figure 2A, however, here FC and NFC juices were marked with different symbols. These two categories of juices showed different



patterns in the PC1 vs PC2 coordinate system, as compared to the LTAC and HTAC classes. The FC and NFC classes are to some extent separated by PC2, which describes 10.73% of the total spectral variation. The PC2 is positively correlated with the spectral band that peaks at about 440 nm and negatively – with the bands below 390 nm, Figure 2D. This observation was in agreement with our previous study, where we have shown the possibility of discriminating between FC and NFC juices using synchronous fluorescence (Włodarska, Khmelinskii & Sikorska, 2018). Based on the present results, we may conclude that different spectral regions are responsible for the discrimination between the LTAC and HTAC categories, and the previously studied FC and NFC categories.

### Multivariate discriminate analysis of synchronous fluorescence spectra

The PLS-DA method was used in order to discriminate between LTAC and HTAC juices. For developing discriminant models, the entire sample set was divided into training and test sets. The training set consisted of 32 samples with the mean TAC value of  $4.86 \pm 3.91$ . The test set consisted of 16 samples with the mean TAC value of  $4.83 \pm 3.84$ . Each of the two sets contained the same number of juices from both categories. The PLS-DA models were developed for the entire uTSFS and the individual SFS. All of the models were optimized using various pre-processing methods and the optimal method was selected individually for each of the models studied. The models' performance was evaluated based on the classification error rate for cross-validation, and external validation. Table 1 shows the error rates for the optimal models obtained for uTSFS and individual SFS.

The PLS-DA model for uTSFS preprocessed using second order derivative and normalization was characterized by the error rates of 0.094 and 0.062, for cross-validation and external validation, respectively. The predictive capability of the models based on the individual SFS depended on the  $\Delta\lambda$  value. The error rates for the cross-validation were in range from 0.062 to 0.125, and in the range from 0.062 to 0.250 for the external validation. The models for SFS measured at  $\Delta\lambda$  of 20, 50 and 120 nm had error rates lower than 0.100 for both cross-validation and external validation. The best model was obtained for the analysis of unit vector normalized SFS measured at  $\Delta\lambda = 120$  nm, and was characterized by the errors of 0.062, for both cross-validation and external validation. The sensitivity and selectivity were evaluated for this model. The model sensitivity for a particular class is defined as the proportion of the samples that were correctly assigned to that class. The selectivity of a model is defined as the proportion of samples of other classes that were correctly rejected by the model. The sensitivity and selectivity of this model for cross-validation was 0.93 for both LTAC and HTAC classes. For the external validation, the sensitivity was 1.00 for LTAC and 0.875 for HTAC class. The selectivity in this case was 0.875 for LTAC and 1.00 for HTAC class, as the sensitivity and selectivity values are symmetrical for any two-class model.

**Table 1. PLS-DA models for the discrimination between apple juices with low (LTAC) and high (HTAC) total antioxidant activity. Misclassification error rates for calibration, cross-validation and external validation**

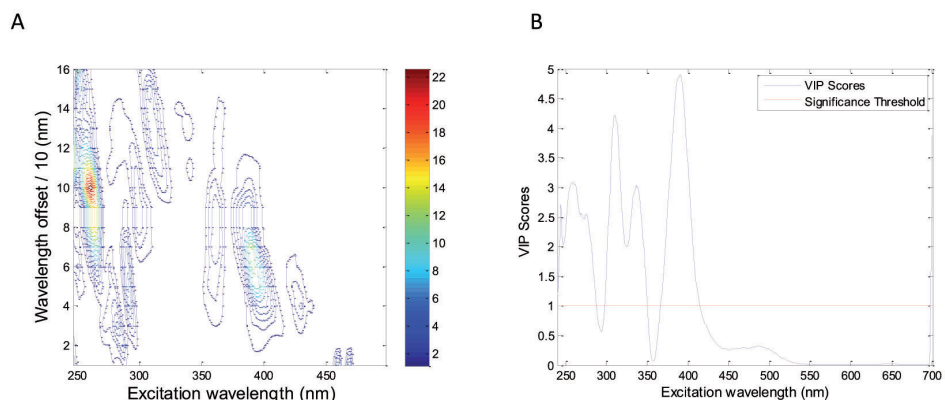
Model	Pre-processing	LV	Error rate		
			Calibration (n = 32)	Cross validation (n = 32)	External validation (n = 16)
uTSFS 10-16 nm	2 <sup>nd</sup> derivative + norm	2	0.062	0.094	0.062
SFS 10 nm	1 <sup>st</sup> derivative + norm	2	0.125	0.125	0.187
SFS 20 nm	2 <sup>nd</sup> derivative + norm	2	0.062	0.094	0.062
SFS 30 nm	2 <sup>nd</sup> derivative + norm	2	0.094	0.094	0.125
SFS 40 nm	1 <sup>st</sup> derivative + norm	2	0.125	0.094	0.125
SFS 50 nm	norm	3	0.062	0.094	0.062
SFS 60 nm	2 <sup>nd</sup> derivative + norm	4	0.062	0.062	0.125
SFS 70 nm	2 <sup>nd</sup> derivative	2	0.062	0.094	0.125
SFS 80 nm	2 <sup>nd</sup> derivative	2	0.062	0.094	0.125
SFS 90 nm	1 <sup>st</sup> derivative + norm	3	0.062	0.125	0.062
SFS 100 nm	1 <sup>st</sup> derivative + norm	4	0.031	0.062	0.250
SFS 110 nm	1 <sup>st</sup> derivative + norm	3	0.031	0.062	0.250
SFS 120 nm	norm	4	0.062	0.062	0.062
SFS 130 nm	1 <sup>st</sup> derivative + norm	5	0.062	0.062	0.125
SFS 140 nm	2 <sup>nd</sup> derivative + norm	3	0.062	0.062	0.187
SFS 150 nm	1 <sup>st</sup> derivative	2	0.094	0.094	0.187
SFS 160 nm	1 <sup>st</sup> derivative	2	0.094	0.094	0.187

Source: own study.

To identify the spectral ranges that significantly contribute to the discrimination between the two studied categories of juices, the Variable Importance in Projection (VIP) method was used.

Figure 3 shows the VIP plots obtained from the PLS-DA analysis of the uTSFS, pre-processed using the second-order derivative and unit vector normalization, and the SFS at  $\Delta\lambda = 120$  nm unit vector normalized. The variables with significant contribution to the discrimination between the categories studied are characterized by the VIP values higher than unity. The analysis of the VIP plot revealed that the spectral range contributing to the model corresponded mainly to the emission bands below 450 nm, which may originate from several phenolic compounds present in apple juices. Recently we have shown that the spectral region above about 450 nm was the most important for the discrimination between the FC and NFC juice categories. This region may correspond to the fluorescence of non-enzymatic browning products (Włodarska, Khmelinskii & Sikorska, 2018). Thus,

different spectral regions contribute significantly to the discrimination between juices with different antioxidant capacity (LTAC and HTAC categories) and the differently processed juices (FC and NFC categories).



**Figure 3. Variable Importance in Projection (VIP) plot for the PLS-DA models: A) for the analysis of uTSFS in the range of  $\Delta\lambda = 10$ -160 nm, pre-processed using the second-order derivative and unit vector normalization, B) for the analysis of SFS measured at  $\Delta\lambda = 120$  nm, pre-processed using the unit vector normalization**

Source: own study

It should be emphasized that the prediction of TAC using fluorescence relied on indirect correlations and exploited the fact that many phenolic compounds responsible for antioxidant properties are fluorescent (Airado-Rodríguez, *et al.* 2009). High positive correlation between the TPC and TAC has been observed in several studies of apple juice antioxidant properties, indicating that such properties are primarily determined by their phenolic contents (Gardner, *et al.* 2000; Gliszczyńska-Święto & Tyrakowska, 2003; Włodarska, *et al.* 2017).

Altogether, the results presented in this paper and those reported by us recently (Włodarska, Khmelinskii & Sikorska, 2018) demonstrated that direct synchronous fluorescence measurements could be useful for controlling different aspects of the apple juice quality, including their authenticity in terms of their commercial category (FC and NFC) and antioxidant properties.

## Conclusions

The study demonstrated the suitability of synchronous fluorescence for distinguishing apple juices with different total antioxidant capacity. Such application was based on the development of discriminant models using the

spectra and the information about the sample membership in the respective category. The model optimization involved selection of the  $\Delta\lambda$  offset for the synchronous fluorescence spectra, and of the spectral pre-processing. The PLS-DA model with the lowest misclassification error rate of 0.062 was obtained for the analysis of normalized SFS measured at  $\Delta\lambda = 120$  nm. The presented results may be important for practical applications, enabling the development of simple, rapid, economical, and environmentally friendly methods for routine sorting of apple juices according to their antioxidant properties.

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## QUALITY OF SOY-BASED MEAT ALTERNATIVES

Iwona Jasińska-Kuligowska<sup>1</sup>, Maciej Kuligowski<sup>2</sup>

### Abstract

Using food products of animal origin is one cause of progressive environmental degradation. More beneficial to the planet is the use of plant origin protein. In the plant kingdom, soybean seeds are one of the richest sources of protein. In this study, the quality of selected soy-based meat alternatives was determined. Chemical properties, including the determination of protein, fat, and total polyphenol content, were evaluated. The fat content in the soybean products was at a similar level of 6.3–9%, with the exception of fried tempeh (14%). The highest protein content was determined for fried and untreated tempeh, 24.6 and 20.7, respectively. The amount of protein in tempeh was 2.2 times higher than that of tofu. We found the most favorable protein-fat ratio in tempeh 2.3 and natto 2.2. The highest amounts of polyphenols were found in natto and miso, and the lowest in tofu. The total polyphenol content in tofu was 12.6 times lower than in natto. Among the tested soybean meat alternatives, tempeh consumption appears to be the most favorable in terms of protein content and protein-fat ratio. Natto (18.3% protein) also seems to be an interesting alternative, especially for consumers looking for foods that are also additionally rich in bioactive components.

**Keywords:** food quality, meat alternatives, sustainable consumption, soy products

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Food Quality and Safety, Poland; [iwona.jasinska-kuligowska@ue.poznan.pl](mailto:iwona.jasinska-kuligowska@ue.poznan.pl)

<sup>2</sup> Poznań University of Life Sciences, Faculty of Food Science and Nutrition, Department of Food Technology of Plant Origin, Poland



## Introduction

The practical implementation of the principles of sustainable development is now a need of the moment and requires a holistic approach to human functioning in the surrounding environment. Intensification of animal production, consumerism, and food waste are largely contributing to progressive degradation of ecosystems (IPCC, 2019). Ensure sustainable consumption and production patterns is one of the European Sustainable Development Goals. Resource efficiency and circular economy actions aim to decouple economic growth from resource use and environmental degradation (European Commission, 2016). At the same time, the constantly increasing population of the world requires adequate food supply, including protein with high nutritional value. Dietary protein is one of the most important nutrients in human nutrition, and its main source is animal products (FAO, 2009; He, *et al.* 2020). It is estimated that in 2025 the world population will reach nearly 8 billion. According to FAO predictions to meet the nutritional needs of the human population, food production should double by 2030 (FAO, 2015; FAO, 2009). A significant problem that hinders the increase of food production is the constantly decreasing area of agricultural land (Buczyńska & Szadkowska-Stańczyk, 2010; IPCC, 2019). Substitution of meat with alternative plant-based products, the production of which may reduce the negative environmental impact of industrial livestock farming, is currently the subject of intense research (Future food: health and sustainability – conference report, IPCC 2019; He, *et al.* 2020). The EAT-Lancet Commission has formulated the definition and assumptions of the healthy reference diet that would provide major health benefits, and also increase the likelihood of attainment of the Sustainable Development Goals (The EAT-Lancet Commission). Consumption of red and processed meat is positively associated with mortality, particularly, due to cardiovascular disease and cancer. The intake of red meat can also worsen the obesity epidemic and increase the risk of joint inflammation. High consumption of processed red meat was found to be associated with an increased risk of colorectal cancer (Bouvard, *et al.* 2015; Larsson & Wolk, 2006; Rohrmann, *et al.* 2013). Recent Zhang, *et al.* (2021) findings highlight processed meat consumption as a potential risk factor for incident dementia.

Meat alternatives can be classified into cultured meat and plant-based meat alternatives. Cultured meat ('in vitro,' 'artificial,' 'laboratory-grown') is produced through the culture of animal stem cells in muscle cells that develop further into tissue. Alternative plant-based meat products are foods mainly composed of proteins of plant origin. Marine organisms and insects are also valuable sources of protein (Becker, 2007; He, *et al.* 2020; Krzywiński & Tokarczyk, 2011; Sun, *et al.* 2021; Tou, Jaczynski & Chen, 2007). Currently, plant-based meat alternatives are mainly produced through thermoplastic extrusion. Some new technologies like electrospinning and 3D printing are also being used. The main and most



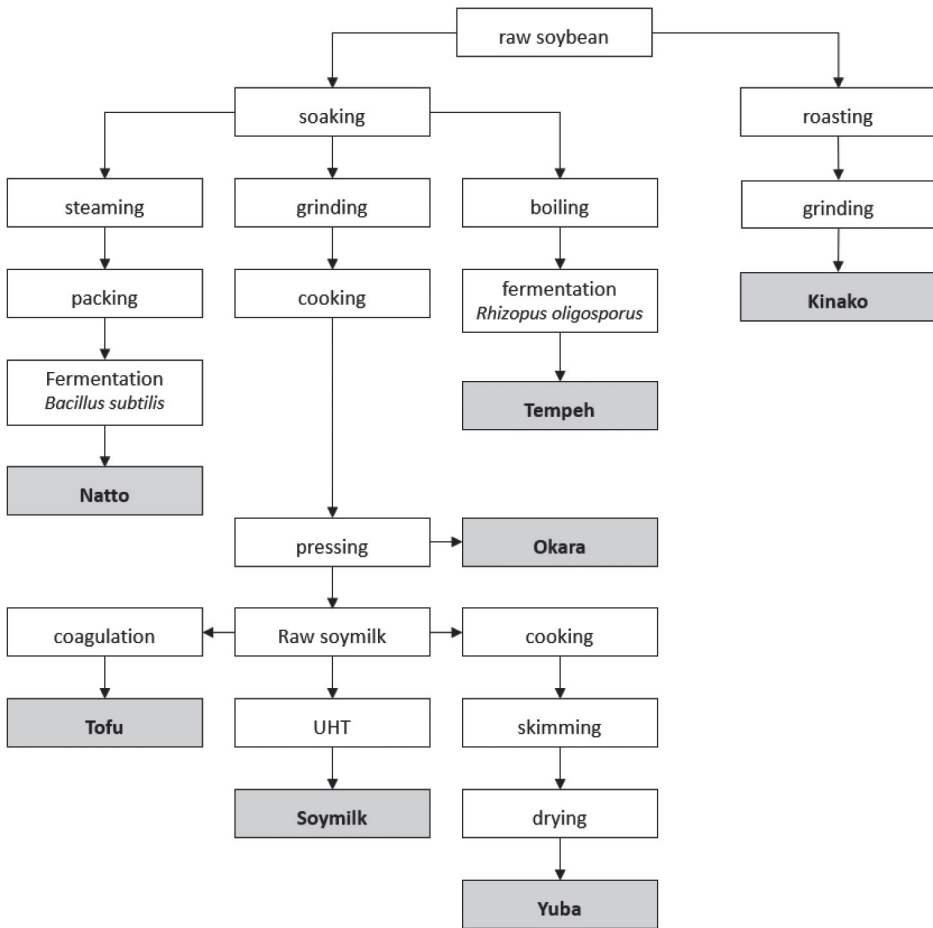
frequently used raw materials are legume seeds – soybeans, peas, lentils, broad beans, and wheat gluten (Hadi & Brightwell, 2021; Sun, *et al.* 2021). Plant-based protein products such as tofu, tempeh or seitan have a long tradition in Asian countries (China, Japan, India, Indonesia). These plant-based products are also commonly used as a protein alternative in vegetarian and vegan diets. (He, *et al.* 2020; Messina & Messina, 2010). In the plant kingdom, soybean seeds are one of the richest sources of protein (Alaswad, *et al.* 2021). Soybean seeds are also sources of isoflavones and other polyphenol components with pro-health activity (Kumar, *et al.* 2020). Isoflavones may contribute to the prevention or inhibition of some diseases. Higher intake of isoflavone in stroke patients was associated with prolonged recurrence-free survival and a reduced risk of stroke recurrence (Chan, *et al.* 2012). The administration of soy isoflavones was also proved to reduce the risk of prostate cancer in Asian populations (Mahmoud, Yang & Bosland, 2014). The most popular soy-based meat alternatives are tempeh, tofu and natto. Figure 1 illustrates the processing of soybeans into commercial soybean products – soymilk, tempeh, tofu, yuba, natto, and kinako.

The objective of this study was to determine the quality of selected soy-based meat alternatives available in Poland. The content of protein, fat and total polyphenols was evaluated in tempeh, tofu, natto, and miso.

## Tempeh

Tempeh (tempe) is a traditional Indonesian food. The earliest reference of tempeh was found in the 1600s, but their tradition is probably much longer. Tempeh is produced by the fermentation of soybeans using *Rhizopus* species, mainly *R. microsporus* var. *oligosporus*, *R. oryzae*, and *R. stolonifer*. Bacteria are also often involved in fermentation and can play an important role in increasing vitamin content. In a typical process, the soybeans are soaked in water, dehulled, and then cooked in boiling water. The prepared soybeans are thoroughly mixed with the starter, wrapped in banana leaves or perforated plastic bags, and left to ferment. These materials created moderate air conditions that are necessary for mold growth. The time of fermentation depends on the temperature and the variety of starters used. Mold growth is vigorous and the entire mass is soon covered and bound by mycelium. This process transforms the loose seeds into solid and sliceable mass. The mold grows not only on the surface but also throughout the fermented mass. During fermentation, the flavor becomes stronger, eventually free ammonia is released, and the initial creamy white color of the cotyledons becomes yellow-dark due to the Maillard reaction. In addition, the surface can change color into gray or even black because of spores produced by the mold. Freshly fermented tempeh has a clean, mushroom-like aroma, sometimes also

defined as a yeast dough. Tempeh is usually consumed fried, boiled, steamed, or roasted. After deep frying, the flavor becomes nut-like. In soups, tempeh reflects the flavors of the other ingredients. The best recognized tempeh is made solely from soybeans, but other tempeh may contain residues after soymilk production (okara) and rarely peanut press cake (residue after oil production). Apart from soybeans, other leguminous and cereals can be used (Ahnan-Winarno, *et al.* 2021; He, *et al.* 2020; Nowak & Kuligowski, 2017).



**Figure 1. Flow diagram illustrating the processing of soybeans to commercial soybean products (soymilk, tempeh, tofu, yuba, natto, kinako)**

Source: based on: (Toda, *et al.* 2000, Nowak & Kuligowski, 2017).

## Tofu

Tofu is the Japanese name for bean curd products, the most popular type of traditional soy protein food. Bean curd has been prepared and consumed in China for thousands of years and a written record of its preparation dates from the second century BC. Tofu production starts with the preparation of soy milk. It is made by soaking soybeans in water, wet-milling, and removing the insoluble parts (named okara) by filtration. After that, soymilk is boiled, then cooled to around 75°C, and the soy proteins are coagulated. Soymilk coagulation is the most important step in the tofu-making process. Calcium sulfate, calcium chloride, magnesium sulfate, and magnesium chloride are many of the different coagulants used on an industrial scale to prepare tofu. Coagulation occurs due to the cross-linking of protein molecules in soymilk with divalent cations. The free supernatant whey is removed, and the curd is transferred to perforated boxes and pressed until a coherent block of curd is obtained. Cooling of the curd is often done for several hours, to cool and leach out excess coagulant and entrapped whey solids. The block is cut into retail-sized portions and wrapped for marketing. Physical properties such as hardness, texture, moisture, and color determine the quality of tofu, especially because of its bland nature. Basic regular tofu is a white, essentially bland, soft product. Their structure can be very different, from soft, gentle gel to a dense, grain-like structure that is more stable than conventional milk-based curd cheeses. Bean curd is a product by itself or a starting material for a variety of related products, including fermented, salted, smoked, dried and fried derivatives (Berk, 1992; He, *et al.* 2020; Moa, *et al.* 2013; Prabhakaran, Perera & Valiyaveettil, 2006).

## Natto

Natto is a traditional Japanese fermented soybean food. To make natto soybeans are soaked, cooked, cooled, and overgrown with *Bacillus subtilis natto* bacteria. The steps used in the production of natto vary according to the raw material, and also the water temperature and soaking time, connected with the ratio of water absorbed, influence the organoleptic quality of the product. Soybeans are washed and soaked in water for 12 to 20 hours. The soybeans are then steamed for 6 hours, although a pressure cooker can be used to reduce the time. The natto fermentation takes about 20–24 hours from the start. During fermentation, the beans become covered with a viscous, sticky fluid/gel that appears to determine, to a large extent, the quality of the finished product. This fluid has the property of forming long, stringy threads. Afterward, the natto is cooled and then aged in a refrigerator for several hours up to one week to allow the development of stringiness. Natto has a gray to tan color, a strong, rather persistent, unique flavor,

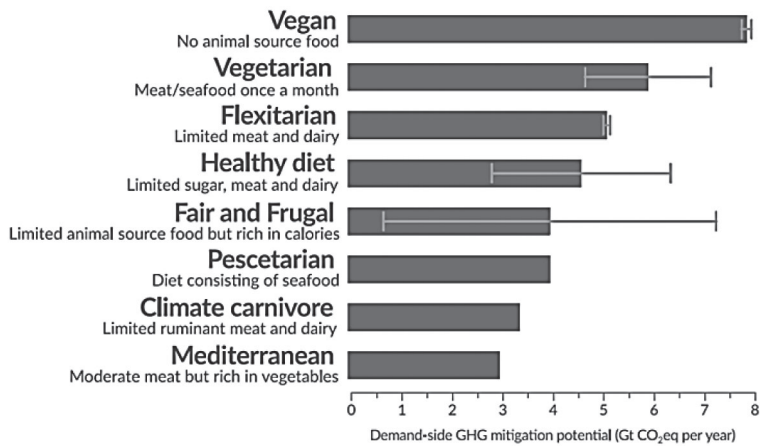
and sometimes a noticeable odor of ammonia. Traditionally, natto is eaten with rice as a sauce, often for breakfast and dinner (Hasim, *et al.* 2015; Jin, *et al.* 2021; Steinkraus, 1983).

## Miso

Miso (fermented soybean paste) is one of the most important Japanese soy foods. The first reference to miso appeared in the ancient Chinese text around 700 BC. Miso is a common Japanese all-purpose high-protein food or seasoning. In Japan, this product has been used primarily as a soup base, but it is also stock for stews, for dips and dressings, and even as a pickling medium. Miso is useful in a vegetarian diet where many of the staples are bland. Made from soybeans by fermentation, with or without the addition of rice or barley, using a mold of *Aspergillus oryzae* and yeast *Saccharomyces rouxii*. *Pediococcus halophilus* and *Streptococcus faecalis* are sometimes also involved in fermentation. The texture of miso varies from smooth and chunky, like soft peanut butter, to the firm texture of cottage cheese.

Each miso has its distinctive flavor and aroma, which for the darker, more traditional varieties is savory and sometimes almost meaty, while for the lighter-colored types it is subtly sweet and delicately refreshing. Although the most common type of miso is made from soybeans, many types of miso are produced, with variations in ingredients, temperature, and duration of fermentation (Katagiri, *et al.* 2020; Murooka & Yamshita, 2008; Ogasawara, Yamada & Egi, 2006; Shurtleff & Aoyagi, 2009; Steinkraus, 1983).

The benefits of more extensive use of soy-based alternatives in the diet include, in addition to health, positive environmental and economic aspects. Reductions in meat consumption can lead to reductions in dietary greenhouse gas (GHG) emissions. The difference in environmental impacts between foods is large; ruminant meats have impacts that are 20 to 100 times those of plant-based foods (Clark & Tilman, 2017). Dietary GHG emissions in meat eaters are approximately at least twice as high as those of vegans (Scarborough, *et al.* 2014; Searchinger, *et al.* 2018). The water, fossil fuel, and phosphate requirement, as well as land use, are several times higher for meat protein production than for soybean-based protein. While the emission of copper is even over 100 times higher (Reijnders & Soret, 2003). In the research conducted by Yue, *et al.* (2017) carbon footprint for meat had the highest value of 6.21 kg CO<sub>2</sub>-equivalent (CO<sub>2</sub>-eq)/kg, and for legumes it was only 0.46. According to the IPCC Report, there is a large mitigation potential of changing diets (Figure 2) (IPCC, 2019).



**Figure 2. Technical mitigation potential of changing diets by 2050 according to a range of scenarios examined in the literature**

Source: based on: (IPCC, 2019).

For both economic and health reasons, as well as environmental reasons, the use of protein sources other than conventional meat in food production is extremely important. The conducted research concerns the use of single cell protein (SCP) obtained from dried cells of microorganisms – bacteria (*Cellulomonas*, *Alcaligenes*), yeasts (*Candida*, *Saccharomyces*), molds (*Trichoderma*, *Fusarium*, *Rhizopus*) (Adedayo, *et al.* 2011; Nalage, *et al.* 2016; Wociór, *et al.* 2010). An important direction is also the use of proteins from marine organisms (krill, algae, spirulina) (Becker, 2007; Tou, Jaczynski & Chen, 2007) and insects (mealworm, crickets, termites) (Bartkiewicz, 2017; Hartmann & Siegrist, 2017; Krzywiński & Tokarczyk 2011; Rumpold & Schlüter, 2013). Cultured meat is also a great meat alternative according to its sustainability benefits and the elimination of animal slaughter. It is predicted to become one of the largest alternative protein markets globally. The nutritional profile of cultured meat can be personalized, and products could be designed to be low in saturated fat, for example. One of the benefits is also that cultured meat is created under sterile laboratory conditions, reducing the risk of foodborne illness. Lab-grown meat is still in the early stages of development, is a promising, but an early stage, technology (Bryant, 2020; Kadim, *et al.* 2015; Stephens, *et al.* 2018).

The wider use of algae, SCP, insects and cultured meat in the food sector is still under development, inter alia due to high production costs, technical difficulties in developing sensory-acceptable products for consumers, or cultural barriers.

## Materials and methods

### Materials

In our study, the quality of four soy-based products was determined. Chemical properties including determination of protein, fat, and total polyphenol content in tempeh, tofu, natto and miso were evaluated. Natto, tempeh (natural and fried) miso, and tofu were obtained from a local market in Poznań, Poland. All reagents were of analytical grade. The Folin–Ciocalteu reagent was purchased from Aldrich-Chemical (St. Louis, MO). Samples were frozen and lyophilized. All analyzes were performed at least in triplicate and the data represent mean values  $\pm$  standard deviation (SD).

### Methods

#### Protein and fat analysis

The content of fat and crude protein was determined according to current standards. Protein was determined with the K-425 SpeedDigester mineralizer and the Büchi K-350 distiller. The nitrogen conversion factor for soy protein in the Kjeldahl method used was 6.25. The fat was extracted using petroleum ether as a solvent with the use of the Randall apparatus model SER 148 from VELS Scientifica. The fat content was determined by the weighting of the extracted lipids (AOAC, 2016; AOAC 2017).

#### Determination of total polyphenols

2.5 grams of lyophilized sample were extracted 2.5 h with 80% ethyl alcohol on a shaker. The mixture was centrifuged at 1780 g for 10 min. Total polyphenols were determined using the Folin–Ciocalteu reagent described by Chandler & Dodds (1983) with Shetty, *et al.* (1995) modification. Gallic acid was used to prepare the standard curve.

#### Statistical analysis

Statistical analysis was performed using Statistica 12.0 StatSoft software. Tukey's multiple means comparison test was used to verify the differences between the samples. The criterion of significance was  $\alpha = 0.05$ .

## Results and discussion

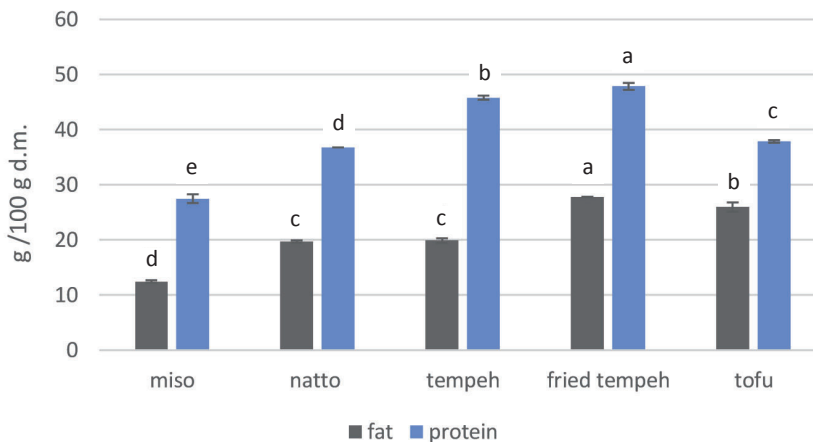
The evaluated soybean products had fat on a similar level from 6.3 to 9% (Table 1). An exception was fried tempeh because of the type of culinary treatment. The increase in the amount of fat in the untreated tempeh was 37%. Thermal processing that involves frying usually displaces water from the raw material. The highest protein content in the tested products was also determined for fried tempeh and seconds for untreated tempeh, followed by natto and in very similar amounts of miso and tofu. The amount of protein in tempeh was 2.2 times higher than that in tofu.

**Table 1. The content of fat and protein in soy products**

g /100 g	miso	natto	tempeh	fried tempeh	tofu
<b>fat</b>	6.31±0.09 <sup>c</sup>	8.45±0.10 <sup>b</sup>	8.96±0.18 <sup>b</sup>	14.28±0.03 <sup>a</sup>	6.36±0.20 <sup>c</sup>
<b>protein</b>	11.78±0.34 <sup>d</sup>	18.65±0.00 <sup>c</sup>	20.64±0.17 <sup>b</sup>	24.59±0.32 <sup>a</sup>	9.28±0.05 <sup>d</sup>

Explanation: a–d – averages in the same line marked with different letters differ significantly ( $\alpha = 0.05$ , Tukey test). Values are the mean  $\pm$  standard deviation of three replicates.

These results of protein and fat content in grams per 100 g of product were compared with their level, converted into 100 g of dry matter (Figure 3). In this presentation, typical for many scientific papers (Kuligowski, *et al.* 2017), tofu ranks second in terms of fat content and third in terms of protein content.



**Figure 3. Fat and protein content (mg/100 g dry mass) of soy products**

Explanation: a–e – averages marked the same letter in the same nutrients have no statistically significant differences ( $\alpha = 0.05$ , Tukey test).

The presentation of the results in grams of the 100 grams of the ready product allows the consumer to evaluate their nutritional value. However, most fermented soy products are a culinary treatment prior to consumption, and this culinary process can drastically alter their nutrient content. It is deceptive to compare, for example, the protein content of soybeans on store shelves with products such as tofu or meat. Soybeans, usually containing around 11% water (Kasankala, Xiong & Chen, 2011; Li-Jun, *et al.* 2004a), have a higher protein content than meat or tofu containing around 70% water (Janiszewski, *et al.* 2015; Li-Jun, *et al.* 2004). Therefore, the ratio of protein to fat in the product can often be a better comparative criterion. This ratio should be high. The most favorable protein-fat ratio was found in tempeh and natto 2.3 and 2.2, respectively, and the least favorable in fried tempeh and tofu, 1.7 and 1.5, respectively. One role of meat replacements is to provide the proper amount of protein. For this reason, the tempeh and natto products seem to be the most attractive among the tested products.

The content of polyphenols was also determined in the tested products. The highest amounts were found in natto and miso and the lowest in tofu (Table 2). The total polyphenols content in tofu was 12.6 times lower in tofu than in natto.

**Table 2. The content of polyphenols in soy products**

mg /100 g	miso	natto	tempeh	fried tempeh	tofu
<b>total polyphenols</b>	271.16±3.54 <sup>b</sup>	313.82±0.22 <sup>a</sup>	107.12±3.22 <sup>d</sup>	115.93±2.62 <sup>c</sup>	24.90±0.30 <sup>e</sup>

Explanation: a–e – averages marked with different letters differ significantly ( $\alpha = 0.05$ , Tukey test). Values are the mean  $\pm$  standard deviation of three replicates.

Polyphenols are considered compounds with a positive influence on the human body (Rasouli, Farzaei & Khodarahmi, 2017). However, they can adversely affect the bioavailability of proteins by interacting with them during culinary processing and digestion (Li, *et al.* 2021).

Variations in the nutrient and bioactive compounds content in soy products can be due to several factors, such as their content in the raw material and the technology used. In natto production, seeds are usually steamed (Jin, *et al.* 2021), which does not create big losses. In the production of tempeh, the seeds are boiled (Ahnan-Winarno, *et al.* 2021) while some soluble compounds can be extracted. The most invasive is the production of tofu, because of wet grinding and cooking (Moa, *et al.* 2013) vitamin B12, and isoflavones in tofu and tempe, as influenced by soybean variety and food processing, particularly fermentation. Principal



results: Raw soybeans contained 2207–2671 lg/kg (dry matter, a large amount of insoluble and thermodegradable ingredients is removed as okara. This could have resulted in a low protein-fat ratio compared to other soy products and low content of polyphenols. In addition, tofu is not fermented and, according to research, this process promotes the release of polyphenols from permanent connections and facilitates their determination (Kuligowski, *et al.* 2017). A high level of total polyphenols has been found in natto. Fermentation by bacteria *Bacillus subtilis* is classified as alkaline fermentation and can lead to very intensive transformations (Moktan, Saha & Sarkar, 2008). Tempeh had a lower total polyphenol content than miso, although molds are involved in both fermentations. This can be explained by the elimination of hulls during the tempeh processes (Ahnan-Winarno, *et al.* 2021). Soy hulls possess a high polyphenol level (Cabezudo, *et al.* 2021).

Among the tested soy products, the consumption of tempeh appears to be the most favorable in terms of protein content and protein-fat ratio. However, due to their sensory values and the possibility of culinary processing, other products can also be considered as a valuable alternative to traditional meat products.

## Conclusions

Meat alternatives have several advantages relative to conventional meat in terms of lower greenhouse gas production, efficiency in land, energy, and water use, animal welfare, and ability to adjust the nutrient composition of the product to individual needs. However, consumers may be cautious about accepting some of the meat-alternatives proposed due to perceptions of ‘artificialness’, inadequate sensory quality, cultural barriers or economic reasons. Therefore, soy-based meat alternatives have great potential to gain acceptance by a large group of consumers. Soy-based meat alternatives are rich in proteins, vitamins, and minerals, but are also sources of isoflavones and dietary fibre. Consumption of dietary fibre can prevent obesity, diabetes, and bowel disease. All of the above factors make soy-based meat alternatives a valuable and future-proof product. One of the additional advantages of traditional soy meat alternatives is that they are relatively low processed compared to modern plant-based texturized products. They also do not contain additional substances, and some of them are based on fermentation processes that can enrich the product with additional nutrients.

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## ASSESSMENT OF THE QUALITY OF UNCONVENTIONAL GLUTEN-FREE FLOURS BASED ON SELECTED PHYSICAL PARAMETERS AND THE SENSORY QUALITY OF COOKIES

Millena Ruszkowska<sup>1</sup>, Patrycja Długosz<sup>1</sup>

### Abstract

The aim of this study was to evaluate the quality of unconventional flours based on the characteristics of selected physical parameters. The study also attempted to assess the sensory quality of products (cakes) made from the tested flours. The test materials were hemp flour (I), cassava tuber flour (cassava starch) (II), coconut flour (III) and flour extracted from domestic crickets (IV). The methodology of the study included assessment of initial water content and activity, evaluation of loose and tapped bulk density, determination of Hausner's ratio and Carr's index, determination of the angle of repose and angle of slide and sensory evaluation. Based on the results, it was found that the flours tested were statistically significantly different in terms of initial water content and activity, tapped density. Based on the sensory evaluation, it was found that the appearance and color of the products made from hemp flour (I) were rated the highest. Products made from coconut flour (III) were characterized by the best smell, taste, and high level of acceptance. On the basis of correlation analysis, it was found that in the case of the produced products only taste and smell determined the degree of acceptance.

### Keywords:

unconventional flours, water content, water activity, flowability, sensory evaluation

<sup>1</sup> Gdynia Maritime University, Faculty of Management and Quality Science, Department of Commodity Science and Quality Management; [m.ruszkowska@wzsj.umg.edu.pl](mailto:m.ruszkowska@wzsj.umg.edu.pl)

## Introduction

Demand for gluten-free products, creates the need for partial or complete elimination of wheat flour from the recipe and contributes to the search by manufacturers of new raw materials, such as unconventional flours. Among raw materials for production of unconventional flours, the following are mentioned: hemp, cassava tubers, coconut and, more and more often, house crickets of the *Acheta domesticus species* or the Cuban cricket (*Gryllus assimilis*). Flours from unconventional raw materials can be high in minerals, vitamins (e.g., A, C, B<sub>6</sub>, B<sub>12</sub>), essential amino acids, essential fatty acids. Therefore, consumption of products with unconventional flours may have beneficial effects on health, e.g., through antimicrobial, anticarcinogenic, hypocholesterolemic, or hypoglycemic effects (Marciniak-Lukasiak, *et al.* 2019).

Edible insects, which are a rich source of protein, fats, vitamins, minerals, and fiber, are a particularly unpopular raw material for flour production (Bartkowicz, 2017). Harnessing the potential of edible insects is not only the availability of a valuable source of nutrients, but also the reduction of greenhouse gas emissions. However, in European countries the use of insects is significantly lower than in Asian or African countries due to reluctance or fear of novelty and cultural barriers. Consequently, modifications are increasingly being made to the way edible insects are processed to make their form acceptable to potential consumers. In particular, this concerns flours or food additives whose form makes consumers more willing to show interest in such unconventional products. This is because they resemble traditional products and are sensorially accepted (Zielińska, *et al.* 2015).

Characterization of physical parameters of unconventional products is particularly important from the point of view of managing their transport and storage and controlling the course of technological process parameters. Particularly important during the transport of products on the process line is the flowability of products, which is determined by such parameters as bulk density and the angle of the bulk and chute (Schulze, 2008). Bulk density is a parameter that determines the degree of filling of packages or equipment, determines the efficiency of transport equipment and allows to determine the magnitude of frictional forces acting on equipment and containers (Schulze, 2008; Tomporowski & Opielak, 2014). The chute angle and the angle of embankment, on the other hand, are properties that determine the size of the storage area, storage capacities, and unloading rate of silos (Peleg, 1978; Tomporowski & Opielak, 2014).

Therefore, the aim of this study was to assess the quality of sample unconventional flours based on the characteristics of selected physic parameters. The study also included sensory evaluation of products made from the tested unconventional flours.



## Material and research methods

Three types of unconventional flours purchased from an organic store in Gdynia were used as research material. The exception was flour from domestic crickets, which was purchased from an online store (Table 1, 2). The products were stored as packaged, in a dry and cool place, according to the manufacturers' recommendation given on the packaging.

**Table 1. Characteristics of the study material**

Value	Product			
	I [hemp flour]	II [cassava starch]	III [coconut flour]	IV [crickets flour]
Energy value [kcal]	312	355	454	344
Fat [g]	8.6	0.02	18.0	5.6
Carbohydrates [g]	3.2	88.0	57.0	5.5
Protein [g]	35.0	0.2	16.0	67.8

Source: own compilation based on producer's declaration.

**Table 2. Parameters of the test material**

Product	Product features
I	gluten-free; vegan; BIO certified; producer country: Poland; country of origin of ingredients: Lithuania;
II	gluten-free; vegan product; BIO certified producer country: Poland; country of origin of ingredients: Cambodia;
III	gluten-free; vegan product; BIO certified; producer country: Poland; country of origin of ingredients: Sri Lanka;
IV	protein product made from 100% commercially ground crickets, bred in clean and hygienic conditions flour does not contain preservatives, artificial colors or flavors; FDA (Food and Drug Administration) approved powder.

Source: own compilation based on producer's declaration.

In the tested products, the initial water content was determined using the drying method at 104°C (Krełowska-Kulas, 1993), and the water activity was determined using the AquaLab 4TE apparatus, version AS4 2.14.0 2017 from Decagon Devices, Inc with an accuracy of  $\pm 0.0003$ , at a temperature of 20°C, taking the material directly from the unit package. Characterization of physicochemical properties of the tested products was carried out on the basis of: evaluation of bulk density

loose and tapped (PN-ISO 8460-1999, Abdullah & Geldart; 1999; Ruszkowska & Wiśniewska, 2017), which are the basic parameters necessary to determine the Hausner coefficient IH and Carr index IC (Ruszkowska, 2012).

The evaluation of the products also included the determination of the chute angle (Leśmian-Kordas, 2001; Ruszkowska & Wiśniewska, 2017) and the determination of the chute angle from smooth and rough surfaces (Tilted Surface Method) using a constant volume of product (Beakawi Al-Hashemi, *et al.* 2018; Cheng, 2018). All determinations including the research methodology were carried out in six replicates.

In this study, an attempt was made to produce a product with the addition of the tested flours and then sensory evaluation of the products was carried out. The following components were used to prepare the dough: 2 eggs, 20 g 18% sour cream, 10 g baking powder, pinch of salt; 250 g butter, 100 g icing sugar. To the prepared base, 100 g of the given flours were added: hemp flour (I), cassava starch (II), coconut flour (III), and house cricket flour (IV). The kneaded dough was chilled for one hour at 8°C, then using molds, cookies were cut from each portion and placed in an oven preheated to 190°C. The baking time was 15 minutes. Sensory evaluation was conducted using a five-point and ten-point rating scale. The evaluation was carried out by a trained team of 12 persons aged between 22 and 45 years. The following features were taken into account: appearance, brittleness, smell, taste, color and the degree of acceptance of the product as a finished product, assuming that it will be available in retail sale at an affordable price for the customer. In order to detect even small differences in subjective evaluation of the degree of acceptance of the product, a scale from 0 to 10 was used, where 0 meant no acceptance of the product, and 10 meant maximum acceptance and willingness to buy the product.

In the statistical study, the verification of the hypothesis on the differentiation of the average level of the examined physicochemical parameters depending on the raw material used was performed by Fisher-Snedecor F-test combined with post-hoc analysis in which the least significant difference (LSD) test was applied. These methods were applied due to the fact that all empirical distributions compared were close to normal distribution, (which was checked by chi-square test of concordance) (Młynarski, 2003). Verification of all hypotheses was performed at the significance level  $\alpha = 0.05$ , based on the test probability value « $p$ ». It was assumed that  $p < 0.05$  indicates significant variation in the dependent variable. The Statistica 13.3 package was used in the analysis. The results of the experiment were also subjected to multivariate discriminant analysis along with canonical analysis (Stanisz, 2007). All empirical distributions of the sensory evaluation results of the produced products did not follow a normal distribution (which was checked by the chi-square test of concordance), therefore, verification of subsequent hypotheses was performed by non parametric tests. Kruskal-Wallis test

for univariate system was performed. The hypothesis of no effect of raw material on the level of organoleptic trait evaluation was rejected when  $p < 0.05$ , and the interpretation of the direction of variation was based on the mean rank value. The values of R-Spearman correlation coefficient were calculated. Statistica 13.3 package was also used in the analysis.

## Results and discussion

Non-bread flours, which also include products obtained from unconventional sources, are raw materials containing all nutrients, whose quantity is a determinant of their quality and determines the suitability of flours for food production. The important factors determining the quality and storage stability of unconventional flours include mainly the content and activity of water, as the main elements shaping the intensity of processes occurring in the product. Thus, the amount of water contained in unconventional flours depends on the water content of the raw material, the intensity of the grinding process and the storage conditions. It is assumed that water content in bread flours should be about 15%, and increased water content may cause quality changes.

Based on the conducted studies on the evaluation of the initial water content in the tested flours obtained from non-conventional sources, it was found that the water content was highest in the cassava starch product (II), significantly lower in the hemp flour product (I), and lowest in the coconut flour (III) and house cricket flour (IV) products (Table 3). Comparing the obtained values of initial water content with the results of the author's study (Ruszkowska, 2012), it was found that cassava starch (II) showed similar water content to wheat flour, while hemp flour (I) on the other hand showed similar water content to soybean flour. The LSD test also showed that all four variants of the evaluated products differed statistically significantly (at  $\alpha = 0.05$ ) in the level of initial water activity (Table 3). Water activity was highest in the cricket flour product (IV) and lowest in the coconut flour product (III).

The value of initial water activity was probably influenced by the degree of water binding in the product resulting from the properties of raw materials. On the basis of the conducted research it was stated that all flours I–IV tested were characterized by low water activity in the range  $0.483 \div 0.359$ , which was a typical feature for loose products.

The knowledge of selected physical parameters of food is a key issue from the point of view of managing their transport and storage. Among the main advantages of powdered food, including unconventional flours, the following are distinguished: convenience of dosing and reduction of storage area (Jedlińska, *et al.* 2012). Particularly important during the transport of products on the processing line is

**Table 3. Water content and activity of the tested products**

Parameter	Parameter value	Product			
		I	II	III	IV
Water content [g/100 g d.m.]	Mean	8.00 <sup>b</sup> ± 0.088	12.52 <sup>c</sup> ± 0.452	4.05 <sup>a</sup> ± 0.040	4.05 <sup>a</sup> ± 0.195
	Min. value	7.88	12.02	3.99	3.78
	Max value	8.10	13.11	4.10	4.22
	p	0.000			
Water activity [-]	Mean	0.471 <sup>c</sup> ± 0.005	0.455 <sup>b</sup> ± 0.003	0.359 <sup>a</sup> ± 0.007	0.483 <sup>d</sup> ± 0.005
	Min. value	0.465	0.449	0.348	0.477
	Max value	0.479	0.458	0.369	0.491
	p	0.000			

Explanation: a–d – equal letter symbols next to the mean values indicate no significant differences between the means in the NIR test.

Source: own study.

the flowability, which is determined by the bulk density and the parameters of the angle of the bulk and the chute (Schulze, 2008). In our study, the LSD test showed that bulk density of loose was highest in hemp flour (I) and cassava starch (II), while it was significantly lower in coconut flour (III) and lowest in house cricket flour (IV) (Table 4).

The LSD test also showed that all the four variants of the products tested differed statistically significantly in the tapped bulk density obtained (at  $\alpha = 0.05$  level). The tapped bulk density was highest in hemp flour (I) and lowest in house cricket flour (IV) (Table 4). Table 4 shows the Hausner ratio and Carr index values for the unconventional flours studied and compares them with the classification proposed by Samborska (Samborska, *et al.* 2011). Hausner's ratio is one of the indicators of cohesiveness, and as its magnitude increases, a more cohesive product is obtained, which is due to an increase in intermolecular forces (Wong, 2000).

The LSD test showed that hemp flour (I) and cricket flour (IV) had significantly higher Hausner ratio than cassava starch products (II) and coconut flour (III). On the other hand, hemp flour (I) and cricket flour (IV) had significantly higher Carr's index value than cassava starch products (II) and coconut flour (III) (Table 4). Based on the classification presented by Samborska (Samborska, *et al.* 2011), it was found that the flours studied I–IV, despite differences in Hausner ratio and Carr's index values, were characterized by medium cohesion and poor flowability.

Another important physical parameter evaluated was the angle of repose and angle of slip, which characterize product properties that determine the size of storage space, storage capacity, and silo emptying rate (Lesmian-Kordas, 2001; Tomporowski & Opielak, 2014).

**Table 4. Characteristics of selected physicochemical parameters**

Parameter	Parameter value	Product			
		I	II	III	IV
Density loose (qL) [g/cm <sup>3</sup> ]	Mean	0.499 <sup>c</sup> ± 0.003	0.498 <sup>c</sup> ± 0.020	0.432 <sup>b</sup> ± 0.008	0.390 <sup>a</sup> ± 0.014
	Min. value	0.494	0.477	0.420	0.374
	Max value	0.503	0,528	0.440	0.410
	p	0.000			
Tapped density (qT) [g / cm <sup>3</sup> ]	Mean	0.703 <sup>d</sup> ± 0.009	0.673 <sup>c</sup> ± 0.013	0.582 <sup>b</sup> ± 0.009	0.553 <sup>a</sup> ± 0.010
	Min. value	0.691	0.658	0.568	0.540
	Max value	0.715	0.692	0.593	0.567
	p	0.000			
HR [-]	Mean	1.408 <sup>b</sup> ± 0.019	1.353 <sup>a</sup> ± 0.025	1.352 <sup>a</sup> ± 0.030	1.417 <sup>b</sup> ± 2.675
	Min. value	1.382	1.315	1.310	1.342
	Max value	1.431	1.378	1.384	1.458
	p	0.005			
Ic [-]	Mean	28.89 <sup>b</sup> ± 0.843	26.35 <sup>a</sup> ± 1.356	26.10 <sup>a</sup> ± 1.213	29.30 <sup>b</sup> ± 2.675
	Min. value	27.80	24.66	24.36	25.72
	Max value	29.88	28.03	27.36	31.16
	p	0.004			

Explanation: a–d – as under Table 3.

Source: own study.

The LSD test showed that hemp flour (I) had a significantly lower slip angle than the other products in groups II–III. On the other hand, the obtained value of slip angle from the metal surface was the highest for coconut flour (III) and the lowest for hemp flour (I). The LSD test showed that the slip angle from the rough surface was highest for cassava starch (II) and lowest for products (I) and (IV), which was probably due to differences in the size of the particles tested (Table 5).

The obtained values of selected physical parameters were also subjected to multivariate discriminant analysis along with canonical analysis. Loose and tapped bulk density and angle of repose of all the samples of unconventional flours tested were the basis of discriminated analysis.

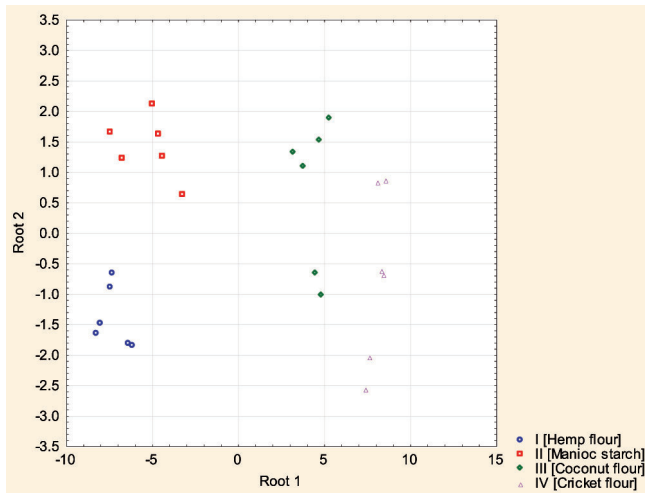
The canonical analysis carried out allowed a graphical presentation of the calculation results (Figure 1), which illustrated the position of the individual samples of the tested products I – IV in the system of two coordinates (elements).

**Table 5. Characteristics angle of repose, and angle of slide of the two surfaces of research**

Parameter	Parameter value	Product			
		I	II	III	IV
Angle of repose (KN) [°]	Mean	72.55 <sup>a</sup> ± 0.348	73.28 <sup>b</sup> ± 0.320	73.25 <sup>b</sup> ± 0.296	73.28 <sup>b</sup> ± 0.345
	Min. value	72.05	72.89	72.86	72.86
	Max value	72.91	73.68	73.55	73.74
	p	0.002			
Angle of slide for of smooth surface [°]	Mean	41.67 <sup>a</sup> ± 4.633	52.50 <sup>b</sup> ± 3.271	60.17 <sup>c</sup> ± 5.776	54.08 <sup>b</sup> ± 3.383
	Min. value	36.00	48.00	52.00	49.00
	Max value	48.00	57.00	67.00	58.00
	p	0.000			
Angle of slide for of rough surface [°]	Mean	64.33 <sup>a</sup> ± 2.805	87.83 <sup>c</sup> ± 3.601	77.67 <sup>b</sup> ± 3.777	67.33 <sup>a</sup> ± 3.615
	Min. value	61.00	83.00	73.00	63.00
	Max value	68.00	93.00	83.00	73.00
	p	0.000			

Explanation: a–e – as under Table 3.

Source: own study.



**Figure 1. Configuration of the tested flours I–IV determined from the loose (qL) and tapped (qT) densities and the angle of repose (KN)**

The configuration in Figure 1 indicated a clear discrimination of samples produced from different raw material. Then the values of the raw coefficients of the discriminant function for the first two elements were calculated (Table 6).

**Table 6. The coefficients of the discriminant function**

Constant	Root 1	Root 2
	108.446	-176.930
Loose bulk density [g/cm <sup>3</sup> ]	-30.849	62.215
Tapped bulk density [g/cm <sup>3</sup> ]	-83.063	-38.076
Angle of repose [°]	-0.578	2.361

Source: own study.

The canonical discriminant functions took, thus, the form:

$$D1 = 108.446 - 30.849 \cdot q_L - 83.063 \cdot q_T - 0.578 \cdot KN$$

$$D2 = -176.930 + 62.215 \cdot q_L - 38.076 \cdot q_T + 2.361 \cdot KN$$

where: D1 – root 1; D2 – root 2.

Table 7 contains the mean values of the canonical variables. Calculating the values of D1 and D2 using the equations presented above and comparing them with the values pledged in Table 6 and the configuration shown in Figure 1 allowed us to identify the raw materials from which the unconventional flours were produced.

**Table 7. Mean of canonical variables**

Product	Root 1	Root 2
I [hemp flour]	-7.25	-1.39
II [cassava starch]	-5.23	1.41
III [coconut flour]	4.40	0.69
IV [cricket flour]	8.09	-0.71

Source: own study.

The mean rank values indicated that: The appearance of the hemp flour product (I) was ranked the highest and that of the house cricket flour the lowest (IV). The crispness of cassava starch product (II) was rated highest and coconut flour product was rated lowest (III). The aroma of the coconut flour product (III) was rated the highest and that of the cassava starch (II) and house cricket flour (IV) the lowest. The flavor of the coconut flour product (III) was rated the highest and that of the cricket flour the lowest (IV). On the other hand, the color of hemp flour product (I) was rated the highest and that of cassava starch the lowest (II). When evaluating the degree of acceptability, the coconut flour product (III) was rated the highest and the cricket flour product was rated the lowest (IV) (Table 8, 9).

**Table 8. Sensory test results – basic statistical measures**

Product	Appearance	Crispness	Smell	Taste	Colour	Acceptance level
	Mean					
I [hemp flour]	4.50	3.33	2.92	3.00	4.42	4.58
II [cassava starch]	4.25	4.17	2.08	4.08	2.17	6.75
III [coconut flour]	3.58	2.08	4.67	4.58	4.17	8.33
IV [cricket flour]	2.08	2.33	2.17	1.92	3.50	2.42

Source: own study.

**Table 9. The variation of sensory quality according to raw material – Kruskal-Wallis test results**

Product	Appearance	Crispness	Smell	Taste	Colour	Acceptance level
	Mean rank					
I [hemp flour]	35.8	29.8	23.0	22.0	35.0	19.4
II [cassava starch]	32.0	39.4	17.2	29.1	8.6	29.9
III [coconut flour]	22.2	12.5	39.6	35.4	31.2	39.2
IV [cricket flour]	8.1	16.3	18.2	11.5	23.3	9.5
	Test results ( $p$ values)					
	0.000	0.000	0.000	0.000	0.000	0.000

Source: own study.

The next stage of the study was to evaluate the correlation between sensory attributes of the products (Spearman's rank correlation matrix) (Table 10). The correlation analysis showed that: the appearance of the products was significantly correlated with crispness and taste (directly proportional correlation). Crispness was significantly correlated with odor and color (inversely proportional correlation).

**Table 10. Correlations between sensory attributes of products (Spearman's rank correlation matrix)**

	Appearance	Crispness	Smell	Taste	Colour	Acceptance level
Appearance	1.000	0.499*	0.050	0.364*	-0.038	0.244
Fragility	–	1.000	-0.296*	0.136	-0.410*	0.034
Smell	–	–	1.000	0.695*	0.295*	0.449*
Taste	–	–	–	1.000	-0.131	0.620*
Colour	–	–	–	–	1.000	0.036
Acceptance level	–	–	–	–	–	1.000

Explanation: symbol \* indicates statistically significant correlation ( $\alpha = 0.05$ ).



Odor was significantly correlated with taste, color and acceptance level (directly proportional correlation). On the other hand, the taste of products made with unconventional poppies I-IV was significantly correlated with the degree of acceptance (directly proportional correlation). On the basis of the obtained results, it was concluded that the degree of acceptance of products made of unconventional flours was determined solely by: taste and smell.

## Conclusions

Based on the assessment of the quality of unconventional flours, based on the characteristics of selected physical parameters, the following conclusions were formulated:

- The tested unconventional flours differed statistically significantly in terms of the initial water content and activity.
- The content and activity of water in flours was probably determined by the parameters of the technological process and storage conditions.
- Flours obtained from unconventional sources differed in their physical properties and statistically significant differences were found in the tapped density values.
- Hemp flour (I) and domestic crickets flour (IV) were characterized by statistically significantly higher values of the Hausner's ratio and Carr's index compared to other unconventional flours tested.
- The tested unconventional flours were characterized low flowability.
- On the basis of the conducted sensory evaluation, it was found that the appearance and color of products made of hemp flour (I) were rated the highest. The best smell, taste and high degree of acceptance were characteristic for products made of coconut flour (III).
- Based on the analysis of correlation, it was found that in the case of manufactured products, only the taste and smell were decisive for the degree of product acceptance.

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## COMPARISON OF THE QUALITY PARAMETERS OF SELECTED TYPES OF ORGANIC CRISPBREAD

Maciej Świtalski<sup>1</sup>, Millena Ruszkowska<sup>1</sup>

### Abstract

The aim of the study was to evaluate the quality parameters of selected types of extruded breads. The research material consisted of 6 types of organic crispbread and the zero product – corn crisps. The research methodology included the determination of water content and activity, determination of water absorption and water solubility indexes, measurement of colour and texture profile (TPA).

The highest water content was observed for product III – quinoa crispbread. Water activity of the tested extrudates did not exceed the value of 0,6. On the basis of color evaluation it was found that the products were characterized by different value of parameters ( $L^*$ ), ( $a^*$ ), and ( $b^*$ ). The tested products were characterized by the value of WAI in range of 461.4–578.36 and WSI ranging from 1.93 to 10.28. Product II (chestnut bread) and product IV (bread with green lentils) were extrudates with the highest hardness. The lowest values of cohesion, chewiness and gumminess were observed for product III. On the basis of research carried out significant differences in the studied parameters were found between the extrudate produced from corn grits and products made of other structure-forming raw materials.

**Keywords:** WSI, WAI, TPA, extrusion

## Introduction

<sup>1</sup> Gdynia Maritime University, Faculty of Management and Quality Science, Poland; [maciej.switalski@sd.umg.edu.pl](mailto:maciej.switalski@sd.umg.edu.pl), [m.ruszkowska@wznj.umg.edu.pl](mailto:m.ruszkowska@wznj.umg.edu.pl)

Bread is the main source of carbohydrates in European countries. Its proper selection in the diet may result in a reduced risk of undesirable weight gain, colorectal cancer, coronary heart disease, insulin resistance or type 2 diabetes (Sandvik, *et al.* 2014). For this reason, in recent years researchers have called for an increase in the consumption of whole-grain bread and there has been an increase in the consumption of bread substitutes, with particular emphasis on fortified products (Michalak-Majewska, *et al.* 2020). What is more, it is not only the proper health value of a product that matters to the modern consumer. Increasing social awareness raises expectations in terms of environmental protection, biodiversity and animal welfare (Runowski, 2009). In response to these trends, dynamic market changes and the efforts of producers offering a wide range of gluten-free products, products with increased nutritional value or made from raw materials originated from organic farming are observed. The research conducted so far indicates the perception of cereal products, and bread in particular, from the point of view of their price, sensory and physico-chemical characteristics: especially freshness, recipe modifications, composition, taste, colour and texture parameters (Jeżewska-Zychowicz & Królak, 2015; Ruszkowska & Bartkowicz, 2017).

Therefore the aim of the study was to evaluate the quality parameters important from the consumer's point of view: colour, texture profile, geometrical density as well as physicochemical properties – water content and activity and water absorption and solubility indexes of selected types of extruded bread produced from raw materials originating from organic farming.

## Material and methods

The research material consisted of 6 different types of extruded crispbread and corn crisps used in study as a reference product (product 0). Detailed information about the products are presented in table 1. The products were purchased from an online organic food store and a supermarket.

The tested products were characterised by different composition (Table 1). Extrudates were characterized by the presence of ingredients of plant origin only. High-starch, structure forming raw material were cereal flours (especially rice flour) and corn grits.

Table 2 shows the nutritional value of products declared by the manufacturer.

**Table 1. Product characteristics**

Product code	Product name	Brand/Producer	Ingredients
0	Corn crisps	Miami/ Eurosnack	Corn grits
I	Organic tigernut crispbread	Le Pain de Fleurs/ Ekibio	Rice flour*, tigernut flour* 25%, sea salt
II	Organic chestnut crispbread	Le Pain de Fleurs/ Ekibio	Rice flour *, chestnut flour* (30%), salt
III	Organic quinoa crispbread	Le Pain de Fleurs/ Ekibio	Rice flour*, quinoa flour (40%)*, unrefined cane sugar * (<2%), salt
IV	Organic green lentils crispbread	Le Pain de Fleurs/ Ekibio	Green lentils flour*50%, rice flour*, sea salt
V	Organic buckwheat crispbread	Le Pain de Fleurs/ Ekibio	buckwheat flour*
VI	Organic black rice crispbread	Le Pain de Fleurs/ Ekibio	Black rice flour* 99,5%, sea salt

Explanation: \* Raw material from organic farming.

Source: own study based on information contained on the unit packaging.

**Table 2. Nutritional value**

Nutritional value (100g of product)	0	I	II	III	IV	V	VI
<b>Energy value</b>	1590 kJ/ 375 kcal	1666 kJ/ 395 kcal	1628 kJ/ 384 kcal	1608 kJ/ 380 kcal	1567 kJ/ 370 kcal	1642 kJ/ 388 kcal	1576 kJ/ 372 kcal
<b>Fat</b>							
<b>Saturated fatty acids</b>	1.8 g 0.3 g	7.2 g 1.5 g	1.6 g 0.4 g	3.5 g 0.5 g	1.2 g 0.4 g	2.7 g 0.6 g	2.6 g 0.6 h
<b>Carbohydrates</b>							
<b>Sugars</b>	80 g 0.5 g	72.0 g 6.2 g	83.5 g 9.3 g	76 g 3 g	69.5 g 1.1 g	75.2 g 1.7 g	74.8 g 0.8 g
<b>Dietary fiber</b>	3.3 g	7.5 g	4.9 g	3.1 g	4.2 g	4.5 g	5.6 g
<b>Protein</b>	8.1 g	6.8 g	6.5 g	9.5 g	18.1 g	13.4 g	9.6 g
<b>Salt</b>	0 g	0.5 g	0.58	0.58	0.6 g	0.01 g	0.6 g
<b>Iron</b>	b.d	3.4 mg	b.d	2.1 mg	4.6 mg	3.8 mg	b.d
<b>Magnesium</b>	b.d	b.d	b.d	113 mg	64.3 mg	199 mg	140 mg

Source: own study based on information contained on the unit packaging.

On the basis of the manufacturer's declaration, it was found that the caloric value of the products varied within a small range (370–395 kcal). The product with the

highest fat content was product I (crispbread with tigernuts), while product IV (bread with green lentils) was the poorest in this energy component (Table 2). The highest carbohydrate content was observed in products 0 (corn crisps) and II (crispbread with chestnut). The last mentioned product was also characterised by the highest sugar content. Buckwheat crispbread (product V) and crispbread with green lentils (product IV) contained the highest amount of protein of all studied extruded products. Among the studied cereal products, a highest dietary fiber content was observed in product I.

The assessment of the research material was carried out under laboratory conditions. The extrudates included in the study were evaluated in terms of the key quality parameters. The first parameters determining the stability and rheological properties of bread were activity and water content. The determination of  $a_w$  was performed using an AquaLab 4TE measuring instrument (version AS4 2.14.0 2017 by Decagon Devices, Inc.) with an accuracy of  $\pm 0.0003$  at 293K (20°C)  $\pm 2.5$  K.

The water content of the product was determined by thermal drying method up to constant mass at 378K (105°C), under normal pressure, according to the methodology proposed by Krełowska-Kułas. The determination was made by dosing about 2 g of bread for each measurement (Krełowska-Kułas, 1993).

Colour measurement was an important part of the methodology. The colour of product was measured using a Konica-Minolta CR 400 colorimeter, in the international CIE Lab system, which consists of the three colour parameters:  $L^*$ ,  $a^*$ ,  $b^*$ . The first parameter specifies brightness ( $L^*$ ), where white is characterised by  $L = 100$ , black by  $L = 0$ . The other elements of the model are the parameter ( $a^*$ ) describing the colour green to red and parameter ( $b^*$ ) describing blue and yellow. In addition, both ( $a^*$  and  $b^*$ ) parameters have wider values compared to ( $L^*$ ) (-120 to 120) (Śmiechowska & Kłobukowski, 2017).

Water absorption (WAI) and solubility (WSI) indexes, which are the basis for the quality assessment of extruded products, were determined on the basis of the methodology of Anderson and articles of Gondek & Ekielski. Samples of extrudates (approximately 2 g) were ground with a laboratory grinder to particles with a diameter of less than 0.3 mm and then filled with 20 ml of distilled water. The solution was shaken, and then centrifuged for 10 minutes in a Jouan B 4i at 12500 revolutions per minute, using an overload of 3 G. The supernatant was decanted from the sediment and the gel obtained was weighed. The obtained filtrate was dried at 110°C until the water was completely evaporated (Anderson, *et al.* 1969; Ekielski, Żelaziński & Florczak 2013; Gondek, Jakubczyk & Wieczorek 2013).

Water absorption index:

and water solubility  $WAI = \frac{\text{soaked sample (gel) mass}}{\text{sample mass}} \times 100\%$

The geometric de  $WSI = \frac{\text{dried supernatant mass}}{\text{sample mass}} \times 100\%$  roportion of volume and mass. A cork borer with a diameter of 27 mm was used to cut the bread samples. The samples were weighed and their dimensions using a calliper were determined. Measurements were made in 9 repetitions. The density of a reference product – corn crisps without additives was determined by the displacement method, in 7 repetitions, using: quinoa grains, two cylinders with a capacity of 250 cm<sup>3</sup> and a laboratory weight scale (Gondek, Jakubczyk & Wieczorek 2013; Siwek, *et al.* 2014).

Due to the high fiber and protein content declared by the manufacturer, which may influence the rheological properties – especially hardness, as well as overall acceptability of the products, the next technical quality parameters assessed were the texture parameters: hardness, adhesiveness, gumminess, chewiness, elasticity and cohesiveness (Obradowić 2015). Mechanical properties testing – TPA (Texture Profile Analysis) was carried out using a Brookfield CT3 Texture Analyzer, using a cylindrical probe with a diameter of 50.8 mm and length of 20 mm and the following measurement conditions: trigger force value – 5 g, distance – 50% penetrate depth, test speed – 0,4 mm/sek, pre-test and post-test speed – 1 mm/sek (Jozinović, *et al.* 2012; Yang, *et al.* 2020). Measurements were made in a minimum of 10 repetitions on samples of 10 mm height for product 0 and 20x20 mm size for bread.

For the determination of texture parameters, outliers outside the interquartile range were found. The statistical analysis of all the results of the laboratory measurements was based on the Fisher-Snedecor F-test combined with a post-hoc analysis, which was the least significant difference (LSD) test. Verification of all hypotheses was performed at the significance level = 0.05, based on the test probability value “*p*”. It was assumed that *p*<0.05 indicates significant variation in the dependent variable.

In order to find out what the strength and direction of the interdependencies between the measured parameters are, the values of the Pearson correlation coefficient were calculated. Then, the null hypothesis about the insignificance of the correlation coefficient was verified using the Student’s t-test with the assumed significance level of  $\alpha = 0.05$ . For the analysis the Statistica 13.3 package was used.

## Results

Table 3 presents the results of the measurement of water content and activity.

**Table 3. Water content and activity**

Product code	Water content [g /100 g s.s.]	Water activity
0	6.63 <sup>a</sup> ±0.043	0.2251 <sup>a</sup> ±0.005
I	7.90 <sup>a</sup> ±0.007	0.4509 <sup>d</sup> ±0.001
II	6.86 <sup>b</sup> ±0.026	0.3936 <sup>c</sup> ±0.001
III	8.49 <sup>a</sup> ±0.026	0.3959 <sup>c</sup> ±0.001
IV	7.35 <sup>c</sup> ±0.064	0.3546 <sup>b</sup> ±0.008
V	8.15 <sup>a</sup> ±0.105	0.350 <sup>b</sup> ±0.002
VI	7.63 <sup>d</sup> ±0.011	0.3372 <sup>b</sup> ±0.005

Explanation: a–f – mean values with the different letter within columns are significantly different at  $\alpha=0.05$ .

Source: own study.

Water as a component of food has a dominant role in its structure and other physico-chemical properties. The parameters of water content and activity are of particular importance for storage stability resulted from the rate of biochemical and microbiological changes (le Maguer; 1987). On the basis of the conducted research, it was found that among the studied crispbreads, the highest water content was characteristic for products III (quinoa bread) and V (buckwheat bread) (Table 3). The lowest water content was observed in the reference product (0) and among the studied breads in product IV (crip bread with green lentils). The lowest value of water activity was characteristic for product 0 (corn crisps). The highest value of this parameter was characteristic for product I (bread with tigernut).

On the basis of the measurements carried out, it was found that none of the products exceeded the value  $a_w = 0.6$ , that allows for the growth of microorganisms and negative biochemical changes. On this basis, it can be concluded on the durability and microbiological safety of extrudates (Zhang, *et al.* 2018).

For the water content parameter evaluated, statistically significant differences were found between all the products tested. For the water activity parameter, products II and III as well as group of products IV, V and VI were statistically equal, the rest of the products were significantly different. Moreover, the statistical analysis indicated into high values of the correlation coefficient between the water activity parameter and geometric density (0.922), cohesion (-0.913), springiness (-0.885) and chewiness (-0.909). The results of the water absorption and solubility indexes determination are shown in Table 4.



**Table 4. Quality parameters of extrudates**

Product code	WAI [%]	WSI [%]	Geometric density [g/cm <sup>3</sup> ]
0	496.90 <sup>b</sup> ±2.34	11.14 <sup>e</sup> ±3.59	0.04499 <sup>a</sup> ±0.003
I	461.40 <sup>a</sup> ±26.44	5.15 <sup>d</sup> ±0.33	0.1259 <sup>e</sup> ±0.009
II	538.39 <sup>c</sup> ±17.01	4.08 <sup>c</sup> ±0.22	0.1143 <sup>c</sup> ±0.003
III	508.86 <sup>b</sup> ±41.49	4.64 <sup>c</sup> <sup>d</sup> ±1.10	0.1209 <sup>d</sup> ±0.012
IV	529.86 <sup>c</sup> ±7.46	3.40 <sup>b</sup> <sub>bc</sub> ±1.09	0.1167 <sup>cd</sup> ±0.004
V	578.36 <sup>d</sup> ±34.64	1.93 <sup>a</sup> ±0.09	0.1052 <sup>b</sup> ±0.006
VI	499.18 <sup>b</sup> ±16.53	2.89 <sup>b</sup> ±0.27	0.1064 <sup>b</sup> ±0.007

Explanation: a–d – as under Table 3.

Source: own study.

Other key parameters of the quality of extrudates are the WAI and WSI indexes, related to the starch gelatinization. Their dependence on the production process e.g. screw speed and process temperature, as well as on the composition of the extrusion mixture, e.g. the fibre content of the product and its moisture content, is confirmed (da Silva Alnes, *et al.* 2018; Gondek, Jakubczyk & Wieczorek 2013). Extrudates produced from blends with higher water content and at slower screw speed are characterised by a higher adsorption index, for WSI the opposite relationship is often observed – low blend moisture content and fast screw speed are factors that increase the value of this parameter (Natabirwa, *et al.* 2018).

The highest value of WAI was observed for product V (buckwheat bread), the lowest for product I (bread with tigernut). There was no unambiguous relation between the fiber content and the WAI value, where product 0 was the poorest in this component and product I contained the most dietary fiber (Table 4). The highest value of the solubility index was characteristic for product 0 (corn crisps), while the lowest for product V. Statistical analysis – the least significant difference test showed that WAI was highest in product V, significantly lower in products II and IV, followed by products 0, III and VI. The significantly lowest level of this parameter was found in product I. No statistically significant differences for the WSI parameter were observed between group of products I and III, group of products II, III and IV, as well as between products IV and VI. These indexes were also correlated with texture parameters. For WAI, a positive correlation was observed with hardness (0.613 and 0.565) and gumminess (0.524) parameters. The water solubility index was characterised by correlation with the parameters of hardness (-0.751 and -0.521), cohesiveness (0.630), springiness (0.748) and chewiness (0.673).

Geometric density is one of the parameters determining the porosity of the extrudate, the lower is its value, the higher porosity the product is observed

(Gondek, Jakubczyk & Wieczorek, 2013). This parameter for the tested bread varied within a narrow range, however the highest difference in density was observed between product I (bread with tigernut) and product 0 (Table 4). This conclusion was confirmed by the statistical analysis. The LSD test showed that the level of this trait was highest in product I, significantly lower in products III, II and IV, followed by products V and VI. However, the significantly lowest level of this trait was found in product 0.

Moreover, a correlation between geometric density for almost all texture parameters studied was observed, especially for: cohesiveness (-0,932), springiness (-0.947) and chewiness (-0.940).

Table 5 defines the values of the parameters ( $L^*$ ), ( $a^*$ ), ( $b^*$ ) which are the colour parameters.

**Table 5. Colorimetric analysis results**

Product code	$L$	$a$	$b$
0	83.46 <sup>a</sup> ±0.01	-2.44 <sup>a</sup> ±0.02	31.24 <sup>a</sup> ±0.03
I	80.40 <sup>d</sup> ±0.15	0.19 <sup>c</sup> ±0.05	15.40 <sup>c</sup> ±0.02
II	72.21 <sup>e</sup> ±0.09	3.62 <sup>e</sup> ±0.03	18.53 <sup>f</sup> ±0.04
III	82.39 <sup>e</sup> ±0.02	-1.32 <sup>b</sup> ±0.03	16.23 <sup>d</sup> ±0.03
IV	74.46 <sup>e</sup> ±1.30	0.34 <sup>c</sup> ±0.07	17.37 <sup>e</sup> ±0.62
V	68.20 <sup>b</sup> ±0.34	3.06 <sup>d</sup> ±0.01	13.50 <sup>b</sup> ±0.06
VI	44.48 <sup>a</sup> ±0.02	8.32 <sup>f</sup> ±0.03	1.79 <sup>a</sup> ±0.02

Explanation: a–f – as under Table 3.

Source: own study.

In the quality assessment of the food products, the parametric interpretation of colour is also of particular importance (Dobrzańska & Cais-Sokolińska, 2014). In addition to its role as an indicator of storage changes or loss of nutrients, it is also a factor that determines the choice of the product by the consumer.

On the basis of the colorimetric measurements carried out, it was found that the highest value of the parameter  $L^*$  (describing brightness) was characterised by products 0 (corn crisps) and III (bread with quinoa). Product 0 was also characterised by the highest proportion of green and yellow colour (Table 5). Product VI (bread with black rice) was characterized by a completely different color, darker, with a greater proportion of red and a smaller proportion of yellow. Statistical analysis showed differences in all color parameters, except for products 0 and III, as well as II and IV for the  $L^*$  parameter and products I together with IV for the  $a^*$  parameter.

Texture is a multidimensional property of food and results from its molecular, microscopic and macroscopic structure. It involves many characteristics such as: hardness, cohesiveness, adhesiveness, springiness, gumminess, chewiness or elasticity and in addition many other important parameters with individual reference to food products (Kubiak & Dolik, 2017). Their simultaneous analysis is possible using the 2-cycle TPA compression test. The key parameter for measuring texture is hardness. This parameter shows the maximum force recorded in a given cycle. Directly related to hardness, cohesiveness is a measure of the compactness and strength of internal bonds in a sample (Brookfield, 2019; Gil, *et al.* 2017). Based on the measurements it was found that the hardest samples were products V (chestnut bread) and II (buckwheat bread), while product 0 had the lowest value of this parameter (Table 6).

**Table 6. Texture Profile Analysis results of crispbread**

Product code	Hardness [g]	Hardness 2 cycle [g]	Cohesiveness	Springiness [mm]	Gumminess [g]	Chewiness [mJ]
0	3187.90 <sup>a</sup> ±451.79	2890.00 <sup>a</sup> ±406.72	0.312 <sup>e</sup> ±0.020	3.274 <sup>e</sup> ±0.11	990.50 <sup>c</sup> ±119.73	32.29 <sup>e</sup> ±3.86
I	6599.67 <sup>b</sup> ±433.17	2763.80 <sup>a</sup> ±433.63	0.062 <sup>b</sup> ±0.011	0.535 <sup>b</sup> ±0.09	424.30 <sup>a</sup> ±96.82	2.27 <sup>a</sup> ±0.83
II	11113.91 <sup>e</sup> ±789.30	5861.33 <sup>c</sup> ±887.41	0.073 <sup>b, c</sup> ±0.014	0.41 <sup>a</sup> ±0.05	770.17 <sup>b</sup> ±174.37	3.07 <sup>a, b</sup> ±0.68
III	7881.11 <sup>c</sup> ±1030.41	3095.20 <sup>a</sup> ±773.21	0.047 <sup>a</sup> ±0.013	0.502 <sup>b</sup> ±0.08	349.80 <sup>a</sup> ±90.98	1.75 <sup>a</sup> ±0.62
IV	9586.80 <sup>d</sup> ±511.44	4882.78 <sup>b</sup> ±237.43	0.086 <sup>c</sup> ±0.08	0.567 <sup>b</sup> ±0.04	812.55 <sup>b</sup> ±136.24	4.59 <sup>b</sup> ±0.78
V	11290.18 <sup>e</sup> ±881.18	6098.33 <sup>c</sup> ±1006.55	0.130 <sup>d</sup> ±0.014	0.910 <sup>d</sup> ±0.11	1369.00 <sup>d</sup> ±202.93	11.64 <sup>d</sup> ±2.00
VI	8209.82 <sup>c</sup> ±831.63	5654.55 <sup>c</sup> ±1059.45	0.122 <sup>d</sup> ±0.020	0.834 <sup>c</sup> ±0.066	992.18 <sup>c</sup> ±166.37	8.16 <sup>c</sup> ±1.85

Explanation: a–e – as under Table 3.

Source: own study.

A decrease in hardness was observed between the first and second cycle of measurements. The most cohesive product were corn crisps (product 0). The lowest cohesiveness was observed for product III (bread with quinoa). Statistical analysis, in terms of the hardness parameter, confirmed that products II and V belonged to one group as well as products III and VI. The LSD test showed that the level of hardness in the second cycle varied to a lesser extent – it was the highest

in products II, V and VI, significantly lower – in product VI, while the significantly lowest level of this characteristic was found in product 0, I and III. No significant differences for the cohesion parameter were observed between products in 3 groups including products I and II, II and IV, as well as V and VI.

Another texture parameter investigated was gumminess, which describes the energy required to overcome the forces of hardness and compactness of the extrudates (sample destruction) (Brookfield, 2019). Its highest value was observed for product V, the lowest for product I and III. Statistical analysis determined that products I and III were characterised by equal gumminess, as well as product II in relation to product IV, and product 0 in relation to product VI.

The last two parameters of the TPA profile were springiness and chewiness. Springiness is defined as a measure of the degree of regeneration of the sample structure, the rate at which the sample returns to its initial state between the first and second test cycle. Chewiness, on the other hand, is a parameter indicating the amount of energy required to grind the sample into a form that allows a bite of food to be swallowed (Brookfield, 2019; Gil, *et al.* 2017). The measurements made with the texture analyser indicated that all tested products had significantly lower gumminess and chewiness than the reference product (product 0). The lowest value of chewiness was observed for product III, while the product with the least gumminess was product I, II and III. No statistically significant differences were found in products I, III and IV for the gumminess parameter as well as in the groups that were made up of the products: I, II, III and II, IV for chewiness parameter.

The general analysis of texture allows us to assume that the reference product, which was familiar to the consumers, was characterised by a different structure in comparison to the examined crispbread. This characteristic of crispbread may result in its non-acceptance by consumers. However, the tested crispbread was characterised by a similar texture profile to other products available on the Polish market (Michalak-Majewska, *et al.* 2020).

## Conclusions

Colour, WAI, WSI, water content and activity as well as texture parameters are important quality parameters of crispbread. Among the studied crispbreads, product II was characterised by the lowest water content, while product III by the highest value of this parameter. The aw values of all products were within the range of 0.6.

The products were characterised by diversified colour. The highest values of the  $\Delta E$  parameter were characteristic for product VI. The brightest colour was observed

for product III, the darkest for product VI. The lowest value of WSI parameter and the highest value of WAI parameter among the investigated bakery products was characteristic for product V, an inverse relation was characteristic for bread I. Moreover, WAI and WSI parameters were correlated with results of TPA. Products II and IV were extrudates with the highest hardness. For product III the lowest values of cohesion, chewiness and gumminess were observed.

On the basis of the research it was found that all types of crispbread differed from the comparative product (corn crisps). The differences were particularly evident in terms of parameters: water content and activity, water solubility index, geometric density, colour as well as texture parameters (especially hardness, cohesion, chewiness and gumminess). Due to the existence of these differences, it is possible that consumers may be neophobic towards organic bread or do not accept it, what should be confirmed or excluded by conducting further scientific work.

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## QUALITY AND SAFETY OF SELECTED CONFECTIONERY PRODUCTS BASED ON THEIR FATTY ACIDS COMPOSITION

Dorota Klensporf-Pawlik<sup>1</sup>, Pola Rolińska<sup>1</sup>, Wojciech Zmudziński<sup>1</sup>

### Abstract

The quality and safety of food products are of great interest of most consumers. Unfortunately, *trans* fatty acids (TFA) and saturated fatty acids, which are strongly correlated with the risk of chronic diseases, especially cardiovascular heart diseases, are still present in our daily diet. As the effect of global works on TFA limitations, on 25 of April 2019 the European Commission has published, regulation which envisages the limits on the amount of *trans* fatty acids, other than *trans* fatty acids naturally occurring in fat of animal origin. The regulation set the maximum limit for industrially produced *trans* fatty acids at 2 grams per 100 grams of fat in food intended for the final consumer. According to Commission Regulation 2019/649 food which does not comply with this, may continue to be placed on the market until 1 April 2021. The hypothesis of this work is that, the use of *trans* fatty acids in food has been sharply decreased, and the current intake of TFA is much lower than in previous years. However, there is a potential risk, that *trans* fatty acids could be replaced in various products with saturated fatty acids (SFA). Therefore, the objective of this study was to determine the TFA and SFA content in several confectionery products, recognized previously as a *trans* fatty acid sources.

**Keywords:** quality, safety, *trans* fatty acids, confectionery products

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Food Quality and Safety, Poland; [dorota.klensporf-pawlik@ue.poznan.pl](mailto:dorota.klensporf-pawlik@ue.poznan.pl)

## Introduction

Nutrition plays an important role among the risk factors of coronary heart disease, which is still recognized as one of a major contribution to mortality in the world. The supply of dietary fats has changed over years in Europe, because of the worldwide recommendations to decrease the consumption of saturated fatty acids (SFA) and increase the use of vegetable oil rich in unsaturated fatty acids. Epidemiological studies have shown that high intake of SFA increased production of LDL-particles, VLDL-cholesterol and platelet aggregation which are correlated with atherogenesis and cardiovascular events (Ristic-Medic, *et al.* 2009). However, vegetable oils were often used in food industry as partially hydrogenated. The process of partial hydrogenation of oils leads to the formation of positional isomers caused by double bond migration, and also geometric isomers formed as *cis* isomers become *trans*. Technological conditions which favor selective hydrogenation, unfortunately also favor formation of *trans* isomers (Cizmeci, *et al.* 2005). *Trans* fatty acids (TFA) have been defined as “all the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated, interrupted by at least one methylene group, carbon-carbon double bonds in *trans* configuration” (Codex Alimentarius, 2006). The major process contributing to the formation of industrial TFA is partial hydrogenation of vegetable oils, aiming mainly to increase their stability and versatility. Recently, the U.S. Food and Drug Administration (FDA) has determined that partially hydrogenated oils (PHO) are no longer Generally Recognized as Safe (GRAS), and after June 2018 the use of PHOs in food will be permitted only after FDA approval (Food and Drug Administration Notice, 17 June 2015). Unfortunately, TFA due to their physical properties such as melting point and textural properties, were successfully used in baking and confectionary industry. Żbikowska and Krygier (2003) stated that fats with *trans* isomers are even essential for baking cakes of good quality. Their positive effect on the overall quality of sponge cakes and puff pastry cakes were also observed (Żbikowska & Krygier, 2005; Żbikowska, Rutkowska & Krygier, 2005). Therefore, complete replacement of *trans* fatty acids cannot be achieved by simply removing the *trans* isomer, in relation to a host beneficial functional properties like giving a proper texture, crispiness and softness.

*Trans* fatty acids have a well-documented adverse effects on development of coronary heart disease. TFAs lead to an increase in highly atherogenic lipoprotein(a), total and LDL-cholesterol and decrease the cardioprotective HDL-cholesterol (Skeaff & Miller, 2009). Elimination of industrially produced *trans* fatty acids (TFA) from the food supply was for a long time the priority target of World Health Organization (WHO) action package. WHO released the “REPLACE” action packaged, calling on governments to remove *trans*-fat from the global food chain by 2023. During the past decades, different policy actions have been implemented by various countries aiming the reduction of TFA intake in their population.



Those actions included mandatory or voluntary TFA labelling, reformulation, and even TFA prohibition (Downs, *et al.* 2017). As the effect of global works on TFA limitations, on 25 of April 2019 also the European Commission has published, regulation which envisages the limits on the amount of *trans* fatty acids, other than *trans* fatty acids naturally occurring in fat of animal origin. The regulation set the maximum limit for industrially produced *trans* fatty acids at 2 grams per 100 grams of fat in food intended for the final consumer. According to Commission Regulation 2019/649 food which does not comply with this, may continue to be placed on the market until 1 April 2021 (Commission regulation (EU) 2019/649). Eliminating such harmful substance from processed foods could prevent hundreds of thousands of heart attacks and deaths annually (WHO). However, the role of saturated fatty acids in consumer health is also still debated. Generally, countries advise people to avoid high intakes of SFA, and if it is possible to replace them by *cis* unsaturated fatty acids. The maximum level SFA intake is 10% of total energy from fat (Brouwer, 2020).

Therefore, the hypothesis of this work is that, the use of *trans* fatty acids in food has been sharply decreased, and the current intake of TFA is much lower than in previous years. However, there is a potential risk, that *trans* fatty acids are replaced in various products with saturated fatty acids (SFA). Therefore, the objective of this study was to determine the TFA and SFA content in several confectionery products and margarines, recognized previously as a *trans* fatty acid sources.

## Materials and methods

### Confectionery products and margarines

Different confectionery products and margarines were subjected to an analysis. Products were commonly accessible brands, as well as brands of discount grocery shops. Products were purchased in local market in the transitional period of Commission regulation (EU) 2019/649. Products were divided into 5 groups such as shortbreads, wafers, chocolate bars, puff pastry and margarines.

### Lipid extraction

Cold extraction with hexane as a solvent was used for the extraction of lipids. As nonpolar and less toxic solvent hexane is commonly used for lipid extraction from different matrices. Confectionery products samples were ground in laboratory mill, homogenized in hexane, and extracted twice for 30 minutes, at room temperature with constant stirring. Hexane extracts were filtered and then the solvent was evaporated on a vacuum evaporator at 40°C. Obtained lipids were stored till further analysis.

## Fatty acids analysis

Fatty acids profile was determined in the lipid extracts by means of gas chromatography. Gas chromatography is the most accurate and common method used for TFA identification and quantification, however required preparation of fatty acids as volatile methyl esters. Sodium methoxide was used for the preparation of fatty acid methyl esters (FAME) (PN-ISO 12966-2011). FAME were analyzed applying the Agilent 7820A gas chromatograph, equipped with a capillary column BPX-70 (60m x 0.25mm x 0.25 $\mu$ m) and flame ionization detection was used. Temperature gradient from 120°C to 230°C, in a total time of 50 min. The carrier gas was helium with constant flow 0.8 ml/min. Injection port was set to 250°C, with the split ratio 50:1, and the detector was at 270°C. The identification of fatty acids methyl esters was done by comparison of retention times of peaks in a sample with those of standard pure compounds. The quantification was based on relative percentage basis.

## The calculated lipid quality indexes

The atherogenic index (AI) and thrombogenic index (TI) were calculated from the fatty acids composition using equations by Ulbricht and Southgate (1991), modified by adding the TFA percentage to equation, including them to the sum of SFA (Vucic, *et al.* 2015):

$$AI = [C12:0 + 4x(C14:0) + C16:0 + TFA]/[\Sigma MUFA + \Sigma n-3 PUFA + \Sigma n-6 PUFA]$$

$$TI = [C14:0 + C16:0 + C18:0 + TFA]/[0.5 x \Sigma MUFA + 0.5 x \Sigma n-6 PUFA + 3x \Sigma n-3 PUFA + (\Sigma n-3PUFA/ \Sigma n-6PUFA)]$$

where:  $\Sigma$ MUFA – sum of monounsaturated fatty acids excluding TFA, TFA – *trans* fatty acids, all fatty acids (lauric, myristic, palmitic, stearic) and sums are expressed as a percentage of total fatty acids.

The hypocholesterolemic and hypercholesterolemic ratio (h/H) was calculated according to Santos-Silva, Bessa & Santos-Silva (2002).

$$h/H = (C18:1 n-9 + C18:2 n-6 + C18:3 n-3)/(C12:0 + C14:0 + C16:0)$$

## Results and discussion

### Fatty acids composition

The fatty acids composition in selected confectionery products and margarines are presented in Table 1. The obtained results indicate that the confectionery products and margarines were characterized by a diversified composition of

fatty acids. Fatty acids composition is one of the most important factor affecting nutritional quality of different food (Orsavova, *et al.* 2015). Chocolate bars (CB) had the most differential profile (Figure 1A), CB samples were characterized by the presence of short chain fatty acids (C6:0 – C8:0), which were not detected in wafer cookies and puff pastry samples at all, but also medium (C10:0 – C14:0) and long chain fatty acids (C16:0 – C24:0). Short chain fatty acids are typical for different chocolates (Ergonul, Ergonul & Seckin, 2010), and covering bars with chocolate glaze could be the reason for its presence. Whereas, the less differential profile was observed in puff pastry samples (Figure 1B). Only four fatty acids were identified in all samples, namely palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n9) and linoleic acid (C18:2 n6). Palmitic acid was the predominant saturated acid in confectionery products and margarines, 20,19% in wafer cookie (WC3) to 52,37% in puff pastry (PP3). Stearic acid content changed from 3,32% in margarines (M2) to 31,59% in chocolate bars (CB4). Oleic acid and linoleic acid are the main unsaturated fatty acids in analysed samples, oleic acid ranged from 24,98% WC2 to 42,79% M1, whereas linoleic acid from 1,44% WC2 to 11,79% M1. The variation in fatty acid composition is mainly related to the type of fat used in the product.

Generally, the dominant group of fatty acids in the analysed samples were saturated fatty acid, followed by monounsaturated fatty acids and polyunsaturated fatty acids, with some exception (Table 2). The percentage participation of SFA in the fat separated from different food products ranged from 37,89% to 64,69%. Lower content of SFA was observed in fat extracted from wafer cookies 26,10% to 37,39%. Kmiecik, *et al.* (2016) reported saturated fatty acids in bars up to 80,30%, and 75,54% in wafers. Confectionery products are recognized nowadays, as a source of saturated fatty acids in daily diet. The high content of SFA is the effect of replacing partially hydrogenated oils with palm oil or fully hydrogenated fats in confectionery production (Cakmak, Guler & Aktumsek, 2010; Norhayati, *et al.* 2011).

Additionally, samples with lower SFA percentage, were characterized by the presence of *trans* fatty acids, ranged from 11,67% to 32,57%, in WC4 and WC2, respectively. Since, 1 April such content is completely not allowed (Commission regulation (EU) 2019/649), and hopefully composition of fat used in production process has been changed. Moreover, margarines which were recognized for years as the main source of TFA, contained only 1,96% or was TFA free product. Typical margarines in 2000 contained around 12% TFA (Bruce, 2020). Although, high content of SFA and TFA, the desirable fatty acids (DFA) content in confectionery products and margarines ranged from 41,33% to 66,68%. DFA is calculated to assess the nutritional value of food, especially that DFA have an important role regarding to biological activity (Chen, *et al.* 2016).

**Table 1. Fatty acids composition [%] in selected confectionery products and margarines**

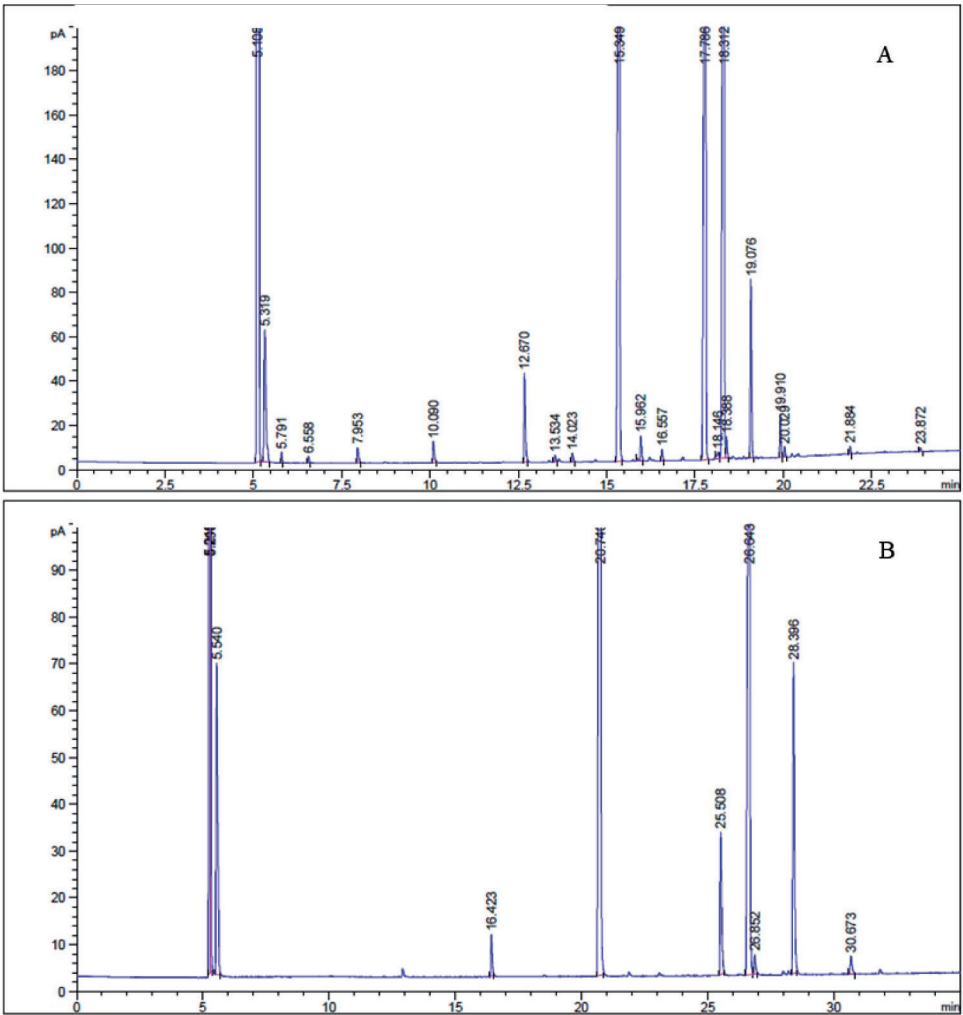
FA [%]	Shortbread				Chocolate bar					Wafer cookies					Puff pastry			Margarine		
	SB1	SB2	SB3	SB4	CB1	CB2	CB3	CB4	CB5	WC1	WC2	WC3	WC4	WC5	PP1	PP2	PP3	PP4	M1	M2
<b>C4:0</b>	nd	0.80 ±0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<b>C6:0</b>	nd	0.91 ±0.02	nd	nd	0.15 ±0.00	0.11 ±0.00	nd	0.21 ±0.01	0.20 ±0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<b>C8:0</b>	nd	0.67 ±0.02	nd	0.55 ±0.02	0.33 ±0.00	0.15 ±0.00	0.13 ±0.00	0.15 ±0.01	0.14 ±0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.48 ±0.01
<b>C10:0</b>	nd	1.89 ±0.04	nd	0.59 ±0.01	0.51 ±0.00	0.30 ±0.00	0.16 ±0.00	0.39 ±0.02	0.38 ±0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.51 ±0.01
<b>C12:0</b>	0.24 ±0.02	2.74 ±0.02	0.60 ±0.01	10.14 ±0.14	3.05 ±0.01	1.64 ±0.00	1.84 ±0.01	0.61 ±0.01	0.56 ±0.00	nd	3.57 ±0.00	nd	nd	nd	nd	nd	nd	nd	2.35 ±0.01	8.44 ±0.03
<b>C14:0</b>	1.24 ±0.10	9.80 ±0.09	1.38 ±0.00	4.44 ±0.05	2.85 ±0.01	2.22 ±0.00	1.07 ±0.02	2.14 ±0.06	2.21 ±0.00	nd	1.86 ±0.00	nd	0.77 ±0.00	0.72 ±0.00	0.98 ±0.00	1.06 ±0.00	1.02 ±0.00	1.05 ±0.00	1.49 ±0.00	3.44 ±0.01
<b>C14:1</b>	nd	0.77 ±0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<b>C16:0</b>	43.08 ±0.53	36.37 ±0.15	42.66 ±0.56	35.45 ±0.28	32.73 ±0.05	36.46 ±0.04	28.15 ±0.17	28.04 ±0.02	30.18 ±0.04	20.29 ±0.06	20.66 ±0.02	20.19 ±0.01	35.91 ±0.00	37.16 ±0.03	49.70 ±0.00	48.91 ±0.04	52.37 ±0.04	50.60 ±0.01	31.17 ±0.05	28.62 ±0.02
<b>C16:1</b>	nd	1.67 ±0.02	nd	nd	0.45 ±0.04	0.38 ±0.03	0.20 ±0.00	0.55 ±0.04	0.66 ±0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<b>C17:0</b>	nd	0.56 ±0.01	nd	nd	0.21 ±0.00	0.18 ±0.00	0.20 ±0.00	0.43 ±0.18	0.28 ±0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
<b>C18:0</b>	4.37 ±0.12	10.41 ±0.08	4.69 ±0.01	4.04 ±0.01	20.99 ±0.02	16.93 ±0.08	31.12 ±0.28	31.59 ±0.14	27.55 ±0.09	13.47 ±0.04	12.74 ±0.02	17.20 ±0.01	8.90 ±0.01	22.78 ±0.01	4.69 ±0.00	4.45 ±0.01	4.78 ±0.00	4.46 ±0.01	4.12 ±0.01	3.32 ±0.01



**Table 2. Sum of SFA, MUFA, PUFA, TFA, DFA and fatty acids ratios**

FA [%]	Shortbread				Chocolate bar					Wafer cookies					Puff pastry				Margarine	
	SB1	SB2	SB3	SB4	CB1	CB2	CB3	CB4	CB5	WC1	WC2	WC3	WC4	WC5	PP1	PP2	PP3	PP4	M1	M2
<b>SFA</b>	49.57 ±0.78	54.57 ±0.25	45.82 ±0.54	55.99 ±0.50	61.64 ±0.04	58.80 ±0.04	63.94 ±0.11	64.69 ±0.09	62.60 ±0.06	34.68 ±0.04	26.10 ±0.02	37.39 ±0.00	45.58 ±0.00	37.89 ±0.03	56.08 ±0.01	58.17 ±0.04	55.07 ±0.03	56.11 ±0.02	42.24 ±0.03	48.71 ±0.03
<b>MUFA</b>	39.26 ±0.57	27.29 ±0.18	39.91 ±0.42	35.35 ±0.39	33.11 ±0.04	35.03 ±0.01	31.80 ±0.06	31.93 ±0.10	33.18 ±0.04	34.45 ±0.03	27.16 ±0.00	29.32 ±0.01	39.52 ±0.06	34.77 ±0.02	35.41 ±0.01	33.63 ±0.06	35.51 ±0.03	34.70 ±0.02	44.00 ±0.02	39.63 ±0.02
<b>PUFA</b>	11.17 ±0.21	4.92 ±0.07	9.58 ±0.13	8.66 ±0.11	5.25 ±0.01	6.18 ±0.05	3.39 ±0.05	3.16 ±0.00	4.01 ±0.02	2.78 ±0.01	1.44 ±0.01	1.66 ±0.00	3.24 ±0.01	4.56 ±0.00	8.51 ±0.00	8.20 ±0.01	9.42 ±0.01	9.19 ±0.01	11.79 ±0.01	11.66 ±0.01
<b>TFA</b>	nd	1.13 ±0.13	nd	nd	nd	nd	0.67 ±0.01	0.22 ±0.01	0.21 ±0.00	28.10 ±0.01	32.57 ±0.01	31.63 ±0.00	11.67 ±0.04	nd	nd	nd	nd	nd	1.96 ±0.05	nd
<b>DFA</b>	54.80 ±0.65	42.63 ±0.13	54.18 ±0.54	48.05 ±0.49	59.36 ±0.06	58.13 ±0.04	66.31 ±0.16	66.68 ±0.24	64.74 ±0.04	50.69 ±0.08	41.33 ±0.01	48.18 ±0.03	51.56 ±0.03	62.11 ±0.03	48.61 ±0.01	46.61 ±0.04	49.38 ±0.03	48.35 ±0.01	59.92 ±0.02	54.61 ±0.05
<b>18:0+18:1/ 16:0</b>	1.01 ±0.02	1.02 ±0.01	1.05 ±0.02	1.11 ±0.02	1.64 ±0.00	1.41 ±0.00	2.23 ±0.02	2.25 ±0.01	1.99 ±0.00	2.36 ±0.01	1.93 ±0.00	2.30 ±0.00	1.35 ±0.00	1.55 ±0.00	0.81 ±0.00	0.73 ±0.00	0.82 ±0.00	0.77 ±0.00	1.38 ±0.28	1.50 ±0.00
<b>SFA/UFA</b>	0.98 ±0.03	1.69 ±0.02	0.93 ±0.02	1.27 ±0.03	1.61 ±0.00	1.43 ±0.00	1.82 ±0.01	1.84 ±0.01	1.68 ±0.00	0.93 ±0.00	0.91 ±0.00	1.21 ±0.00	1.07 ±0.00	0.96 ±0.00	1.28 ±0.00	1.39 ±0.00	1.23 ±0.00	1.28 ±0.00	0.76 ±0.00	0.95 ±0.00
<b>SFA+TFA/ UFA</b>	0.98 ±0.03	1.73 ±0.02	0.93 ±0.02	1.27 ±0.03	1.61 ±0.00	1.43 ±0.00	1.84 ±0.01	1.85 ±0.01	1.69 ±0.00	1.69 ±0.00	2.05 ±0.00	2.23 ±0.00	1.34 ±0.00	0.96 ±0.00	1.28 ±0.00	1.39 ±0.00	1.23 ±0.00	1.28 ±0.00	0.79 ±0.00	0.95 ±0.00

Explanation: SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, TFA – trans fatty acids, DFA – desirable fatty acids, UFA – unsaturated fatty acids.



**Figure 1. Chromatogram of fatty acids methyl esters separation of fat extracted from chocolate bar (A) and puff pastry (B)**

**Lipid quality indexes.** In order to evaluate potential effect of fatty acids on the incidence of pathogenic phenomena, atherogenic index (AI), thrombogenic index (TI) and hypocholesterolemic/hypercholesterolemic index (h/H) were calculated (Table 3). The lowest AI index was observed for margarine M1 0.74, whereas the highest 2.25 for wafer cookie WC2 and 2,34 for shortbread SB2. The AI shows the relationship between the sum of the main SFA and that of important unsaturated fatty acids. Moreover, lowest value of TI was observed in margarine M2 1.30 and the highest 4.74 for WC2. The TI indicates the tendency to form clots in the blood vessels, defined as relationship between prothrombogenic SFA and

antithrombogenic MUFA, n-6 PUFA and n-3 PUFA (Ghaeni, Ghahfarokhi & Zaheri, 2015). Elevated values of AI and TI are the effect of high content of saturated and *trans* fatty acids in analysed samples, which are responsible for health issues. Myristic acid (C14:0) is considered to be 4-times more atherogenic than the other saturated fatty acids, thus the coefficient of 4 is assigned to it. Additionally, n-3 PUFA are more antiatherogenic than others PUFA and are assigned with coefficient 3 (Pikul, *et al.* 2008). The smaller the AI and TI, the greater the protective potential of coronary artery disease. Therefore, in terms of consumer health safety, the AI lower than 1.0 and TI lower than 0.5, in the diet are recommended (Fernandes, *et al.* 2014). It was found that, among analysed samples only six samples had AI lower than 1.0, but none of them had TI lower than recommended. Suggesting adverse effect of such products on human safety and health. Additionally, the ratio between hypocholesterolemic and hypercholesterolemic fatty acids indicated the effect of specific fatty acids on cholesterol metabolism, ranged from 0.63 SB2 to 1.76 WC1. Higher h/H values are considered more beneficial for consumer health.

**Table 3. Nutritional quality indexes of confectionery products**

Confectionery products		AI	TI	H/H
Shortbread	SB1	0.96±0.03	1.93±0.06	1.12±0.03
	SB2	2.34±0.02	3.18±0.02	0.63±0.01
	SB3	0.99±0.02	1.97±0.05	1.09±0.03
	SB4	1.44±0.03	2.00±0.04	0.87±0.02
Chocolate bar	CB1	1.23±0.00	2.85±0.00	0.97±0.00
	CB2	1.14±0.00	2.62±0.00	1.00±0.00
	CB3	0.99±0.00	3.35±0.02	1.12±0.00
	CB4	1.07±0.01	3.40±0.00	1.11±0.01
	CB5	1.07±0.00	3.12±0.01	1.10±0.00
Wafer cookie	WC1	1.30±0.00	3.32±0.00	1.76±0.00
	WC2	2.25±0.00	4.74±0.00	1.01±0.00
	WC3	1.67±0.00	4.46±0.00	1.44±0.00
	WC4	1.13±0.00	2.68±0.01	1.15±0.00
	WC5	0.96±0.00	3.08±0.00	1.04±0.00
Puff pastry	PP1	1.22±0.00	2.52±0.00	0.86±0.00
	PP2	1.35±0.00	2.78±0.00	0.78±0.00
	PP3	1.18±0.00	2.42±0.00	0.89±0.00
	PP4	1.25±0.00	2.56±0.00	0.85±0.00
Margarine	M1	0.74±0.00	1.32±0.00	1.59±0.00
	M2	0.99±0.00	1.30±0.00	1.27±0.00



## Conclusions

Confectionery products and margarines are nowadays very popular. The type of fat used in their production affect their quality and safety for consumers. Elimination of *trans* fatty acids in food products forced by the EU Regulation 2019/649, influenced the fatty acid profile in products available on the market. However, based on obtained results, the confectionery products and margarines available on the market had too high content of SFA, with only low proportion of polyunsaturated fatty acids. Therefore, there is a strong need for reformulation of such types of food, and replace at least part of SFA and remove TFA to the recommended values.

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## INFLUENCE OF SELECTED FACTORS ON THE QUANTITY AND QUALITY OF WHEY SEPARATED IN THE PRODUCTION OF TVAROG – ACID CURD CHEESE

Jolanta Król<sup>1</sup>, Aneta Brodziak<sup>1</sup>

### Abstract

The aim of the study was to assess acid whey separated during traditional manufacture of tvarog – Polish acid curd cheese. Two variants of starter cultures were used in the study. The tvarog was made from bulk milk obtained from two breeds of cows (Polish Holstein-Friesian and Simmental) during two production seasons (spring/summer and autumn/winter). Forty whey samples were analysed. The following parameters were determined in each sample: content of crude protein, fat, lactose, and dry matter, pH value, content of fat-soluble vitamins. The volume of whey obtained was determined as well. The breed of cow, season, and starter cultures were shown to influence the amount of whey separated as well as its chemical composition. The quantity of whey separated was significantly lower, which is technologically more favourable, in the production of tvarog from the milk of Simmental cows and in the spring/summer, when the cows grazed in the pasture. The starter culture also influenced the separation of whey, with less whey obtained when starter variant 2 was used. Whey obtained in the spring and summer, irrespective of the cow breed, had lower protein and fat content, with higher content of vitamins. The starter cultures did not affect the composition of the whey.

**Keywords:** whey, cheese, tvarog, chemical composition, vitamins

<sup>1</sup> University of Life Sciences in Lublin Department of Quality Assessment and Processing of Animal Products, Poland, [jolanta.krol@up.lublin.pl](mailto:jolanta.krol@up.lublin.pl)

## Introduction

Whey is one of the most important by-products in the dairy industry, obtained mainly in the manufacturing process of cheese, including curd cheese. The scale of whey production is on a steady increase; it has been estimated that approx. 9 million tons of cheese are produced in the European Union annually, which translates to an average production of 50 million m<sup>3</sup> of whey a year. Also in Poland, the volumes have been growing in recent years along with the production of cheese (Table 1). The estimated production of whey in Poland in 2020 reached over 1,700 thousand tons, including 310 tons of dried whey (IERiGŻ-PIB, 2021).

**Table 1. Production of whey and cheeses (thousand tons) in Poland in the years 2016–2020**

Product/Year	2016	2017	2018	2019	2020
<b>Whey in total</b>	1301	1795	1650	1720	1750
<b>Dried whey</b>	283	289	321	308	310
<b>Acid curd cheeses (tvarog)</b>	449	459	470	480	490
<b>Rennet cheeses</b>	329	338	352	373	370

Source: IERiGŻ-PIB, 2021.

In the past, whey was treated as a burdensome waste that had a significant, negative effect on the surrounding environment. It was often released into rivers and water reservoirs leading to excessive pollution. This resulted in thinning of the aqueous flora and fauna as decomposition of untreated whey consumes huge amounts of oxygen that water organisms need to survive (Michalska, *et al.* 2013). Currently, however, whey is increasingly recognised as a valuable raw material fit for further processing. Its usefulness for processing is determined by the content of whey proteins and lactose. Content of whey proteins plays an important role during fractioning and concentrating of whey by employing membrane techniques. Therefore, it is possible to manufacture a wide range of whey-based products, from whey protein concentrates (WPC) to whey protein isolates (WPI) (Brodziak, Król & Litwińczuk; 2012; Król, *et al.* 2011a; Siemianowski & Szpendowski, 2010; Śliwa, Sikora & Ogonowski, 2011). Membrane techniques of whey fractioning facilitate separation of ingredients based on their molecular mass. Depending on the membrane density, the following membrane processes can be distinguished: reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), and microfiltration (MF). The latter process is sufficient to separate fat and bacteria. Ultrafiltration allows the separation of proteins while in order to separate lactose, nanofiltration is required. Dehydration and simultaneous demineralisation of whey is achieved in the process of reverse osmosis. The method of ultrafiltration facilitates

concentration of whey protein and can yield products with different content thereof and varied share of other ingredients (Smithers, 2008). Products of the process include whey protein concentrate that can contain up to 86% of whey protein. They are most often used to standardise protein content in defatted milk powder and as additives improving the quality and functional properties of other products (Karolini-Skaradzińska, *et al.* 2010). Whey protein isolates (WPI) are characterised by even higher levels of minimum protein content, i.e. over 90%. They can be used in protein supplements for athletes or body builders as well as various food additives (Glibowski, 2004; Szczurek, 2008). The other direction is the use of lactose obtained from whey. Lactose is available in three forms – technical lactose, and dedicated for food or pharmaceutical industries (Dajnowiec, *et al.* 2012).

The aim of the paper was to evaluate the quantity and quality of sour whey obtained in the process of curd cheese production, relative to the cattle breed (Polish Holstein Friesian, Simmental), production season (spring-summer, autumn-winter), type of starter cultures used (lyophilised culture, traditional starter).

## Material and methods

Whey originating from the process of curd cheese (tvarog) production conducted under laboratory conditions was analysed. Two variants of starter cultures were used in the study: starter variant 1, i.e. freeze-dried DVS starters used to directly inoculate milk with lactic acid bacteria (Flora Danica by Chr. Hansen, Denmark), and starter variant 2, a traditional working starter obtained using freeze-dried inoculants of pure cultures of mesophilic lactic acid bacteria. The starters differed primarily in terms of their content of *Lactococcus lactis subsp. diacetylactis* strains that are responsible for the synthesis of compounds affecting scent and taste properties as well as CO<sub>2</sub> production. The first of the starters contained microorganisms of the *Lactococcus lactis subsp. diacetylactis* type at the level of 5–40%, and the second at – 5–30%. The tvarog was made from bulk milk obtained from two cow breeds (Polish Holstein-Friesian and Simmental) during two production seasons (spring/summer and autumn/winter). Incubation took place at the temperature of 30–32°C for 12 h to reach pH of approx. 4.6. The whey was then allowed to drain for 2 h. Forty whey samples were analysed. The following parameters were determined in each sample: content of crude protein, fat, lactose, and dry matter (with the Infrared Milk Analyzer, Bentley Instruments) and active acidity (pH value) using pH-meter (Elmetron CP-401, Poland). The concentrations of selected fat-soluble vitamins, i.e. A, D<sub>3</sub> and E, in the milk, whey and cheese were determined by reversed-phase high performance liquid chromatography (RP-HPLC) using a ProStar Varian liquid chromatograph equipped

with a fluorescence detector. Samples were prepared by extracting fat according to the Röse-Gottlieb method as modified by Hewavitharana, van Brakel & Harnett (1996). Compounds were separated on a Pursuit XRs 3-C18 column (Varian, USA) with a length of 150 mm and a diameter of 4.6 mm. The mobile phase was a mixture of acetonitrile, methanol, water and dichloromethane (Sigma, Germany), and the flow rate was set at 1 ml/min. Reference substances were analysed under identical conditions. Standard solutions of vitamins were used for this purpose: vitamin E ( $\alpha$ -tocopherol) with  $\geq 97\%$  purity (HPLC), vitamin D<sub>3</sub> (cholecalciferol) with  $\geq 98\%$  purity (HPLC), and vitamin A (retinol)  $\geq 99\%$  (Sigma, Germany). Qualitative identification of each substance was based on analysis of retention times read from individual chromatograms using Star 6.2 Chromatography Workstation software (Varian, USA). Quantitative analysis was performed by the external standard method. A statistical analysis of the results was performed using StatSoft Inc. Statistica ver. 13.1 (Dell 2016), using one-way and multi-way analyses of variance (ANOVA). The significance of the differences between the means for the groups was determined by Mann-Whitney test at a level of  $p$  ( $\alpha$ ) = 0.05. The results are presented as the means  $\pm$  SD.

## Results and Discussion

Significantly ( $p \leq 0.01$ ) lower whey run-off, i.e. more beneficial from the technological perspective, was observed when producing curd cheese from the milk of Simmental cows (Table 2). The run-off observed for the milk from Holstein Friesian cows was 7% higher. In the opinion of many authors (Król, *et al.* 2012; Skryplonek & Jasińska, 2016), the addition of whey proteins helps to reduce the separation of whey from the curd, which is due to the proteins' ability to bind water. This suggests that the milk obtained from Simmental cows contained higher amounts of whey proteins when compared to Holstein Friesian milk. At the same time, Dmytrów (2015) concluded that the amount of whey produced was significantly affected by the type of starter culture used. In the author's opinion, the phenomenon can be explained by the fact that bacteria present in curd cheese starters, due to varied acidity of the medium, dictate the shrinkage of the casein curd, thus determining the amount of whey separated. The starter cultures used in the present study significantly ( $p \leq 0.05$ ) affected the whey release. Higher amounts of whey were obtained with the use of Flora Donica starter (starter culture 1). In this respect, starter cultures 2 proved more beneficial as the relevant whey separation was approximately 3% lower. No significant impact of the season on the amount of whey produced was observed. In a study by Król, *et al.* (2019), it was demonstrated that the addition of whey protein concentrate (WPC), regardless of the starter used, significantly influenced the amount of whey obtained, with the largest and clearest quantities reported for samples without

the addition of WPC. As the amount of WPC increased, the volume of whey was smaller and it became more turbid. It is notable that higher (significantly at  $p \leq 0.01$ ) differences in the quantity of whey obtained were recorded in samples where the Flora Danica starter was used. Skryplonek & Jasińska (2016) observed whey release in a quantity corresponding to 1/3 of the volume of the raw material. In our own study, the same reached over 50%.

The correct pH levels of whey created in the process of acidic milk coagulation when producing curd cheese should be between 4.5 and 4.7 (Wronkowska, *et al.* 2012). Hence, the results obtained in the present study can be treated as optimum (Table 2). The water content in the whey was fairly stable at slightly over 93%. Statistically significant differences in terms of the same were only recorded between the respective seasons. Whey obtained in the production of curd cheese during the spring-summer season contained significantly more ( $p \leq 0.05$ ) water than that obtained in the autumn-winter season. The registered water content was similar to that reported by other authors (Siemianowski, *et al.* 2013) who placed it at 93.58%. As estimated by Oziemkowski (1993), the average composition of sour whey includes between approx. 94 and 95% water. The production season significantly differentiated the content of fat ( $p \leq 0.01$ ) and protein ( $p \leq 0.05$ ) in the whey. The content of fat and protein was two times lower in whey obtained after draining curd cheese produced from spring-summer milk.

**Table 2. Quantity and basic physicochemical parameters of whey with regard to the analysed factors**

Parameter	Breed		Season		Starter cultures*	
	Holstein Friesian	Simmental	autumn/winter	spring/summer	1	3
Obtained quantity (ml)	591 <sup>B</sup> ±61	554 <sup>A</sup> ±50	578 ±51	569 ±66	581 <sup>b</sup> ±37	565 <sup>a</sup> ±73
Active acidity (pH value)	4.62 ±0.18	4.60 ±0.16	4.67 <sup>b</sup> ±0.17	4.52 <sup>a</sup> ±0.12	4.69 <sup>B</sup> ±0.12	4.52 <sup>A</sup> ±0.17
Water (%)	93.54 ±0.26	93.41 ±0.35	93.32 <sup>b</sup> ±0.30	93.69 <sup>a</sup> ±0.17	93.48 ±0.33	93.47 ±0.30
Fat (%)	0.25 ±0.09	0.30 ±0.11	0.35 <sup>B</sup> ±0.10	0.17 <sup>A</sup> ±0.04	0.27 ±0.09	0.28 ±0.06
Protein (%)	0.46 ±0.04	0.46 ±0.07	0.49 <sup>b</sup> ±0.03	0.44 <sup>a</sup> ±0.06	0.48 <sup>b</sup> ±0.06	0.43 <sup>a</sup> ±0.04
Lactose (%)	4.58 ±0.19	4.54 ±0.18	4.60 ±0.22	4.55 ±0.13	4.56 ±0.22	4.57 ±0.23

Explanation: \* Starter cultures: 1 – freeze-dried DVS starter, 2 – traditional starter.

a, b, A, B – differences statistically significant within the factor: a, b – at  $p \leq 0.05$ ; A, B – at  $p \leq 0.01$ .

It resulted from the lower level of these compounds in milk dedicated for processing. No significant differences in this respect were observed between the respective cow breeds. The used starter cultures did not significantly affect the content of fat in the whey. However, milk treated with starter cultures 1 produced whey containing significantly more protein as compared to starter culture 2 (0.48% vs. 0.43%). As observed by Oziemkowski (1993), the fat content in sour whey was between 0.1 and 0.2%, and the protein content between 0.8 and 1%. In turn, in other experiments (Siemianowski, *et al.* 2013) the protein content in whey was reported at 0.41% and was similar to that recorded in the present study. Lactose, corresponding to the largest percentage component of whey, was not statistically significantly affected, oscillating between 4.50 and 4.60% for all variables, which corresponded to results reported by other authors (Siemianowski, *et al.* 2013) who estimated the lactose content in sour whey at 4.68%. Król, Brodziak & Litwińczuk (2011b) studied the impact of the cattle breed (Polish Holstein Friesian black and white, Polish Holstein Friesian red and white, Simmental, Jersey) on the chemical composition of rennet whey. It was found to on average contain 1.45% of total protein, with the highest levels thereof recorded for whey from the milk of Jersey cows (1.66%). The highest content of lactose was observed in the whey from Polish Holstein Friesian red and white cows' milk (4.51%), and the lowest for the black and white cows (4.27%). The fat content was reported between 0.04% (Polish Holstein Friesian red and white) and 0.06% (Simmental and Jersey). As reported by Zadow (1992), protein content in rennet whey oscillated between 0.8 and 0.9%, while Zander & Zander (2007) estimated it at 0.62%. In turn, the lactose content in whey was between 3.54 and 4.18% according to Räsänen, *et al.* (2002).

Table 3 presents the content of lipophilic vitamins in the sour whey obtained. It was observed that the concentration of vitamins A, D<sub>3</sub>, and E in whey varied, depending particularly on the season during which the milk used in the curd cheese production was obtained. In the case of each respective vitamin, significantly ( $p \leq 0.01$ ) higher concentrations measured in the whey obtained from curd cheese drainage were recorded for the spring-summer season. Breed significantly influenced the content of vitamins D<sub>3</sub> and E. Significantly higher ( $p \leq 0.01$ ) content thereof was observed in the whey from Holstein Friesian cows' milk. The whey produced during drainage of curd cheese produced from Simmental milk contained 5% less vitamin D<sub>3</sub> and 9% less vitamin E. No statistically significant differences in terms of the vitamin content were observed relative to the respective starter cultures used. It is difficult to compare the obtained values of vitamin content in sour whey to other reports due to the lack of relevant data in the available literature.



**Table 3. Content of lipophilic vitamins in the whey with regard to the analysed factors**

Parameter	Breed		Season		Starter cultures*	
	Holstein Friesian	Simmental	autumn/winter	spring/summer	Holstein Friesian	Simmental
Vitamin A (mg/l)	0.089 ±0.007	0.085 ±0.012	0.082 <sup>A</sup> ±0.07	0.094 <sup>B</sup> ±0.008	0.088 ±0.010	0.086 ±0.009
Vitamin D <sub>3</sub> (µg/l)	0.117 <sup>B</sup> ±0.008	0.111 <sup>A</sup> ±0.006	0.110 <sup>A</sup> ±0.005	0.118 <sup>B</sup> ±0.008	0.114 ±0.007	0.114 ±0.008
Vitamin E (mg/l)	0.288 <sup>B</sup> ±±0.022	0.262 <sup>A</sup> ±0.023	0.263 <sup>A</sup> ±0.024	0.291 <sup>B</sup> ±0.018	0.275 ±0.027	0.275 ±0.024

Explanation: \* Starter cultures: 1 – freeze-dried DVS starter, 2 – traditional starter.

a, b, A, B – differences statistically significant within the factor: a, b – at  $p \leq 0.05$ ; A, B – at  $p \leq 0.01$ .

## Conclusions

In summary, it can be concluded that the breed of cows, system and season of production, as well as the starter cultures used, had a bearing on the amount of whey produced, its chemical composition and the content of lipophilic vitamins. It was demonstrated that lower whey separation, preferable from the technological perspective, was observed during the production of curd cheese from the milk of Simmental cows under the traditional system, as well as using starter cultures 2 – i.e. the curd traditional starter. It should be emphasised that the quality of whey is directly correlated with the milk quality. It is difficult to indicate unequivocally which whey is characterised by the highest quality, taking into consideration all factors simultaneously. Whey from the autumn/winter season contained the highest level of the components of dry matter, mostly including protein ( $p \leq 0.05$ ) and fat ( $p \leq 0.01$ ). With regard to the environmental aspect, whey containing less dry matter, from the spring/summer season on the basis of starter cultures 2 would be the best. However, the whey with higher protein and lactose content, i.e. obtained in the autumn/winter season and using the starter cultures 1, would be more appropriate for the further food processing. Generally, it should be emphasised that the aim in the cheese production, including tvarog, is maintaining the highest level of dry matter compounds in the product.

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## THE EFFECT OF FAT CONTENT ON QUALITY OF HOMEMADE NATURAL YOGHURT

Jacek Lewandowicz<sup>1</sup>

### Abstract

Yoghurts are an important constituent of human diet, and their annual consumption in Poland is estimated at 6–8 kg per person. Consumers are favourable towards innovations developed on the yoghurt market, the most desired products are those with new uncommon flavours or health-promoting properties. Moreover consumers seek for low calorie products, especially those produced on the basis of skimmed milk without addition of sucrose and/or milk powder. Therefore the aim of the work was to evaluate the effect of fat content on physicochemical and rheological properties of natural yoghurt. The experimental material consisted of natural yoghurts prepared on basis of skimmed milk 0%, 0.5%, 2.0%, 3.2%, whole milk 3.8% and evaporated milk 5.0%. Investigated products were evaluated in terms of rheological properties, universal texture profile (TPA), acidity (°SH) and syneresis.

It was found that the content of fat used for production of natural yoghurt considerably affects its rheological properties. Other physicochemical properties are affected to significantly smaller extent. Obtained results show that production of low calorie yoghurt with desired sensory properties may be limited, thus indicating that use of additives such as modified starch that can act both as a rheology modifier and emulsion stabilizer can be beneficial.

**Keywords:** natural yoghurt, low fat, rheological properties, TPA, quality design

<sup>1</sup> Poznań University of Technology, Institute of Logistics; [jacek.lewandowicz@put.poznan.pl](mailto:jacek.lewandowicz@put.poznan.pl)

## Introduction

Yoghurt according to Codex Alimentarius is a milk product obtained by fermentation of milk using suitable microorganisms (symbiotic cultures of *Streptococcus thermophilus* and any species of *Lactobacillus*) that results in reduction of pH and coagulation (FAO/WHO, 2003). The raw material for production of yoghurt may consist of whole, semi-skimmed or skimmed milk, and/or reconstituted milk powder (Mojka, 2013), however the amount of fat in ready product should not exceed 15% (FAO/WHO, 2003). The use of certain food additives is also justified for production of natural yoghurt i.e. stabilizers and thickeners (FAO/WHO, 2003), however not permitted by the European law (Commission Regulation No 1129/2011). Therefore for production of natural yoghurt addition of evaporated milk, milk powder or milk proteins may be considered, that serve similar technological functions (Kowalka, Pikul & Oziemkowski, 2000). Those substrates are relatively expensive (Glibowski & Krępacka, 2006), not permitted by certain international standards such as Demeter (Wichrowska & Wojdyła, 2014) as well as not welcome by many consumers (Kulczyński, 2020).

Yoghurt are important constituent of human diet, characterized by stable consumption, that is estimated at over 6 kg per person annually in Poland (Kowalczyk & Szymański, 2017; Wichrowska & Wojdyła, 2014; Wyrzykowski, 2019). The most popular on the market are flavored yoghurts, especially those with addition of fruits (Wichrowska & Wojdyła, 2014), however products containing: seeds, herbs or vegetables are also available (Wajs & Stobiecka, 2020). Nevertheless the highest growth is observed in the segment of natural yoghurts, as those products are previewed as healthier alternative (Wichrowska & Wojdyła, 2014). New products are constantly developed as consumers seek for innovations characterized by new extraordinary tastes as well as health promoting properties (Kowalczyk & Szymański, 2017; Lewandowicz, Le Thanh-Blicharz & Śmigielska, 2019).

Consumption of yoghurt is associated with improvement of diet quality (Cifelli, Agarwal & Fulgoni III, 2020) and numerous health benefits such as lowering inflammatory biomarkers (Yuan, Singer & Moore, 2021), prevention of cardiovascular disease (Cifelli, Agarwal & Fulgoni III, 2020) or several gastrointestinal diseases (Szwedziak & Antczak 2016). For this reason research towards development of yoghurt with health promoting properties are often performed, partially noteworthy are those: with reduced fat content (Mleko, 1996; Saleh, *et al.* 2020) or fortified with bioactive substances (Jovanović, *et al.* 2020; Lewandowicz, Le Thanh-Blicharz & Śmigielska, 2019; Stankiewicz, 2009) or mineral salts (Śmigielska, 2016; Żbikowska & Żbikowski 2011). Therefore the aim of the work was to determine the influence of fat content on rheological properties and texture of natural yoghurt.

## Materials and methods

The experimental material consisted of 6 natural yoghurts varying in fat content. Sterilized milk (SM “MLEKPOL”) with fat content of 0.0%, 0.5%, 2.0%, 3.2%, and 3.8% respectively as well as condensed milk prepared by reconstitution of whole milk powder (SM “MLEKPOL”) with fat content of 5.0% was used for production of yoghurt. Natural yoghurt (SM „Jana”) containing among others Bifidobacterium BB-12® and Lactobacillus acidophilus La-5® was used as a starter culture. All reagents used were of analytical grade unless otherwise stated.

Yoghurts were prepared in 160 cm<sup>3</sup> portions using 10 cm<sup>3</sup> of natural yoghurt and 150 cm<sup>3</sup> of milk. Fermentation was performed using yoghurt maker JG3523 (Severin Elektrogeräte GmbH) during 12 hours. After the fermentation yoghurts were matured for 24 hours at temperature of 4±2°C. Samples were coded with three character codes according to their fact content (Table 1).

**Table 1. Nutrition per 100 ml of unfermented product**

Sample	Fat	Protein	Carbohydrates
Y00	0.3	3.4	5.1
Y05	0.7	3.4	5.1
Y20	2.1	3.4	4.9
Y32	3.3	3.3	4.9
Y38	3.8	3.3	4.9
Y50	4.9	4.8	7.0

Source: calculated based on manufacturer statements.

Explanation: Y00 – yoghurt produced with 0.0% fat skimmed milk, Y05 – yoghurt produced with 0.5% fat semi-skimmed milk, Y20 – yoghurt produced with 2.0% fat semi-skimmed milk, Y32 – yoghurt produced with 3.2% fat semi-skimmed milk, Y38 – yoghurt produced with 3.8% fat whole milk, Y50 – yoghurt produced with 5.0% fat condensed milk.

Syneresis was determined by centrifugation. Yoghurt samples of roughly 10 cm<sup>3</sup> were placed into 15 cm<sup>3</sup> scaled test tubes and centrifuged with acceleration of 250 x g during 30 minutes. Synereis was calculated in percent according to the following formula:

$$\text{Syneresis} = \frac{V_y - V_w}{V_y} \cdot 100\% \quad (1)$$

where:  $V_y$  – volume of yoghurt [cm<sup>3</sup>],  $V_w$  – volume of separated whey [cm<sup>3</sup>].

Titrateable acidity was determined by neutralization of accurately weighted 25 g yoghurt sample using 0.25 mol/dm<sup>3</sup> sodium hydroxide solution using phenolphthalein as indicator. Obtained results are expressed as Soxhlet-Henkel degrees (°SH) as well as lactic acid content using 1°SH = 0.0225% of lactic acid conversion ratio (Wichrowska & Wojdyła, 2014).

Universal Texture Profile (Texture Profile Analysis) was determined with TA-XT2 texturometer (Stable Micro Systems) Standard “double bite test” was performed with aluminium cylindrical head (35 mm diameter) in 68 mm diameter vessel on depth of 20 mm with speed of 0.5 mm/s. Fracturability (N), Hardness (N), adhesiveness (N·s), cohesiveness, springiness, resilience and gumminess [N] were determined.

Rheological properties were determined using RotoVisco1 rheometer (Haake Technik GmbH). Before the measurement samples were thermostated at 20°C and relaxed in measuring cylinder for 5 min. Data collection and calculations were made using RheoWin 3.61 software. Measurements were performed under following conditions: Z20 DIN Ti coaxial measurement system, 1-600-1 s<sup>-1</sup> shear rate range and time of 2 min. Obtained flow curves were described with Ostwald de Waele (2) and Casson (3) equation.

$$\tau = K \cdot \dot{\gamma}^n \quad (2)$$

where:

$\tau$  – shear stress Pa,  $K$  – consistency index Pa·s<sup>n</sup>,  $\dot{\gamma}$  – shear rate [s<sup>-1</sup>],  $n$  – flow behavior (dimensionless)

$$[\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta_c} \cdot \sqrt{\dot{\gamma}}] \quad (3)$$

where:

$\tau$  – shear stress Pa,  $\tau_0$  – field stress Pa,  $\eta_c$  – Casson plastic viscosity Pa·s,  $\dot{\gamma}$  – shear rate s<sup>-1</sup>.

All analyses were performed in triplicate (on two independent trials) and the results are presented as mean ± standard deviation. Experimental data was studied using one way analysis of variance and Tukey’s post hoc test. Cluster analysis (CA) was conducted based on Ward’s method and Euclidean distance was used as a measure of similarity. The statistical analyses were performed using Statistica 13.3 software (Dell Software Inc.).

## Results and discussion

Titration acidity and lactic acid content of investigated natural yoghurts is presented in Table 2. All analyzed products met the requirement of Codex Alimentarius which sets the minimal amount of lactic acid (titrable) at 0.6%, which corresponds to almost 27°SH (FAO/WHO, 2003). All yoghurts except the one prepared with milk powder (Y50) were characterized by comparable values of lactic acid content ranging between 0.96-0.99. Similar values were reported by Wichrowska & Wojdyła (2014) for various commercial natural yoghurts that were characterized by lactic acid content between 0.81–1.08. Slightly lower values (0.89-0.93) were reported by Lewandowicz, Le Thanh-Blicharz & Śmigielska (2019) for natural yoghurts prepared at laboratory scale with and without addition of inulin. Considerably higher acidity of Y50 is related to better availability of nutrients, and especially carbohydrates that were present at level over 1/3 higher to compared to any other sample.

Yoghurt is thermodynamically unstable dispersed system. The first reason for that is the fact that milk and is an oil in water emulsion. Secondly, during fermentation formed lactic acid causes proteins to coagulate thus forming thixotropic sol/gel structure (Lewandowicz, Le Thanh-Blicharz & Śmigielska 2019). Syneresis study (Table 1) revealed significant differences between investigated samples. Yoghurts produced with skimmed (Y00) or substantially semi-skimmed milk (Y05) were characterized by very poor stability below 50%. The increase of fat content in milk to 2.0% (Y20) resulted in improvement of yoghurt stability by reduction of syneresis by half. Further increase of fat content (Y32, Y38 and Y50) resulted in reduction of syneresis to levels observed for commercial products i.e. below 10% (Wichrowska & Wojdyła 2014).

Universal texture profile exposed significant influence of fat content on texture of natural yoghurt (Table 3). Along with the increase of fat content one may notice increase in such parameters as fracturability, hardness, adhesiveness and gumminess as well as decrease of cohesiveness and resilience. However, the above statement is only true for products based on semi-skimmed or whole milk. Yoghurt produced with skimmed milk (Y00) was characterized by higher values of hardness, adhesiveness and gumminess than the one produced with 0.5% fat semi-skimmed milk. This indicates that at low levels fat makes the yoghurt texture more liquid/sol like.

Therefore obtain more solid/gel like structure higher content of fat is required. It should be noted that addition of milk powder (Y50) leads to improvement of texture profile parameters, but also along with those negatively perceived by most consumers in products like yogurt i.e. adhesiveness and gumminess. This indicates that standardization of yoghurt dry mass should be performed along with sensory trials.



**Table 2. Titratable acidity and syneresis of natural yoghurts**

Sample	Titratable acidity [°SH]	Lactic acid [g·100 g <sup>-1</sup> ]	Syneresis [%]
Y00	43.9 ± 0.3 <sup>a</sup>	0.99 ± 0.01 <sup>a</sup>	53.1 ± 1.3 <sup>c</sup>
Y05	43.7 ± 0.6 <sup>a</sup>	0.98 ± 0.01 <sup>a</sup>	52.4 ± 0.1 <sup>c</sup>
Y20	43.1 ± 0.8 <sup>a</sup>	0.97 ± 0.02 <sup>a</sup>	26.5 ± 2.1
Y32	42.6 ± 0.4 <sup>a</sup>	0.96 ± 0.01 <sup>a</sup>	5.0 ± 0.0 <sup>b</sup>
Y38	43.0 ± 0.3 <sup>a</sup>	0.97 ± 0.01 <sup>a</sup>	7.5 ± 2.5 <sup>ab</sup>
Y50	57.1 ± 0.7 <sup>b</sup>	1.28 ± 0.02 <sup>b</sup>	2.5 ± 0.0 <sup>a</sup>

Explanation: data are expressed as mean value ± standard deviation. Values denoted with the same letter within a column (a, b) do not differ significantly ( $p > 0.05$ ).

Yoghurts are pseudoplastic, thixotropic non-Newtonian fluids (Dołhańczuk-Śródka, *et al.* 2015). All investigated samples did not deviate from that characteristics (Table 4.). Rheological examination of yoghurt samples confirmed previous observations regarding both the influence of fat content as well as the lack of it.

**Table 3. Universal texture profile of natural yoghurts**

Sample	Fracturability	Hardness	Adhesiveness	Springiness	Cohesiveness	Resilience	Gumminess
Y00	nd	1.44 ± 0.07 <sup>ab</sup>	-0.82 ± 0.04 <sup>a</sup>	0.95 ± 0.01 <sup>a</sup>	0.51 ± 0.02 <sup>a</sup>	0.07 ± 0.01	0.74 ± 0.05 <sup>ab</sup>
Y05	nd	1.14 ± 0.06 <sup>a</sup>	-0.52 ± 0.03 <sup>a</sup>	0.95 ± 0.01 <sup>a</sup>	0.57 ± 0.03 <sup>b</sup>	0.13 ± 0.01	0.64 ± 0.05 <sup>a</sup>
Y20	1.11 ± 0.05 <sup>a</sup>	1.54 ± 0.07 <sup>bc</sup>	-5.95 ± 0.18	0.96 ± 0.01 <sup>a</sup>	0.52 ± 0.01 <sup>ab</sup>	0.03 ± 0.01 <sup>a</sup>	0.81 ± 0.05 <sup>ab</sup>
Y32	1.33 ± 0.07 <sup>ab</sup>	1.82 ± 0.08 <sup>c</sup>	-9.47 ± 0.15	0.96 ± 0.01 <sup>a</sup>	0.51 ± 0.02 <sup>a</sup>	0.02 ± 0.00 <sup>a</sup>	0.93 ± 0.07 <sup>bc</sup>
Y38	1.61 ± 0.06 <sup>b</sup>	2.22 ± 0.09	-11.88 ± 0.28	0.94 ± 0.01 <sup>a</sup>	0.47 ± 0.02 <sup>a</sup>	0.02 ± 0.00 <sup>a</sup>	1.05 ± 0.08 <sup>c</sup>
Y50	4.78 ± 0.24	5.06 ± 0.21	-32.18 ± 0.79	0.94 ± 0.01 <sup>a</sup>	0.47 ± 0.02 <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>	1.87 ± 0.11

Explanation: nd – not detected; data are expressed as mean value ± standard deviation. Values denoted with the same letter within a column (a, b) do not differ significantly ( $p > 0.05$ ).

Employed Ostwald de Waele model was characterized by good fitting to the experimental data as indicated by coefficient of determination values between 0.94-0.97. Calculated equation constants (K and n) were relatively low when

compared many semi-solid food products (Le Thanh-Blicharz & Lewandowicz, 2020), what may be associated with destruction of yoghurt clot prior application of sample to rheometer. Nevertheless Lewandowicz, Le Thanh-Blicharz & Śmigielska (2019) reported much lower values of consistency index for natural yoghurts with addition of inulin. On the other hand those samples were characterized by significantly higher values of flow behavior index (0.437-0.514). This indicates that Ostwald de Waele model can be useful for rapid comparison of yoghurt samples within the same trial, but inconvenient to compare between different studies.

Casson model was characterized by much worse fitting to the experimental data when compared to Ostwald de Waele. That was especially evident in case of samples characterized by significantly different rheological properties i.e. Y00 and Y50. Surprisingly samples different mostly in terms of yield stress constant, whereas variation between Casson plastic viscosity was much lower. The latter constant separated investigated yoghurts into two groups – those containing very low amount of fat <0.7% and the rest that contained more than 2.1%.

Thixotropy which is the measure of rheological instability increased along with viscosity of the sample. This phenomenon is typical for most food products. Based on calculated values of area of hysteresis loop in comparison to rest of rheological parameters it is difficult to assess the effect of fat content on rheological stability of yoghurt. Nevertheless relatively small differences in the values of this parameter indicate rather insignificant effect.

**Table 4. Rheological equation parameters and thixotropy of natural yoghurts**

Sample	Ostwald de Waele model			Casson model			Thixotropy Pa·s <sup>-1</sup>
	K Pa·s <sup>n</sup>	n -	R <sup>2</sup>	τ Pa	η <sub>c</sub> Pa·s	R <sup>2</sup>	
Y00	5.4 ± 0.9 <sup>a</sup>	0.246 ± 0.015	0.95	10.5 ± 1.1 <sup>ab</sup>	0.006 ± 0.001 <sup>a</sup>	0.87	5644 ± 345 <sup>ab</sup>
Y05	2.7 ± 0.5 <sup>a</sup>	0.330 ± 0.015 <sup>a</sup>	0.97	6.5 ± 0.9 <sup>a</sup>	0.009 ± 0.001 <sup>ab</sup>	0.92	4414 ± 374 <sup>a</sup>
Y20	4.0 ± 0.1 <sup>a</sup>	0.335 ± 0.005 <sup>a</sup>	0.97	9.8 ± 0.4 <sup>ab</sup>	0.013 ± 0.001 <sup>c</sup>	0.92	6809 ± 117 <sup>bc</sup>
Y32	5.2 ± 0.7 <sup>a</sup>	0.305 ± 0.010 <sup>a</sup>	0.97	11.9 ± 1.2 <sup>b</sup>	0.012 ± 0.001 <sup>bc</sup>	0.92	7401 ± 542 <sup>c</sup>
Y38	5.8 ± 0.1 <sup>a</sup>	0.305 ± 0.002 <sup>a</sup>	0.97	13.2 ± 0.4 <sup>b</sup>	0.014 ± 0.001 <sup>c</sup>	0.92	8006 ± 95 <sup>c</sup>
Y50	24.0 ± 3.1 <sup>b</sup>	0.196 ± 0.012	0.94	42.1 ± 3.4	0.013 ± 0.002 <sup>c</sup>	0.84	16325 ± 1181

Explanation: K – consistency index, n – flow behavior index, R<sup>2</sup> – coefficient of determination, τ – yield stress, η<sub>c</sub> – Casson plastic viscosity; data are expressed as mean value ± standard deviation. Values denoted with the same letter within a column (a, b) do not differ significantly (*p* > 0.05).

Hierarchical cluster analysis (Figure 1) strengthened the thesis regarding the similarities and dissimilarities between samples indicated in previous experiments. The most similar group were samples produced by mildly semi-skimmed milk – Y20 and Y32 and also closely related to them sample prepared with whole milk – Y38. Second group was formed from samples with very low fat content i.e. Y00 and Y05. Yoghurt produced from milk powder that was characterized with clearly different physicochemical properties was assigned by the farthest linkages to any other product.

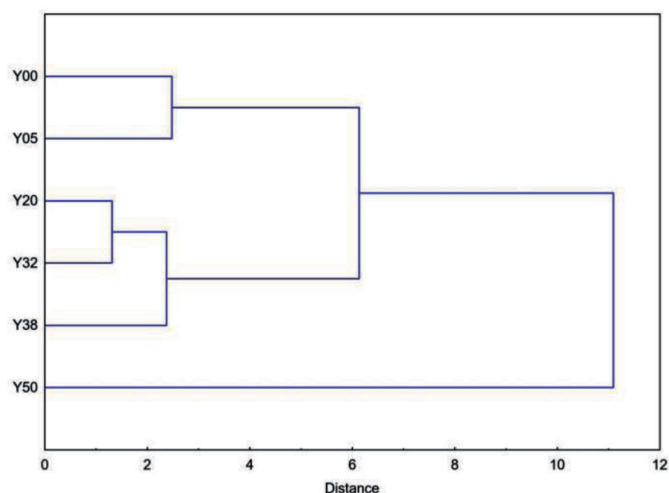


Figure 1. Dendrogram of hierarchical cluster analysis of natural yoghurts

## Conclusions

Content of fat in milk used for production of homemade natural yoghurt has significant influence on its physicochemical properties. Decrease of fat content leads to progressive reduction of viscosity and stability along with more liquid like texture. However for products with fat content below 2.0% it recommended to use fully skimmed milk as it exhibits more desired rheological and texture promoting properties. Nevertheless production of low-fat and non-fat homemade yoghurt should be assisted with addition of hydrocolloids in order maintain desired properties of final product. Moreover, excessive use of milk powder in production of homemade yoghurt to enhance its viscosity and water binding capacity may lead to development of undesired sticky texture and high acidity.

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## EVALUATION OF THE MICROBIOLOGICAL QUALITY OF INNOVATIVE FERMENTED MILK BEVERAGES ON THE TRI-CITY MARKET

Jadwiga Stankiewicz<sup>1</sup>

### Abstract

Promotion of the positive effect on health is one of the reasons why consumers are interested in new assortments of fermented milk beverages. Also, higher nutritional awareness of buyers, the syntax of producers to search for product innovations in this sector of the dairy industry.

The aim of the research was to assess the microbiological quality of innovative fermented milk drinks available on the Tri-City market. A total of 30 samples of these products, with different compositions, from 7 producers were tested. In the material tested, the total number of aerobic mesophilic bacteria, the number of lactic acid bacteria, the number of coagulase-positive staphylococci and the presence of *Escherichia coli* and *Salmonella* were determined. The analyzes were carried out using the traditional plate method with flooding inoculation, according to the applicable methodological standards, on the day of purchasing the products. Over 30% of the tested samples did not meet the criteria for the presence of the number of these microorganisms at the appropriate level ( $10^7$  cfu/g). The presence of *Escherichai coli* and *Salmonella* was not found in any of the tested samples, while the presence of coagulase-positive staphylococci was noted in 10% of the tested material.

### Keywords:

microbiological quality, fermented milk beverages, innovative fermented milk drinks

<sup>1</sup> Gdynia Maritime University, Faculty of Management and Quality Science, Department of Quality Management, Poland; [j.stankiewicz@wznj.umg.edu.pl](mailto:j.stankiewicz@wznj.umg.edu.pl)

## Introduction

Fermented milk drinks, due to their special properties, are a valuable supplement to the daily human diet (Wajs & Stobiecka, 2020). Considering the frequency of introduction of innovative products, the most dynamic segment of the food products market is fermented milk beverages, especially yoghurts (Mojka, 2013).

Implementation of new production technologies, broadening the trade offer as well as increasing competitiveness on the market are the effects of satisfying the growing demands of consumers. According to CSO data, in the first three quarters of 2018, the average monthly consumption of yoghurt per person per household was 0.53 kg and was almost 2% lower than in the same period of 2017. According to the Nielsen research group, the dairy market in Poland is worth more than 20 billion PLN, which constitutes about 16% of the entire fast-moving consumer goods market (FMCG from English: fast moving consumer goods). Dairy products are the largest segment of the food products market from a consumer market perspective (Kociubińska, 2021, *Rynek jogurtów i pozostałych produktów*, 2019). At the same time, however, this area of the dairy industry has increasing competition from year to year in the form of plant-based products. Ariadna Panel's September 2020 survey results showed that milk alternatives are the most popular category among plant-based dairy substitutes. Almost 40% of Poles buy these products at least occasionally (6% buy plant milk more often than cow's milk, and 3% choose plant milk only). Every fifth Pole occasionally buys plant-based yoghurt substitutes, and 14% of consumers occasionally choose plant-based cheese or ice cream alternatives – these are the people who are already diversifying their diet with plant-based equivalents of traditional dairy (*Roślinne alternatywy nabiału...*, 2021). For a modern consumer, quality and safety of purchased food is a very important issue. A visible trend is the choice of certified, GMO-free, lactose-reduced and organic products from local processing plants and enriched with ingredients with health-promoting properties (Baby, Antony & Vijayan, 2018; Górska, 2021; Petcu, *et al.* 2020; Piekut, 2021; Puksza & Platta, 2017; Rana & Paul, 2017; Sembratowicz & Rusinek, 2015; Świąder, Kulawiak & Chen 2020; Vuksan, *et al.* 2016). Microbiological quality of the final product is determined by microbiological purity of the raw material, hygiene of production and the quality of additional ingredients used. Among the microflora of milk, psychrophilic microorganisms of the *Pseudomonas*, *Alcaligenes*, *Proteus* and *Acinetobacter* genus deserve special attention. They have the ability to produce heat-resistant exoenzymes, lipases and proteases that retain their activity even in heat-treated milk (Mojka, 2013, Molenda, 2010). Microbial contamination of milk with human pathogenic microflora may be *Streptococcus* spp., *Campylobacter* spp., *Corynebacterium diphtheriae*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Shigella* spp. i *Salmonella* spp. (Matusiak, 2017).

Flavourings added to heat-processed milk may also be a source of contamination for the finished product. An example of such an adverse effect was the mass poisoning of chocolate syrup infected with *Yersinia enterocolitica* bacteria, used as a flavouring in UHT milk (Government of the United States..., 2011). At the same time, there is a growing product-awareness among consumers as they tend to reach for products with simple ingredients, reduced sugar content and reduced energy value.

One of the factors determining the dietetic, prophylactic and therapeutic properties of yoghurts is the presence of live starter cultures bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) throughout their declared shelf life. According to the requirements of the FAO/WHO Codex Alimentarius Standard (Codex 2003), the number of characteristic microflora of yoghurt must be at least  $10^7$ cfu in 1g/1ml of the product throughout the shelf life of the yoghurt.

In Poland, the legal document regulating the presence of microorganisms in milk and milk products is the Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for consumption means. This Regulation 'lays down microbiological criteria for certain micro-organisms and the implementing rules to be complied with by food business operators when implementing the general and specific hygiene measures for foodstuffs referred to in Article 4 of the Regulation (EC) No 853/2004'. This document covers microbiological quality parameters in relation to two aspects – food safety and process hygiene indicators. In the context of process hygiene indicators for the dairy industry, the criterion for the presence of *Enterobacteriaceae*, *E. coli* and coagulase-positive staphylococci was considered (Rozporządzenie WE, 2073/2005). However, more complete data on the microbiological requirements of the product can be obtained on the basis of data contained in the Regulation of the Minister of Health from 13 January 2003 on the maximum levels of chemical and biological impurities that may be present in food, food ingredients, authorised additives, processing aid substances or on the surface of food, which specifies the permissible number of coli bacteria and *Salmonella* (Rozporządzenie Ministra Zdrowia, 2003, Sokołowska, *et al.* 2011). Significant contamination of raw milk with coli forms may indicate lack of milking hygiene, while in dairy products they are the competitive microflora in relation to technological bacteria strains (Ziajka, 2008). Results from RASFF (Rapid Alert System for Feed and Food) data show reports of *Salmonella* in raw milk and raw milk products each year. These bacteria remain viable in milk for a long time even after the fermentation process, however, regardless of the survival rate of the cells of this pathogen, its presence in the final product may be due to the so-called cross-contamination, at any stage of the food chain. Sources of *Salmonella* in dairy products can be: raw milk, carrier workers, water that does not meet hygienic criteria, and additional ingredients used in production (Ziaro & Zaręba,



2020). Other microbial contaminants of milk and dairy products reported in RASFF in the period between 1983 and 2019 are microorganisms such as *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, aerobic bacteria and moulds (Godlewska, 2019). Dairy products, especially those derived from raw milk or milk that has been heat-treated at temperatures below pasteurisation temperature can also be a source of *Escherichia coli* O157. Epidemiological data indicate that the cause of human food poisoning cases by *E. coli* O157:H7 was secondary contamination of milk or milk products (Ziarno & Zaręba, 2020a).

*Staphylococcus aureus* is most commonly isolated from products such as rennet ripened cheeses, cottage cheeses, poultry and delicatessen products. Staphylococci are a group of microorganisms commonly found in the area associated with dairy production. The presence of enterotoxigenic staphylococci, both coagulase-positive and coagulase-negative, was confirmed in the milk of ruminants, especially goats and sheep. The vegetative form of this microorganism dies during heat treatment, however, the ability to produce enterotoxins that persist for a long time after technological treatments such as pasteurisation, drying or smoking may pose a risk to consumers. Staphylococcal food poisoning (SPF) is a disorder associated with the consumption of food contaminated with staphylococcal enterotoxins (Podkownik, *et al.* 2015).

Taking the above into consideration, a study was undertaken to assess the microbiological quality of innovative fermented milk beverages available on the Tri-City market.

## Material and methods

The study material consisted of 30 samples of fermented milk beverages ( $n = 30$ ) from retail chains in Tri-City from seven different producers. Products with different compositions were microbiologically analysed. In this publication they are identified by the symbols A ( $n = 5$ ), B ( $n = 6$ ), C ( $n = 3$ ), D ( $n = 3$ ), E ( $n = 6$ ), F ( $n = 3$ ) and G ( $n = 4$ ). According to producers' declarations, all tested samples had milk and live cultures of lactic fermentation bacteria in their composition. The A samples included bio yoghurts with forest fruit, cherry and blueberry flavours, as well as natural strawberry-flavoured high-protein drinks. Fermented milk drinks labeled as B samples had chia seeds, blueberries, dates, flaxseed, pear, and kiwi in their composition. All samples marked as C according to the manufacturer's declaration had an increased protein content. The D samples contained white chocolate and salted caramel in their composition. Dietary fibre and the addition of vegetables such as beetroot, kale and carrot were included in the fermented milk drinks marked with the letter E. According to the manufacturer's declaration of samples F, the products were enriched with fruits such as melon, strawberry,

pomegranate, raspberry, kiwi and strawberry. Samples G, on the other hand, were characterised by a lack of lactose in the beverage composition; however, they were supplemented with coconut, pineapple, blackberry, raspberry and black currant. The total number of aerobic mesophilic bacteria (OLD) was determined on nutrient agar from Merck, the number of staphylococci on Baird-Parker RPF selective medium from bioMérieux, the number of lactic acid bacteria (LAB) on MRS AGAR selective medium from Merck and the presence of *Escherichia coli* and *Salmonella* on VRB AGAR selective medium from Merck. Aerobic mesophiles were incubated at 30°C for 72 hours, staphylococci, lactobacilli and *Salmonella* and *Escherichia coli* at 37°C for 48 hours. Microbiological analyses were performed using the traditional plate method, in duplicate. The tests were carried out each time within ½ to 1 hour after purchase of the samples at the points of sale where they were kept refrigerated. Transport of samples was done with respect to the cold chain. All analysed samples were tested within their shelf life. Microbiological tests were performed in accordance with PN-A-86034-02:1993, PN-A-86034-03:1993, PN ISO 15214:2002, PN-EN ISO 6579-1:2017, PN-EN ISO 6888-2:2001 and PN-EN-ISO-4833-1:2013. To analyse the obtained results of the study, elements of descriptive statistics were applied, i.e., the values of average and standard deviations using an Excel 2010 spreadsheet.

## Results and discussion

For microbiological tests were selected products that distinguished in the market offer by such features as: organic, eco, non-GMO, vege (samples A, E, G), higher milk protein content (samples A, C, D), addition of fiber, cereals, vegetables, dried fruits, chia seeds (samples B), reduced lactose content (samples F), and reduced energy value (samples A, C).

The development of the population of aerobic mesophilic bacteria in the studied samples is shown in Table 1. The total number of aerobic mesophilic bacteria in the studied material ranged from 7.36 log cfu/ml to 8.69 log cfu/ml. The obtained results showed that the highest number of aerobic mesophilic bacteria was characteristic for the samples from producer E. The population of these microorganisms ranged from 8.34 to 8.69 log cfu/ml of the tested product. The highest values of aerobic mesophil count were recorded in fermented milk drinks enriched with fibre and vegetables. Samples G with the non-GMO designation showed a similar level of the number of these microorganisms, and among them both those enriched with fruit additives and natural ones. The lowest number of aerobic mesophilic bacteria was observed in samples F, which contained only lactose-free fermented milk drinks.

**Table 1. Population of mesophilic aerobic bacteria in the tested samples of fermented milk beverages (log cfu/ml)**

Producer	N	M	Min	Max	SD
A	5	8.17	7.36	8.64	0.43
B	6	8.41	8.19	8.52	0.11
C	3	8.26	8.08	8.36	0.13
D	3	8.21	7.66	8.65	0.41
E	6	8.49	8.34	8.69	0.15
F	3	7.51	7.36	7.62	0.44
G	4	8.43	8.22	8.54	0.12

Explanation: N – number of samples, M – arithmetic mean, Min – minimum value, Max – maximum value, SD – standard deviation.

Source: own study.

An important indicator of the quality of products containing lactic acid bacteria strains is the presence of the number of these microorganisms in 1 ml. According to FAO/WHO guidelines, the minimum number of these bacteria present in the product, which should ensure a dietary, prophylactic and therapeutic effect, should be  $10^7$  cfu in 1 ml (Codex Standard 2003). Table 2 shows the data obtained in this study on the abundance of lactic fermentation bacteria populations in fermented milk beverages. The analysed samples had numbers of these microorganisms ranging from absent to 8.64 log cfu/ml. The highest number of lactic fermentation bacteria was characteristic for G samples producer (average value 8.17 log cfu/ml). Slightly lower numbers of LAB was found in samples designated as B and E, with average values of 8.06 log cfu/ml and 8.08 log cfu/ml, respectively. The results of Kycia and Krysiński's study proved that yoghurts made from goat's milk were characterised by the numbers of technological microflora at a normative level in almost 95% of the tested samples (Kycia & Krysiński, 2014). Steinka and Kukułowicz, in a study of dietary dairy products, found that flavor-enriched samples had higher levels of *Lactobacillus* compared to natural products (Steinka & Kukułowicz, 2011). No similar relationship was reported in the present study. The evaluation of consecutive samples of milk fermented beverages in terms of the number of lactic fermentation bacteria showed that the material from producers A, C, D and F did not meet the criteria for the number of these microorganisms. Among samples A, only one sample showed LAB levels below  $10^7$  cfu/ml (6.34 log cfu/ml). Whereas, each sample of material from producers C and D had lactic acid bacteria counts that did not meet the criteria set out in the FAO/WHO reference documents. The numbers of these bacteria were 6.49 log cfu/ml for samples C and 6.34 log cfu/ml for samples D respectively. In the test material from producer F, there was a complete absence of LAB in all tested samples. The

absence of lactic fermentation bacteria in samples F may have been due to the fact that these were lactose-free beverages. In their production, specific starter cultures are used that produce increased amounts of  $\beta$ -D galactosidase (Ohlsson, *et al.* 2017, Sokołowska, 2019). Lactic acid bacteria have the ability to produce lactic acid, acetic acid and propionic acid, lowering the pH of the environment, thus disrupting the growth of many potentially pathogenic microorganisms. Bacteriocins produced by some *Lactobacillus* strains show antimicrobial activity against *Staphylococcus aureus*, *Salmonella* or *E. coli*. Active LAB strains contribute to the elimination or inhibition of pathogenic microflora. The absence of these microorganisms in F samples may have created conditions for the growth of coagulase-positive staphylococci in these beverages (Jałosińska, 2015).

**Table 2. Population of lactic acid bacteria in the tested samples of fermented milk beverages (log cfu/ml)**

Producer	N	N <sub>0</sub>	N <sub>k</sub>	M	Min	Max	SD
A	5	0	1	7.47	6.34	7.91	0.59
B	6	0	0	8.06	7.64	8.64	0.37
C	3	0	3	6.49	6.44	6.56	0.05
D	3	0	3	6.34	6.23	6.46	0.09
E	6	0	0	8.08	7.99	8.19	0.08
F	3	3	3	0	0	0	0
G	4	0	0	8.17	8.14	8.20	0.03

Explanation: N – number of samples, N<sub>0</sub> – number of samples where no presence of lactic acid bacteria was found, N<sub>k</sub> – number of samples not meeting the LAB presence criteria, M – arithmetic mean, Min – minimum value, Max – maximum value, SD – standard deviation.

Source: own study.

The presence of coagulase-positive staphylococci was also determined by analysing the material tested for microbiological safety. Commission Regulation (EC) No 1447/2007 of 5 December 2007 on microbiological criteria for foodstuffs does not lay down permitted limits for the presence of coagulase-positive staphylococci in fermented milk drinks as a safety criterion. The limit of numbers of these microorganisms in the group of dairy products was set only for cheeses produced from milk subjected to thermal treatment at temperatures lower than pasteurisation ( $10^0$  to  $10^2$  cfu/g of product). However, an indication for testing products for enterotoxins is a coagulase-positive staphylococcus count exceeding  $10^5$  cfu/g of product. The obtained results showed that only in the samples of lactose-free fermented milk beverages (samples F) the presence of this group of microorganisms was recorded. It was possible that there were no

active LAB strains that acted antagonistically to coagulase-positive staphylococci in these samples. The number of coagulase-positive staphylococci ranged from 1.30 log to 1.78 log cfu/ml, so the results of the study on the numbers of these microorganisms did not exceed permissible values. Studies on the survival of *Staphylococcus aureus* in fermented milk beverages show that they are capable of slow growth in the acidic environment of these dairy products (Steinka, 2020). Her analysis of staphylococcal populations in fermented milk drinks depending on the flavour additive did not exceed 10 cfu per ml (Steinka, 2008). In contrast, the population size of *Staphylococcus aureus* isolated from yoghurt milk in the study of Belickova, et al. reached much higher levels of 2.43 to 3.54 log cfu/ml (Belickova, et al. 2001). *Staphylococcus aureus* can be a secondary contaminant of dairy products because it is found in humans, in dust and in the air. The bacterium finds opportunities to grow in fermented milk beverages when raw milk is improperly stored or pasteurized. However, enterotoxins produced by *Staphylococcus aureus* are one of the most common causes of food poisoning in humans. About 21 varieties of thermostable toxins produced by this microorganism are known (Argudín, Mendoza & Rodicio, 2010; Matusiak, 2017). *Salmonella* and *Escherichia coli* were another group of microorganisms determined in the study as an indicator of microbiological safety of food products. According to Szczawiński, et al., *Salmonella* and *Escherichia coli* may be present in yogurt depending on the type of product, its pH and storage temperature (Szczawiński, et al. 2014). All tested samples of fermented milk beverages were free of *Escherichia coli* and *Salmonella*. Similar results were obtained by Jakubowska and Matusevičius when they made a qualitative assessment of natural yoghurts available in retail trade. The data of these authors showed that the examined fermented milk beverages did not show the presence of moulds, yeasts and *Enterobacteriaceae* (Jakubowska & Matusevičius, 2018).

## Conclusions

- More than 30% of the tested samples did not meet the criteria for lactic fermentation bacteria counts at the correct level ( $10^7$ cfu/ml).
- The presence of coagulase-positive staphylococci was recorded in 10% of the material tested, which were samples of lactose-free fermented milk drinks.
- *Escherichia coli* and *Salmonella* were not found in any of the tested samples.

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## PRODUCT INNOVATIONS ON THE EXAMPLE OF COFFEE

Przemysław Dmowski<sup>1</sup>, Maria Śmiechowska<sup>1</sup>, Piotr Bykowski<sup>1</sup>,  
Wiktoria Eliaasz<sup>1</sup>

### Abstract

Innovation is considered an important factor in the competitiveness of enterprises. In theory and in economic practice, innovations can be divided into product, process, organizational, marketing and ecological innovations. An example of such innovations can be products made with the use of by-products arising e.g. during coffee brewing.

Hence, the aim of the study was to present possibilities of using coffee and by-products of its processing as a material for designing and manufacturing innovative products. To achieve the aim of the study, the analysis and synthesis method was applied on the basis of data from a literature review and a pilot questionnaire study conducted on a randomly selected group of 80 students from three Polish universities. The research tool was an electronic survey questionnaire in the form of a Google document, posted on websites. A moderate potential for using coffee and its by-products as innovative products was demonstrated among the surveyed group of respondents. The exception was fuels produced from coffee grounds. The results also indicate that the surveyed students are moderately optimistic about implementing innovative products in general.

**Keywords:** innovation, coffee, product, quality

<sup>1</sup> Gdynia Maritime University, Faculty of Management and Quality Science, Department of Commodity Science and Quality Management; [p.dmowski@wznj.umg.edu.pl](mailto:p.dmowski@wznj.umg.edu.pl); [m.smiechowska@wznj.umg.edu.pl](mailto:m.smiechowska@wznj.umg.edu.pl); [p.bykowski@wznj.umg.edu.pl](mailto:p.bykowski@wznj.umg.edu.pl); [wk.eliasz@gmail.com](mailto:wk.eliasz@gmail.com)

## Introduction

The term “innovation” derives from the Latin terms *innovatio* and *innovare* which mean renewal and creating something new, respectively. The basic definition of innovation is an action or sequence of activities leading to the introduction or improvement of a product or service by an enterprise to the market, provision of new services and undertaking new economic activity. Since the term innovation usually refers to the creation of something new, innovation is most often defined as the process of transforming existing possibilities into new ideas and putting them into practice (Fundusze Europejskie na innowacje, 2015). The key issues in defining the concept of innovation are also the categories of change, novelty, reform or an idea perceived as new, previously unknown. Hence, innovations are new, creative changes used in economic activity, which are accumulated, thus enlarging and improving the existing technology. Furmanek (2017), on the other hand, states that innovations are various facts, processes and phenomena of technical, organizational, social or psychological nature. Schumpeter was considered the precursor of the theory of technical and technological innovations, while a significant change in its perception was introduced by Peter Ferdinand Drucker. Drucker introduced also many concepts and theories related to changes in behaviour in society, giving them new meanings. He identified the theories on innovation with new opportunities introduced by enterprises. He argued that if a company does not innovate, it inevitably grows old and goes bankrupt (Klimczuk-Kochańska, 2016; Kozłowska, 2016). Śmiechowska & Newerli-Guz (2020) think similarly, claiming that innovation is an important factor in the competitiveness of enterprises. They state that in the literature on the subject we can find many different definitions of innovation. However, the key element of the concept of innovation that distinguishes it from an invention or discovery is implementation. While the invention or discovery may remain only in the design phase, or possibly go to the prototype phase, the innovation must be implemented and constitute a product offer.

All the presented concepts regarding innovation confirm that the enterprises undertaking innovative activities pursue new goals, being also more competitive on the market and more recognizable. An important issue is also the conscious management of innovation in the company, because the real market success is determined by breakthrough innovations on the global scale, which define the needs of recipients. For this purpose, it may be easier to introduce digital solutions, that will optimize the process of developing and implementing innovations (Furmanek, 2017; Raquel, *et al.* 2016).

In theory and in business practice, innovations can be divided into product, process, organizational, marketing and ecological innovations (Kozłowska, 2016). Product innovations should be characterized by significantly reduced energy

consumption in the process of production and during product application. They should be also adapted to the current trends. This type of innovations should give new functional features to the products. Product innovations are mainly based on the new technologies or on the combination of previous technologies with new ones in the production process, through the use of new knowledge in the field or the use of novel materials. The main idea of such products is to facilitate a human use of the given product or service and to adapt it to the needs of consumers, following current trends. An example of such innovation can be the development of mobile phones (Furmanek, 2017).

The next type of innovations are process (technological) innovations consisting mainly in the implementation of the changes in the manufacturing methods. These methods include the implementation of technologies that significantly reduce the amount of human work, shortening the implementation time of the individual production processes and limiting the number of the potential non-conformities, thus leading to the significant reduction in the costs of the organization's functioning. Use of new software for order processing, warehousing, accounting, delivery identification and control, and computerization of the quality control process are examples of process innovations. Another example is the creation of new production technology installations, automation of production lines and the use of real-time sensors (Osiadacz, Chalabala & Książek, 2020).

Another type of innovation is organizational innovation, which consists in developing enterprise management methods, taking into account new jobs, establishing cooperation with stakeholders and introducing modern management solutions. The main aspects considered when organizational innovations are introducing new methods in the principles of operation, new methods of division of tasks and decision-making powers, new methods in the field of relations with the environment and new approach to basic activities in the organization, such as: organizing, planning, controlling, making decisions, motivation and an appropriate strategy (Zawada & Herbuś, 2015).

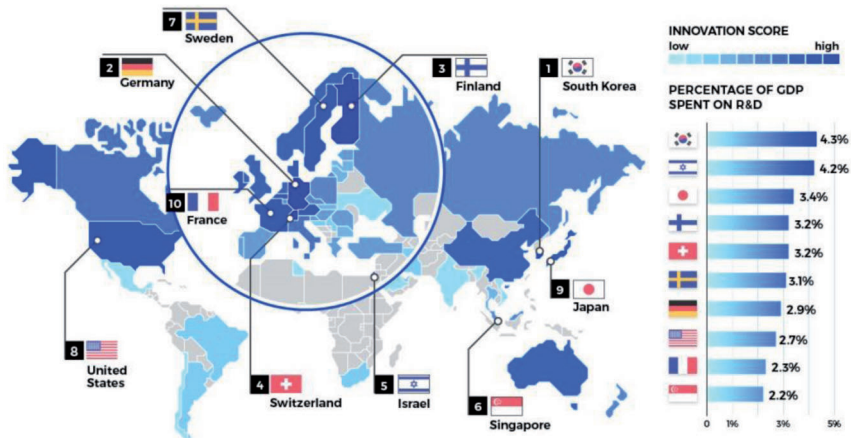
On the other hand, marketing innovations consist in introducing novel marketing strategies in the company, including changes in the design of a given product in terms of the appearance of the packaging, advertising promotion and establishing a new pricing strategy. These innovations can relate to specific marketing methods. They are aimed at drawing consumers' attention to a given product by changing its visual features or promoting a new product, which gives the company an improvement in sales in order to gain new market segments. An example of such innovation may be the development and application of a new trademark and the introduction of a personalized information system exemplified by loyalty cards or membership cards (Szul, 2016; European funds for innovation, 2015).

Today, ecological innovations (called eco-innovations) are very popular and constantly changing. Their goals are to eliminate negative impacts on the environment, thus leading to sustainable development. Such innovations lead to integrated solutions aimed at reducing the use of resources and energy, while maintaining or increasing the quality of the product or service. The aim of such innovations is also to modernize the industrial-age society, seeking to exert pressure on society through pro-ecological applications, such as reducing the consumption of natural resources, reducing the environmental and indirect impact of harmful substances on humans, and reducing energy intensity (Rutkowska-Podołowska & Pakulska, 2016; Matrepazi & Zabaniotou, 2020).

Currently, the issue of innovation is at the centre of interest in many fields, disciplines and scientific areas. Innovations should be complementary, i.e., product innovations should be accompanied by process innovations (Hullova, *et al.* 2019). It is also a topical economic and social issue. The importance of these issues is evidenced, for example, by substantial financial resources that the European Union allocates under the multi-annual programs in the field of research and innovation to strengthening, *inter alia*, industrial innovations, by investing in key technologies, searching for solutions to the most important social problems, such as, for example, counteracting waste or finally by ensuring that scientific discoveries will be used to produce useful goods with real commercial potential. Under the Horizon 2020 program, in the field of research and innovation, implemented in 2014–2020, the EU allocated 80 billion Euro for this purpose. In addition, in the developed Reconstruction Plan for Europe, combining the long-term EU budget with Next Generation EU, a total of about EUR 1.8 billion has been allocated to the recovery of the European economy after the COVID-19 crisis, of which approximately 50% will be earmarked for, e.g., research and innovation and the fight against climate change, including reducing waste. The issues of the innovation and the elimination of waste also appear in the 6 Priorities of the European Commission prepared for the years 2019–2024. As part of one of these priorities – the European Green Deal, an action plan was developed to enable, *inter alia*, more efficient use of resources thanks to the transition to a clean and circular economy, making Europe the first climate neutral continent with a modern and resource-efficient economy (Recovery plan for Europe, 2021).

According to the statistical data, South Korea is the most innovative economy in the world (Figure 1), contributing as much as 4.3% of GDP to research and development.

Nowadays, a growing part of society pays attention to innovations used in food products and packaging (Śmiechowska & Newerli-Guz, 2020; Thompson & Moughan, 2008). New lifestyles, higher incomes, and consumer awareness of quality and ecology create demand for designing and marketing diverse and innovative food products. According to Eurostat data, in 2020 Poland was one of



**Figure 1. The most innovative countries globally (2016–2020)**

Source: <https://www.visualcapitalist.com/the-10-most-innovative-economies-in-2019/> [16.06.2021].

the main food producers in Europe. The share of domestic producers in the entire EU food sector was about 10%, and food production accounted for about 16% of total sold production. However, when it comes to innovation, the food sector is more stable compared to other sectors, such as high-tech. Nevertheless, several areas related to food and the food industry have undergone significant changes in the last decades (Guine & Ramalhosa, 2016). These changes are currently related to, among others, the use of by-products such as paper, bamboo, sugarcane, cereal bran, seaweed, oils and coffee grounds for the production of innovative sustainable packaging for food products and the products themselves.

Hence, the aim of this article was to present the possibilities of using coffee and coffee processing by-products as materials in designing and production of innovative utility products.

## Product innovations on the example of coffee

An example of product, process and eco-innovation can be products manufactured using by-products from coffee brewing. Ecologically, this allows for a significant reduction in waste, positively impacting economic, environmental and social benefits, thus shaping eco-social and eco-innovative attitudes (Matrapazi & Zabaniotou, 2020). In London alone, approximately 200,000 tonnes of coffee by-products are generated annually, for which appropriate uses must be found. Hence, there is a worldwide effort to develop efficient and environmentally friendly technologies for the management of these by-products. Much of it has been used in the cosmetics industry as additives to products that increase skin elasticity and

improve microcirculation in skin tissues. The by-products of coffee brewing have also found application in the production of supplements stimulating metabolism, removing fat deposits and thus having slimming and detoxifying effects, as well as in gardening as a valuable fertilizer stimulating growth, especially of coniferous trees.

The article focuses mainly on the so far not very popular, innovative use of the so-called coffee grounds, such as, among others, frames for glasses, dishes made of grounds, tool and object holders made of grounds, decorative elements made of coffee, furniture and sportswear made of grounds. On the other hand, a classic example of process innovation could be the use of coffee brewing by-products as coffee biofuel (McDonell, 2018). As mentioned above, innovative coffee-based products include sunglasses frames designed by the Ukrainian brand Ochis (Figure 2) and mugs and cups for consuming more coffee, designed by German designer Julian Lechner (Figure 3).



**Figure 2. Sunglasses rims made of coffee grounds**

Source: <https://tenisufki.eu/arttykul/ukrainska-marka-ochis-produkuje-okulary-przeciwsloneczne-z-fusow-po-kawie> [17.06.2021].



**Figure 3. Cups produced from coffee grounds**

Source: <https://sznyt.pl/2018/07/23/kawa-podana-w-kawie-berlinski-start-sprzedaje-kubki-zrobione-z-fusow/> [17.06.2021].

The eyeglass frames made from a combination of natural coffee grounds and flax, according to the manufacturer, are 100% biodegradable and easily biodegradable when discarded, and can become a natural fertilizer to moisten the soil after a year. The reusable and waterproof dishes, which are dishwasher safe according to the designer's recommendations, are also undoubtedly an innovative product (Takalainen, 2019). Tool holders are also innovative and interesting, as well as lamps, vases, and candle holders (Figure 4 and Figure 5).

The operation of the gripper, made from coffee grounds, is that a balloon is filled with coffee, pressing and deforming around the object to be lifted, and then a vacuum sucks air from the balloon to stiffen the grip on the object. When the vacuum is released, the balloon becomes soft again and the gripper releases the object (Quick, 2010). On the other hand, lamps, vases and candlesticks designed by Raul Lauri from Melbourne are multisensory in nature and are classified in

terms of applied art. Another classic example of art using the by-products of coffee brewing is Fernando Mosca, a Spanish artist who has been working on coffee art projects for years, and his work includes paintings and decorative elements as well as furniture designed by „Re-work” (Takalainen, 2019).



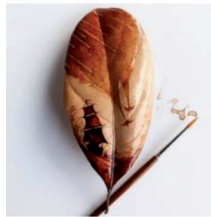
**Figure 4. Tool gripper with coffee grounds**

Source: <https://newatlas.com/universal-robotic-gripper/16729/> [17.06.2021].



**Figure 5. Lamps produced from coffee grounds**

Source: <http://designteka.pl/lampy-pachnace-kawa.html> [17.06.2021].



**Figure 6. A decorative element from coffee**

Source: <https://inhabitat.com/6-amazing-products-made-almost-entirely-from-recycled-coffee-grounds/> [17.06.2021].



**Figure 7. Furniture containing coffee ground made by “Re-work”**

Source: <https://inhabitat.com/re-worked-brews-up-furniture-from-recycled-coffee-grounds/> [17.06.2020].

Created by British designers from the company “Re-wor””, dealing with the design and manufacture of products that use coffee grounds, the furniture consists of 99% recycled materials and the remaining 1% of flame retardant and natural pigment (Takalainen, 2019).

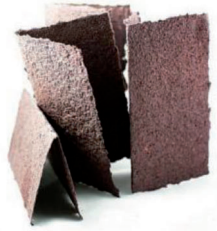
Another innovative solution is 3D printing (Figure 8) and paper articles (Figure 9) created by the Japanese company “3Dom”. An innovative solution is undoubtedly the conversion of coffee grounds into the ink used in printers called “Riti Coffee Printer”. This original idea was born from the fact that the current printers are very material-intensive and most parts are thrown away after use, in line with the assumptions of eco-innovation, solving the problems related to waste, turning them into by-products, thus giving them a second life (Takalainen, 2019).





**Figure 8. The printer “Riti Coffee Printer” by “3Dom”**

Source: <https://ifworlddesignguide.com/entry/59696-riti-printer> [17.06.2021].



**Figure 9. Paper made from coffee grounds**

Source: <https://printerest.com> [17.06.2020].

An innovative solution is also the use of by-products of coffee brewing to the production of clothing (Figure 10) and footwear (Figure 11).



**Figure 10. Coffee fibers**

Source: [https://www.materialconnexion.online/products\\_services/spotlight/Singtex/](https://www.materialconnexion.online/products_services/spotlight/Singtex/) [17.06.2021].



**Figure 11. Sports shoes made with the addition of coffee grounds**

Source: <https://sourcingjournal.com/footwear/footwear-brands/ren-recycled-coffee-sneakers-161240/> [17.06.2020].

Clothing and footwear are made of the materials obtained in the recycling process. For the production of fabrics and footwear, the technology of producing yarn from used coffee grounds is used. The materials produced in this way are resistant to the odours and UV radiation, thanks to which they work very well in sports clothes.

An innovative solution worth noting is undoubtedly the already mentioned biofuel production based on coffee by-products (Sanjib Kumar Karmee, 2017). The production process of such biofuel, to put it simply, consists in mixing coffee



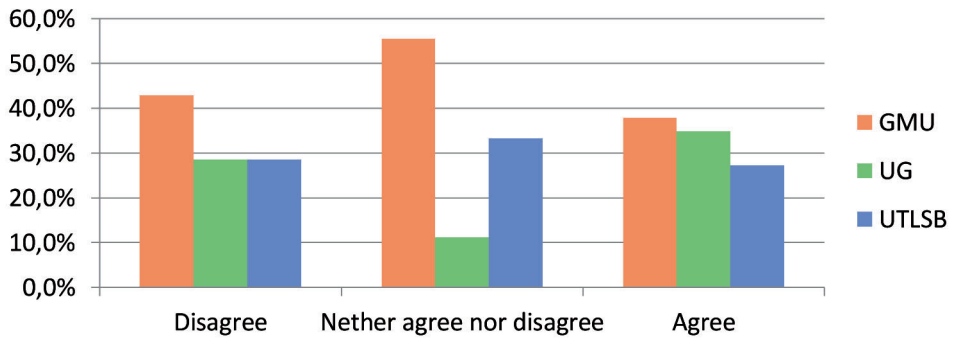
grounds with animal and vegetable fats, which, according to the manufacturer's claims, reduces environmental emissions by up to about 15%. Such fuel, consisting of 80% diesel and 20% coffee grounds biofuel, can be used not only in motor vehicles, but also in transport or home heating. The future of such solutions is also linked to work conducted by researchers at the Ulsan National Institute of Science and Technology in South Korea, who have shown that by modifying and using coffee grounds, a new product can be produced that can provide a simple and inexpensive way to remove harmful greenhouse gases from the atmosphere. The research shows that the new material from coffee by-products can absorb and store methane from the atmosphere (Takalainen, 2019).

However, all the solutions outlined above need to be accepted by global societies in order to make a significant impact on sustainability issues. Therefore, the next stage of the research was to find out the opinion of a selected group of students on coffee-based product innovations.

## Product innovations on the example of coffee

The aim of this study was to find out the opinions of students from three selected Polish universities on product innovations based on coffee. The research tool was a survey questionnaire developed in electronic form and posted on the corresponding Google browser web page. The subject of the pilot study was a group of students from Polish universities, including 33 respondents from Gdynia Maritime University (GMU), 25 respondents from University of Gdańsk (UG) and 22 respondents from University of Technology and Life Sciences in Bydgoszcz (UTLSB). A chi-square test was used to determine statistically significant differences in the range of answers given depending on the academic center from which the respondents came. Statistical analyses were verified at the significance level performed at  $\alpha = 0.05$ .

When asked to interpret the concept of innovativeness, the vast majority of respondents indicated an answer concerning a product or service created with the use of modern technologies. According to the respondents, an innovative product is also a product characterized by high quality and usefulness (about 50% of indications on average) and products and/or services that do not have a negative impact on the environment (about 25%). In turn, as attributes of product innovation, students from the surveyed universities clearly indicated that the main attribute of innovation is the guarantee of development of a given organization (about 50%), and thus the increase of its competitiveness in the industry (about 36%). Another important aspect indicated by students from the surveyed centre's was that innovative products should be in line with trends and customer needs (Figure 12).



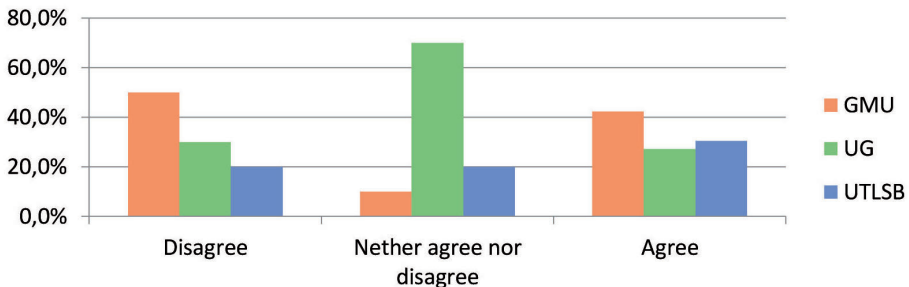
**Figure 12. Students' opinions on the compatibility of innovations with trends and customer needs**

Explanations: GMU – Gdynia Maritime University; UG – University of Gdańsk; UTLSB – University of Technology and Life Sciences in Bydgoszcz.

Source: own study.

When analysing the distribution of responses depending on the university where the respondents studied, no significant differences were found. Both those agreeing ( $p > 0.05$ ) and disagreeing ( $p > 0.05$ ) with the statement in this question were statistically the same in each university. The only exception was the attitude of those who were undecided ( $p < 0.05$ ). As far as the question of whether an innovative product should be in line with trends and meet consumer needs is concerned, the highest number of persons who could not clearly state their opinion was among GMU students, and the lowest among UG students.

According to students from the surveyed universities, innovative products should also be characterized by interesting and original design (Figure 13).



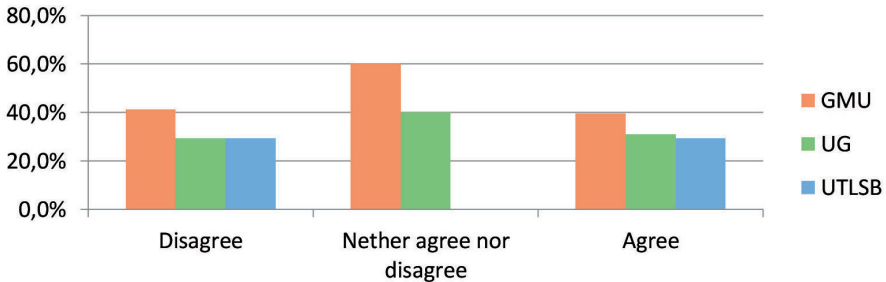
**Figure 13. The importance of the appearance of innovative products in the opinion of the students surveyed**

Explanations: GMU, UG, UTLSB as in Figure 12.

Source: own study.

According to students, especially in the opinion of GMU students, innovative products should be characterized, above all, by an appropriate level of quality, original design and should arouse widespread interest. On this issue, the most undecided respondents were UG students, for whom this was not a significant determinant of product innovation.

One of the innovative products made from the by-products of coffee brewing are coffee mugs (Figure 14).



**Figure 14. Opinions of surveyed students on the use of coffee mugs made from the by-products of coffee brewing**

Explanations: GMU, UG, UTLSB as in Figure 12.

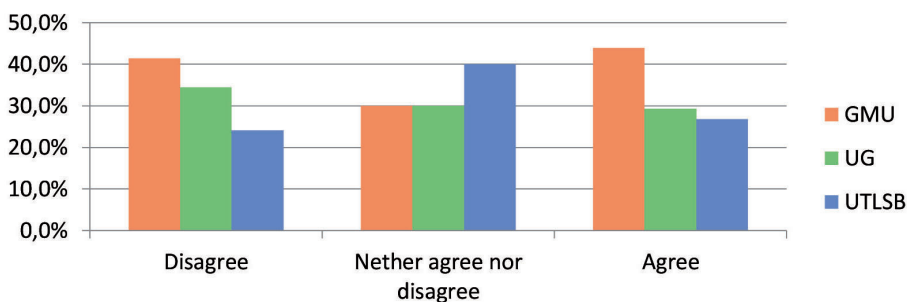
Source: own study.

Respondents' opinions on this product were very similar ( $p > 0.05$ ). Regardless of the university from which the respondents came, the number of students declaring use or dislike of cups made from recycled coffee grounds was evenly distributed. Students from GMU, UG, and UTLSB showed the greatest distance from such cups. Perhaps this indicates that recycled products are still not very popular in our country and thus not fully known by the respondents.

Similar trends were observed for the eyeglass frames that were made using by-products of coffee brewing (Figure 15).

Eyeglass frames manufactured from coffee grounds enjoyed similar acceptance as coffee mugs. Although opinions were divided, especially among GMU students, where a similar number of positive and negative opinions were recorded (about 40% each). Analysing the results obtained, it can be assumed that students are not fully convinced to reach for products, such as mugs or glasses frames, prepared on the basis of coffee brewing residues.

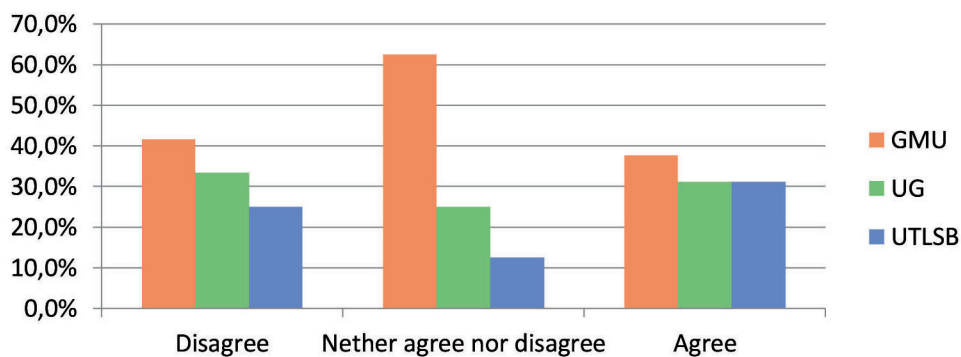
A similar trend was observed for the use of by-products in biofuel production (Figure 16).



**Figure 15. Opinions of surveyed students on the use of coffee grounds for eyeglass frames**

Explanations: GMU, UG, UTLSB as in Figure 12.

Source: own study.



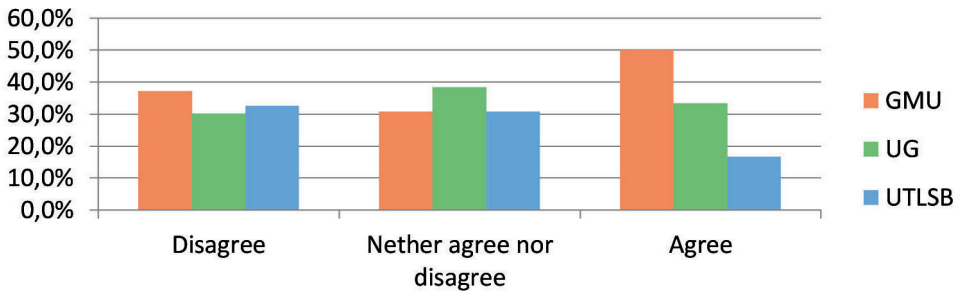
**Figure 16. Students' attitudes towards the production of biofuels with the use of coffee grounds**

Explanations: GMU, UG, UTLSB as in Figure 12.

Source: own study.

The greatest understanding for biofuels derived from coffee was shown by GMU students – about 40%, but it should be noted that the largest part of them also did not have a clear-cut opinion on this issue (over 60%). On the other hand, among the students of UG and UTLSB the opinions were divided and very close to each other. No statistically significant correlation was found between the answers of this group ( $p > 0.05$ ). The ambiguous support of the surveyed students for this type of innovation may be explained, on the one hand, by the constantly increasing fuel prices and the need to use biofuels as an admixture to standard fuels, and on the other hand, by their distrust in the quality of such fuels.

A different situation was observed in the case of acceptance of coffee preparation by-products for production of ecological shoes (Figure 17) and sportswear (Figure 18).

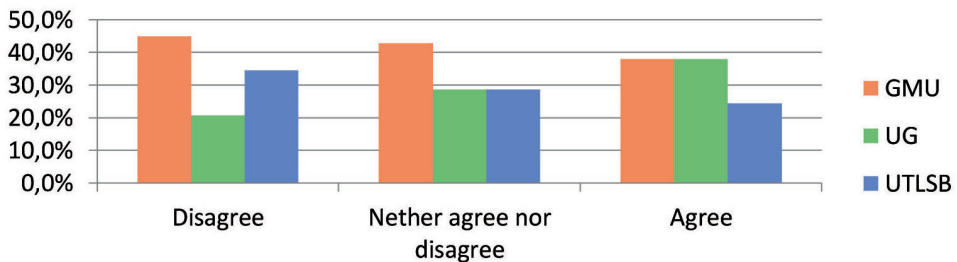


**Figure 17. Students' attitudes towards sports shoes made of coffee by-products**

Explanations: GMU, UG, UTLSB as in Figure 12.

Source: own study.

As with the previous products, athletic shoes made from coffee roasting by-products were most popular among students from UG, where about 50% of the responses approved of this type of solution. Students from the University of Gdansk were more sceptical (about 30%), and the most sceptical were the surveyed students from UTLSB ( $p < 0.05$ ). Among the surveyed group of students from UTLSB, only 15% of the respondents declared that they would be willing to use this type of footwear.



**Figure 18. Students' attitudes towards sportswear made of coffee by-products**

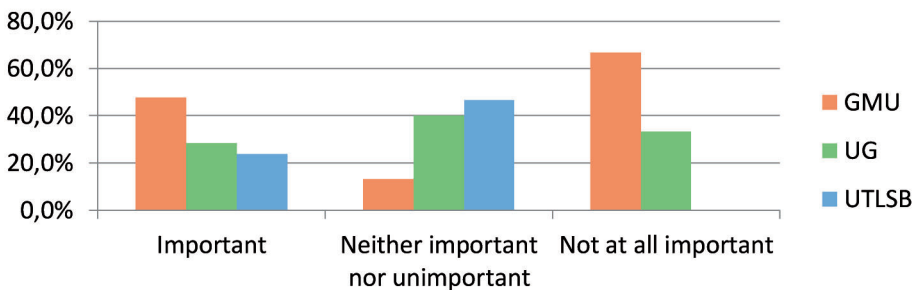
Explanations: GMU, UG, UTLSB as in Figure 12.

Source: own study.

A different situation was observed in the case of innovative sportswear made from coffee brewing by-products. The attitudes of the students surveyed were mostly negative or neutral, especially among GMU students. Among this group, negative or neutral attitudes were presented by more than 40% of the respondents. Thus, it can be concluded that students of the analysed universities showed limited approval for the production and use of sportswear containing coffee brewing by-products.

Students were also asked about their perception of the need for innovative product development. The chances for the development of innovative products were assessed by the respondents at the level of about 50%. The most optimistic were students of the Maritime Academy in Gdynia. Among this group of students the need for development and implementation of innovative products was important for about 50% of the respondents. A more sceptical approach to innovation was presented by students of UG. In turn, the UTLSB students surveyed showed the most neutral attitudes. Of this group, as many as 30% did not consider innovation development to be an important issue (Table 19).

Analysing the obtained results, it can be concluded that there is a moderate climate for the development of innovative products among the surveyed respondents. This may be worrying, especially in the context of the fact that currently Poland belongs to the European countries with a low level of R&D expenditures, which in 2016 amounted to 0.97% of GDP, and in 2018 – 1.21%, with the European average of 2.12% (Ziętek-Kwaśniewska, 2020; Żuk & Szpitter, 2018). The presented attitudes towards product innovation in general and product innovation based on coffee by-products may exclude the possibility of achieving the planned amount of R&D expenditures in 2021 of about 2%.



**Figure 19. Prospects for the development of innovations in the opinion of the students**

Explanations: GMU, UG, UTLSB as in Figure 12.

Source: own study.

## Conclusions

To date, many solutions have been developed for innovative products prepared from by-products of the coffee brewing process. These have a wide range of applications but are not fully known to most of the public.

Respondents' evaluation of innovative coffee-based products varied widely. The students surveyed most often indicated biofuels as the most acceptable products,

mainly from an environmental point of view. Less popular among respondents were sportswear and footwear. Concerns in this regard can be justified by the fact that coffee or coffee grounds are not a durable material that could be used as footwear or clothing for everyday use, especially in the context of respondents' expectations, for whom innovative products should be characterized by an appropriate level of quality and durability.

The obtained results of the study indicate that the surveyed students are moderately optimistic about the implementation of innovative products produced on the basis of coffee by-products. The obtained results of the study indicate that the surveyed students are moderately optimistic about the implementation of innovative products produced on the basis of coffee by-products. The attitudes of the surveyed students toward this group of products can be considered very conservative, which may cause difficulties in their potential introduction to the market and use by the analysed group of respondents.

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## ASSESSMENT OF SELECTED MICROBIOLOGICAL HAZARDS OF UNPASTERISED AND UNFILTERED CRAFT BEERS IN POLAND

Michał Świtalski<sup>1</sup>, Jadwiga Stankiewicz<sup>1</sup>, Agnieszka Rybowska<sup>1</sup>

### Abstract

Observed over the last five years a significant increase in the number of “beer premieres” in Poland creates the need for increased attention to safety and quality of craft beers. Particular attention should be paid to unpreserved (neither unpasteurised nor unfiltered) beers, among which a greater probability of the undesirable microflora occurrence is observed.

The aim of the study was analysis of selected microbiological hazards of unpasteurized and unfiltered craft beer available in Polish market. The study determined the number of yeast as a technological microflora, the number of *Lactobacillus acidophilus* as a threat to the quality of the product and the number of filamentous fungi, coliforms, *Escherichia coli* and *Salmonella* as a microflora which is a threat to consumer health. 25 beer samples from 14 breweries were tested. In 21 of 25 samples *Lactobacillus acidophilus* were found. The pathogenic microflora were found in 15 of examined beers. The contamination of beer by *Escherichia coli* deserves special attention due to many samples being contaminated by this pathogen. The results of the research carried out may provide significant knowledge for producers of unpreserved beers and draw their attention to the possibility of occurrence of undesirable microflora in their products.

### Keywords:

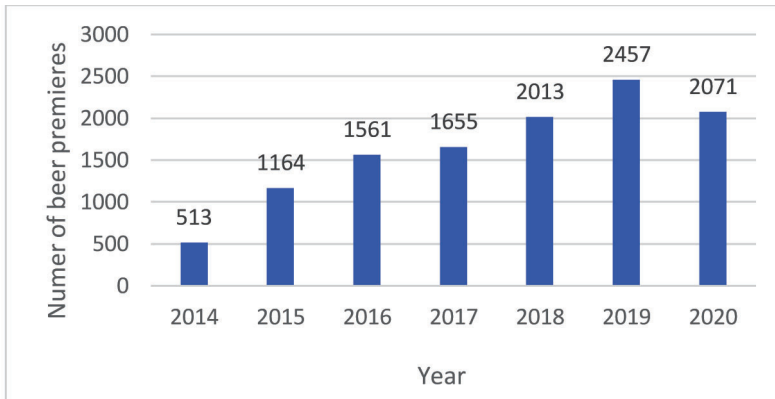
craft beer, unpreserved, microbiological hazards, product quality, consumer safety

<sup>1</sup> Gdynia Maritime University, Faculty of Management and Quality Science, Poland; *michal.switalski@sd.umg.edu.pl*; *j.stankiewicz@wznj.umg.edu.pl*; *a.rybowska@wznj.umg.edu.pl*

## Introduction

From the point of view of the consumer and producer, food quality and safety are key features of a food product (Grudowski, *et al.* 2016). The Regulation of the European Parliament and of the Council of 28 January 2002 states that no dangerous food may be placed on the market. Within the meaning of the Regulation, a food that is harmful to health or unfit for human consumption is considered to be dangerous (Regulation (EC) No 178/2002). Obtaining safe food is possible i.a. due to the prevention of chemical, physical and microbiological hazards. The latter are the result of the presence of pathogenic microorganisms, toxins or parasites occurring, for example, through improper production processes, food preparation or handling (Grudowski, *et al.* 2016). The presence of undesirable microflora is likewise observed in the brewing industry. In the past, the consumption of this beverage took place within a month or even a shorter period for 80–90% of cases. Today, this time has been extended and is from 2–3 months. Contamination are detected sporadically by laboratories, and the development of microflora may occur 3–6 weeks after bottling, which makes it difficult to control the product (Żyrek, 2008).

Changes concern also beer market in Poland. An evident increase in the number of beer premieres and the tendency of establishing new breweries can be observed there (Figure 1). A large number of beer premieres also produced by craft brewers and the fact that a significant part of the breweries established in recent years are craft breweries, often with less quality control capabilities than breweries belonging to a large group justifies raising the subject of safety and quality of craft beers on the Polish market. The topic of this study concerns the possibility of contamination of unpasteurised and unfiltered craft beer.



**Figure 1: Number of beer premieres in Poland (2014–2019)**

Source: own compilation based on data provided by (Groń 2014, 2015, 2016, 2017, 2018, 2020).

## Beer contamination and preservation possibilities

Characteristics of beer such as low pH, anaerobic conditions, the presence of alcohol, high concentration of carbon dioxide, aseptic effects of bitter substances derived from hops and a low amount of nutrients (glucose, maltose, maltotriose, amino acids consumed mostly by growing yeast) cause difficult conditions for the development of many microorganisms (Lewis, 2001; Satora & Tuszyński, 2004; Wiśniewska, 2017). Despite the presence of these conditions, a specific organisms having the opportunity to grow in this type of environment has developed. Those are called “microorganisms harmful to beer”. This group includes both bacteria and wild yeast (Wiśniewska, 2017). As in any food product, undesirable microflora can pose both a risk to the health of the consumer and the quality of the product through its chemical and organoleptic changes (Gawęcki & Krejpcio, 2014; Jagodziński, 2016). The presence of unwanted microflora may also cause stuck fermentation, affect the efficiency of fermentation, the taste of the beer and the stability of the product that may cause financial losses for the brewery (Hill, 2015).

To prevent adverse effects associated with the activity of microflora, beer is pasteurized regardless of the type of packaging (Szostak-Kot, 2010). Pasteurization is a procedure aimed at increasing the microbial stability of beer. For this beverage it is carried out in flow, before filling bottles (68–72°C for 50 s) or after filling bottles (60–63°C for 20 min) (Mitek & Słowiński, 2006).

The pasteurization temperature does not have to be high enough to destroy the heat-resistant bacteria that create the spores because they are not able to grow in beer. The use of a minimum amount of heat is aimed at minimizing changes in the taste of the product and reducing energy consumption. However, this treatment will not solve the problems of poorer beer quality resulting from inadequate hygiene before the preserving process (Hill, 2015). The thermal treatment of the beer accelerates the chemical reactions and thus reduces the stability of taste and may result in “cooked” off-flavors. In addition, pasteurizers in line or for small packages are expensive capital items in comparison with filtration units (Hill, 2015).

Another treatment reducing the number of bacterial contaminants in beer is the beer filtration technology. To obtain visually clear beer, filters able to remove particles smaller than one micron in size are used. However, the need for continuous replacing of sterile filter media make the operating costs higher than pasteurization. All filtration processes make an impact on product quality, resulting in removing the color, foam stability, bitterness or “mouthfeel”. It is not excluded that filtration technology will be more profitable for small breweries, and pasteurization technology for larger ones (Hill, 2015).

However, not all beers undergo pasteurisation or microfiltration processes. This beverage can be divided into preserved and unpreserved beer. Preserved beer is considered to be a beverage that have extended physicochemical and

microbiological stability as a result of additional technological processes such as pasteurization, microfiltration or stabilization. Unpreserved beer is not subjected to any treatment aimed at extending its durability (PN-A-79098:1995).

The aim of this study was analysis of selected microbiological hazards of unpasteurized and unfiltered craft beer available in Polish market.

## Material and methodology

The research material consisted of 25 samples of unpreserved (neither unpasteurized nor unfiltered) beers from 14 breweries (which were identified by letters from A to N). Beers were diversified in terms of beer styles, alcohol content and raw materials used in production. The examined samples are presented in the Table 1.

The mean pH of the test samples was 4.1 with a standard deviation of 0.5. The minimum and maximum values were 2.88 and 5.0, respectively. The average alcohol content declared by the manufacturer was 5.2% vol. (minimum 3.80, maximum 7.0%) with a deviation of 0.9% (Table 2).

Most of the analyzed samples were beers that had been stored on a store shelf at the point of sale. The tested samples included beers containing raw materials and additives such as. min. fruit juices and fruits, herbs and spices, as well as beers without such additives. Lactose was present in 12% of the samples. More than half of the tested beers had an alcohol content greater than 5% vol (Table 3).

The study determined the number of yeast as a technological microflora, the number of *Lactobacillus acidophilus* as a microflora which is a threat to the quality of the product and the number of filamentous fungi, coliforms, *Escherichia coli* and *Salmonella* as a microflora which is a threat to consumer health. VRB, MRS agar, YGC, nutrient agar and media composed of lactose, bile and brilliant green were used as culture media. Incubation was carried out as follows: for fungi at 25°C for 5 days, for *L. acidophilus* at 37°C for three days, for bacteria from the *Enterobacteriaceae* family at 37°C for 24 ± 2h. Depending on the determined microorganisms, dilutions from 10<sup>0</sup> to 10<sup>-4</sup> were made.

Descriptive statistics were used to analyze the research results. The numerical data, i.e. the number of *L. acidophilus*, *E. coli* and *Salmonella*, were recoded into qualitative data (presence or absence of microorganisms). For the variables compliant with the normal distribution, the arithmetic mean, standard deviation and the minimum and maximum value were considered the appropriate measure characterizing these variables. Variables inconsistent with this distribution were characterized by the median, the minimum and maximum value, and the interquartile range. Using the Yates chi-square test ( $\chi^2$ ), the significance of the relationship between the

**Table 1. Examined samples of beer**

No	Beer style	Manufacturer/principal	Alcohol content (% vol.)	pH	Additional ingredients <sup>1</sup>
1	White IPA <sup>2</sup>	A	6.3	4.21	curacao, coriander, chamomile
2	APA <sup>3</sup>	A	4.9	4.15	–
3	IPA with raspberries	B	5.2	3.62	raspberries, lactose
4	Pale Ale	C	5.2	4.11	–
5	IPA	D	6.2	4.34	–
6	Weizen	E	4.2	3.98	–
7	British Ale	E	4.8	4.18	–
8	American Wheat with lemongrass	F	4.8	4.02	lemongrass
9	AIPA <sup>4</sup>	G	6	4.23	–
10	Witbier	D	4.5	3.82	indian coriander, curacao peel
11	Lager	H & I	5.2	4.75	–
12	Pale Wheat beer	J	4.1	4.25	–
13	Milk Stout	E	6	4.68	lactose
14	American Lager	K	5.2	4.86	–
15	APA	H	5.1	4.65	–
16	Berliner Weisse with raspberries	D	3.8	3.08	raspberries
17	AIPA	L	6.2	4.29	–
18	Pale Ale with amber	L	5	3.7	amber
19	Passion fruit Berliner Weisse	K	3.8	3.32	passion fruit juice
20	Dry Stout	L	5.5	4.25	–
21	Sour Ale with gooseberry	M	4.1	2.88	gooseberry juice
22	Coffe black IPA	K	7	4.22	coffee, salt
23	APA	K	4.7	3.94	lactose, lemon juice
24	Tropical Lager	G	5.5	4.31	concentrated mango juice
25	AIPA	N	6.9	3.86	–

Explanation: 1 – listed on the label excluding hops, water, malt and cereal raw materials, 2 – IPA – India Pale Ale, 3 – APA – American Pale Ale, 4 – AIPA – American India Pale Ale.

Source: own compilation based on food labels data.

presence of undesirable bacteria in the beer and the alcohol content (>5% and ≤5% vol.), the method of storage (store shelf and store fridge) as well as the presence in the composition of certain ingredients was estimated. Moreover, quantitative data, after checking the assumptions of this test (compliance with the normal distribution and homogeneity of variance in subgroups), were further analyzed with the Student's t-test for independent groups. The number of bacteria was log transformed (log 10). The level of significance in the analyzes was  $\alpha = 0.05$ . The statistical package Statistica 10 was used for the calculations.

**Table 2. Descriptive statistics for quantitative variables characterizing the research material**

variable	N	M	SD	Me	Min.	Max.	IQR
pH	25	4.1	0.5	4.18	2.88	5.0	0.43
vol. % alcohol	25	5.2	0.9	5.20	3.80	7.0	1.30

Explanation: N – number of samples, M – arithmetic mean, Me – median, SD – standard deviation, Min. – minimum value, Max. – maximum value, IQR – interquartile range.

Source: own study based on statistical analysis.

**Table 3. Descriptive statistics for qualitative variables characterizing the research material**

Variable	Value of the variable	N	%
storage method	shop shelf	16	64
	shop refrigerator	9	36
additional ingredients <sup>1</sup>	yes	12	48
	no	13	52
non-fruit ingredients <sup>2</sup>	yes	6	24
	no	19	76
presence in the composition of fruit or fruit juice	yes	6	24
	no	19	76
presence of lactose	yes	3	12
	no	22	88
alcohol content	≤ 5 % vol.	11	44
	> 5% vol.	14	56

Explanation: <sup>1</sup> – all ingredients listed on the label excluding hops, water, malt and cereal raw materials; <sup>2</sup> – spices, herbs, coffee, salt, amber, lactose.

Source: own study based on statistical analysis.

## Results

The research showed the presence technological, as well as, saprophytic and pathogenic microorganisms in beer samples. No filamentous fungi were found in the tested samples (Table 4).

**Table 4. Descriptive statistics for qualitative variables characterizing the tested beer samples in terms of the presence of microflora**

Variable	Value of the variable	N	%
presence of yeast	yes	21	84
	no	4	16
presence of <i>Lactobacillus acidophilus</i>	yes	21	84
	no	4	16
presence of <i>Salmonella</i>	yes	3	12
	no	22	88
presence of <i>E. coli</i>	yes	14	56
	no	11	44
presence of coliform bacteria	no	25	100

Source: own study.

### Yeast (technological microflora)

The presence of living yeast was not found in following tested samples: Lager beer (11), pale Wheat beer (12), American Lager (14) as well as American Pale Ale (23) (Table 5). IPA (5) contained a population of  $10^1$  cfu/ml.  $10^2$  cfu/ml were found in samples of Witbier (10), Milk Stout (13), passion fruit Berliner Weisse (19) and tropical Lager (24).  $10^3$  cfu/ml was present in AIPA (9) and coffee black IPA (22) beers. The remaining samples were characterized by the number of  $10^4$  cfu/ml. The presence of living yeast in most of the samples indicates that, according to the producer's declaration, these beers were unpreserved.

**Table 5. Number of yeasts in tested samples**

Sample №	Number of yeasts [cfu /cm <sup>3</sup> ]	Sample №	Number of yeasts [cfu /cm <sup>3</sup> ]
1	$>3*10^4$	14	absent
2	$>3*10^4$	15	$6.7*10^4$
3	$>3*10^4$	16	$1.9*10^4$
4	$2.4*10^4$	17	$3.7*10^4$
5	$5*10^1$	18	$2.5*10^4$
6	$3.6*10^4$	19	$7*10^2$
7	$4*10^3$	20	$1.1*10^4$
8	$3.9*10^4$	21	$1.0*10^2$
9	$4.7*10^3$	22	$4.2*10^3$
10	$9*10^2$	23	absent
11	absent	24	$8.0*10^2$
12	absent	25	$3.3*10^4$
13	$2*10^2$	-	-

Source: own study.



## **Lactobacillus acidophilus (saprophytic microorganisms) in tested samples**

The presence of *Lactobacillus* was not found in 3 samples out of 25 (Table 6). These were Lager (11), Pale Wheat (12) and APA (23) beers. The highest number of *L. acidophilus* –  $1 \cdot 10^6$  cfu/ml was determined in dry stout (20). The lowest presence of these bacteria-  $10^1$  cfu/ml was determined in the IPA (5). The number of  $10^2$  cfu/ml was characteristic for Witbier (10), Milk Stout (13), passion fruit Berliner Weisse (19) and Sour Ale with gooseberry (21).  $10^4$  cfu/ml was found among beer styles: white IPA (1), APA (2), British Ale (7), American Wheat with lemongrass (8), AIPA (9), Berliner Weisse with raspberries (16), AIPA (17) and tropical Lager (24). The number of  $10^5$  cfu/ml was determined in IPA with raspberries (3), Weizen (6), APA (15) oraz AIPA (25). The presence of these bacteria do not pose a risk to the consumer health but to the quality of the product.

**Table 6. Number of *Lactobacillus acidophilus***

Sample №	Number of <i>Lactobacillus acidophilus</i> [cfu /cm <sup>3</sup> ]	Sample №	Number of <i>Lactobacillus acidophilus</i> [cfu /cm <sup>3</sup> ]
1	$8.8 \cdot 10^4$	14	absent
2	$2.6 \cdot 10^4$	15	$5.2 \cdot 10^5$
3	$2.1 \cdot 10^5$	16	$4.4 \cdot 10^4$
4	$6.0 \cdot 10^3$	17	$7.0 \cdot 10^4$
5	$1.0 \cdot 10^1$	18	$2.8 \cdot 10^5$
6	$2.4 \cdot 10^5$	19	$9.0 \cdot 10^2$
7	$4.3 \cdot 10^4$	20	$1.0 \cdot 10^6$
8	$4.1 \cdot 10^4$	21	$5.0 \cdot 10^2$
9	$5.4 \cdot 10^4$	22	$7.0 \cdot 10^2$
10	$8.0 \cdot 10^2$	23	absent
11	absent	24	$1.3 \cdot 10^4$
12	absent	25	$4.5 \cdot 10^5$
13	$1.0 \cdot 10^2$	–	–

Source: own study.

The presence of lactobacillus in samples number 16, 19 and 21 should not be evaluated as contamination, as these bacteria may constitute a technological microflora acidifying the product.

Number of *L. acidophilus* did not belong to the variables consistent with the normal distribution (Table 7). Statistical analysis showed that *L. acidophilus* achieved the

highest numbers in comparison to other microorganisms, and the number of colonies in individual samples was varied. The median of number of this strain was 26000 cfu/cm<sup>3</sup>, the interquartile range was 87.500, and the lowest and highest values were then 0 and 1000000 (1\*10<sup>6</sup>) cfu/cm<sup>3</sup>. The log transformation of the data including the number of these microorganisms in the samples showing the presence of this pathogen made it possible to obtain variables consistent with the normal distribution, therefore it was possible to determine the arithmetic mean, standard deviation as well as the minimum and maximum values, which were respectively log 4.2, 1, 3, 10.4 and 6.0 cfu/cm<sup>3</sup>.

**Table 7. Descriptive statistics for quantitative variables characterizing the tested beer samples in terms of the presence of *Lactobacillus acidophilus***

Variable	N	M	SD	Me	Min.	Max.	IQR
number of <i>L. acidophilus</i> [cfu /cm <sup>3</sup> ]	25	123520.4	232152.7	26000	0.0	1000000	87500.00
log <sub>10</sub> of number of <i>L. acidophilus</i> [cfu/cm <sup>3</sup> ]	21	4.2	1.3	4.63	1.04	6.0	2.37

Explanation: N – numer of samples, M – arithmetic mean, Me – median, SD – standard deviation, Min. – minimum value, Max. – maximum value, IQR – interquartile range.

Source: own study.

**Table 8. Percentage of samples contaminated with *L. acidophilus* depending on selected variables**

Variable	Value of the variable	Presence of <i>Lactobacillus acidophilus</i>	
		yes	no
alcohol content	≤ 5 % vol.	81.82%	18.18%
	> 5 % vol.	85.71%	14.29%
storage method	shop shelf	81.25%	18.75%
	shop refrigerator	88.89%	11.11%
additional ingredients	yes	91.67%	8.33%
	no	76.92%	23.08%
non-fruit ingredients	yes	100.00%	0.00%
	no	78.95%	21.05%
presence in the composition of fruit or fruit juice	yes	83.33%	16.67%
	no	84.21%	15.79%
presence of lactose	yes	66.67%	33.33%
	no	86.36%	13.64%

Source: own study.

Although the selected variables and their values differentiated the proportion of beers contaminated with *Lactobacillus acidophilus*, there was no statistically significant relationship between presence of *L. acidophilus* and alcohol content declared by the manufacturer ( $\chi^2Y = 0.082$ ;  $p = 0,7751$ ), presence of *L. acidophilus* and storage method ( $\chi^2Y = 0.005$ ;  $p = 0.9456$ ), presence of this bacteria and presence of additional ingredients ( $\chi^2Y = 0.210$ ;  $p = 0.6465$ ), presence of *L. acidophilus* and non-fruit ingredients ( $\chi^2Y = 0.345$ ;  $p = 0.5568$ ), presence of this microorganism and presence of fruit ingredients ( $\chi^2Y = 0.345$ ;  $p = 0.5568$ ) or presence of *L. acidophilus* and lactose ( $\chi^2Y = 0.001$ ;  $p = 0.9732$ ; Table 8).

### Number of *Salmonella* (pathogenic microorganisms) in tested samples

Among the 25 samples, *Salmonella* were found in 3 of them. These samples were Milk Stout (13), passion fruit Berliner Weisse (19) and Berliner Weisse with raspberries (16). In sample number 16 the highest number of this pathogen were found –  $4.6 * 10^1$  cfu/ml. In the 22 samples tested, no *Salmonella* were found (Table 9).

Table 9. Number of *Salmonella*

Sample №	Number of <i>Salmonella</i> [cfu /cm <sup>3</sup> ]	Sample №	Number of <i>Salmonella</i> [cfu /cm <sup>3</sup> ]
1	absent	14	absent
2	absent	15	absent
3	absent	16	$4.6 * 10^1$
4	absent	17	absent
5	absent	18	absent
6	absent	19	$5 * 10^0$
7	absent	20	absent
8	absent	21	absent
9	absent	22	absent
10	absent	23	absent
11	absent	24	absent
12	absent	25	absent
13	$1 * 10^0$	–	–

Source: own study.

A much lower number of this microorganism compared to *L. aidophilus*, as well as a lower percentage of beers contaminated with this pathogen are confirmed

by statistical analysis. The median and the inter-quartile range concerning the number of *Salmonella* was 0, and the minimum and maximum values were 0 and 46 cfu /cm<sup>3</sup> (Table 10).

**Table 10. Descriptive statistics for quantitative variables characterizing the tested beer samples in terms of the presence of *Salmonella***

Variable	N	M	SD	Me	Min.	Max.	IQR
number of <i>Salmonella</i> [cfu /cm <sup>3</sup> ]	25	2.080	9.2056	0	0.0	46.0	0

Explanation: N – numer of samples, M – arithmetic mean, Me – median, SD – standard deviation, Min. – minimum value, Max. – maximum value, IQR – interquartile range.

Source: own study.

Also in the case of beers contamination with *Salmonella*, a different percentage of contaminated beers was observed depending on the analyzed variables (Table 11). Despite this, there was no statistically significant relationship between presence of *Salmonella* and the alcohol content declared by the manufacturer ( $\chi^2Y = 0.050$ ;  $p = 0.8234$ ), storage method ( $\chi^2Y = 0.290$ ;  $p = 0.5902$ ), presence of additional ingredients ( $\chi^2Y = 1.705$ ;  $p = 0.1916$ ), non-fruit additives ( $\chi^2Y = 0.101$ ;  $p = 0.7512$ ) or presence of fruit ingredients ( $\chi^2Y = 1.263$ ;  $p = 0.2610$ ).

**Table 11. Percentage of *Salmonella* contaminated samples depending on selected variables**

variable	value of the variable	presence of <i>Salmonella</i>	
		yes	no
alcohol content	≤ 5 % vol.	7.14%	92.86%
	> 5 % vol.	18.18%	81.82%
storage method	shop shelf	6.25%	93.75%
	shop refrigerator	22.22%	77.78%
additional ingredients	yes	25.00%	75.00%
	no	0.00%	100.00%
non-fruit ingredients	yes	16.67%	83.33%
	no	10.53%	89.47%
presence in the composition of fruit or fruit juice	yes	33.33%	66.67%
	no	5.26%	94.74%

Source: own study.

## Number of *Escherichia coli* and coliform bacteria (pathogenic microorganisms) in tested samples

The highest number of *E. coli* was found in the Berliner Weisse with raspberries (16) and it was more than  $3 \cdot 10^3$  cfu/ml (Table 12). In the samples number 6, 8, 19, 21 and 24 the presence of this pathogen was found in abundance of  $10^1$  cfu/ml. The presence of this pathogen ( $10^0$  cfu/ml) were found in Lager (11), Pale Wheat beer (12), APA (15), AIPA(17), Pale Ale with amber (18), Dry Stout (20) and AIPA (25).

**Table 12. Number of *Escherichia coli* and coliform bacteria**

Sample №	Number of coliform bacterias [cfu/cm <sup>3</sup> ]	Number of <i>E. coli</i> [cfu/cm <sup>3</sup> ]	Sample №	Number of coliform bacterias [cfu/cm <sup>3</sup> ]	Number of <i>E. coli</i> [cfu/cm <sup>3</sup> ]
1	absent	absent	14	absent	absent
2	absent	absent	15	absent	$3 \cdot 10^0$
3	absent	absent	16	absent	$>3 \cdot 10^3$
4	absent	absent	17	absent	$1 \cdot 10^0$
5	absent	absent	18	absent	$2 \cdot 10^0$
6	absent	$3 \cdot 10^1$	19	absent	$9.1 \cdot 10^1$
7	absent	$1 \cdot 10^1$	20	absent	$3 \cdot 10^0$
8	absent	$2 \cdot 10^1$	21	absent	$1.8 \cdot 10^1$
9	absent	absent	22	absent	absent
10	absent	absent	23	absent	absent
11	absent	$2 \cdot 10^0$	24	absent	$1.0 \cdot 10^1$
12	absent	$2 \cdot 10^0$	25	absent	$4 \cdot 10^0$
13	absent	absent	–	–	–

Source: own study.

*Escherichia coli* contamination can be compared with the limits for unpreserved beer are set by the Regulation of the Minister of Health of 13 January 2003 on the maximum levels of chemical and biological contaminants that may be found in food, food ingredients, allowed food additives, processing aids or on the surface of food. However, this regulation is deemed to be revoked. The maximum limits of coliform bacteria contamination set by the Regulation of the Minister of Health are presented in Table 13.

The regulation sets a limit value (m) of 0 for 0.1 ml of the sample and a maximum value (M) of 0 for 0.01 ml of the sample. The value of m was exceeded by the

following beer samples: Weizen (6), British Ale (7), American Wheat with lemongrass (8), Passion fruit Berliner Weisse (19), Sour ale with gooseberry (21) and Tropical Lager (24). The M value of coliform bacteria was exceeded by Berliner Weisse with raspberries (16). The above means that the results of these seven samples should be considered unsatisfactory.

**Table 13. Maximum limits of coliform bacteria contamination set by the Regulation of the Minister of Health of January 13, 2003**

Foodstuff				Limit in 1 ml	
Beer	Type of contamination	n <sup>1</sup>	c <sup>2</sup>	m	M
unpreserved	coliform bacteria	5	1	0 (0.1 ml)	0 (0.01 ml)
preserved		5	0	0 (10 ml)	–

Explanation: <sup>1</sup> – n – number of sample units; <sup>2</sup> – c – maximum number of sample units that may exceed „m” with a value below „M” without rejecting the batch.

Source: based on (Dz.U. z 2003 r. Nr 37, poz. 326).

In the tested samples, *E. coli* was characterized by a higher number than *Salmonella*, as well as a greater differentiation between the number in individual samples. The median was 1.5 cfu/cm<sup>3</sup>, and the interquartile range was 7 cfu/cm<sup>3</sup>. The minimum and maximum values were 0 and 91 cfu/cm<sup>3</sup> respectively (this analysis did not take into account the uncountable value – the number of beer № 16). Log transformation of the data including the number of *E.coli* in samples containing this pathogen allowed for obtaining variables consistent with the normal distribution. The arithmetic mean was log 0.8, standard deviation log 0.6, and the minimum and maximum values were log 0.0 and log 2.0 cfu/cm<sup>3</sup> respectively (Table 14).

**Table 14. Descriptive statistics for quantitative variables characterizing the tested beer samples in terms of the presence of *Echericha coli***

Variable	N	M	SD	Me	Min.	Max.	IQR
number of <i>E.coli</i> [cfu/cm <sup>3</sup> ]	24	8.2	19.3	1.5	0.0	91	7.00
log <sub>10</sub> of numer <i>E. coli</i> [cfu /cm <sup>3</sup> ]	13	0.8	0.6	0.60	0.0	2.0	0.95

Explanation: N – number of samples, M – arithmetic mean, Me – median, SD – standard deviation, Min. – minimum value, Max. – maximum value, IQR – interquartile range.

Source: own study.

Also for samples analyzed for *E. coli* contamination, a different value of the percentage of contaminated samples was observed depending on the analyzed variable (Table 15). However, as in the above cases, there was no statistically significant relationship between the presence of *E.coli* and the alcohol content declared by the manufacturer ( $\chi^2Y = 1.183$ ;  $p = 0.2767$ ), as well as between presence of *E.coli* and beer storage method ( $\chi^2Y = 1.502$ ;  $p = 0.2204$ ), this microflora presence and additional ingredients ( $\chi^2Y = 0.315$ ;  $p = 0.8592$ ), non-fruit additives ( $\chi^2Y = 0.658$ ;  $p = 0.4172$ ) or presence of *E.coli* and fruit ingredients in a beer composition ( $\chi^2Y = 0.017$ ;  $p = 0.8949$ ).

**Table 15. Percentage of *E. coli* contaminated samples depending on selected variables**

Variable	value of the variable	presence of <i>E. coli</i>	
		yes	no
alcohol content	≤ 5 % vol.	42.86%	57.14%
	> 5 % vol.	72.73%	27.27%
storage method	shop shelf	43.75%	56.25%
	shop refrigerator	77.78%	22.22%
additional ingredients	yes	50.00%	50.00%
	no	61.54%	38.46%
non-fruit ingredients	yes	33.33%	66.67%
	no	63.16%	36.84%
presence in the composition of fruit or fruit juice	yes	66.67%	33.33%
	no	52.63%	47.37%

Source: own study.

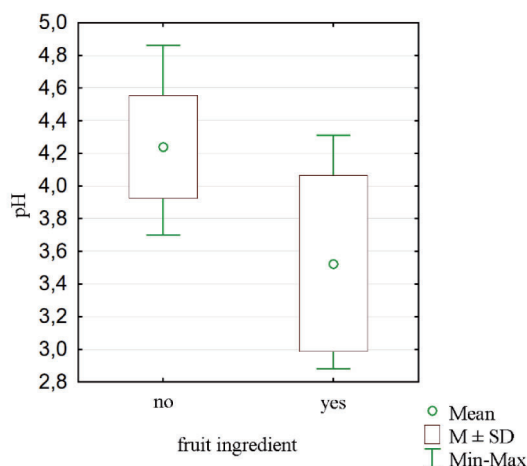
Despite the fact that no significant relationships were found between the occurrence of certain undesirable microorganisms and analyzed variables, the search for the relationship between the additives and the characteristics of a given beer was undertaken.

It was shown that in beers with the addition of fruit or fruit juice, the average pH was 3.5, while the pH of the beers without such additives was 4.24. The difference between the means was statistically significant (Table 16).

**Table 16. Descriptive statistics for the relationship between pH and the presence of a fruit ingredient**

Presence in the composition of fruit or fruit juice	N	M	SD	t	p
yes	6	3.5250	0.5392	4.071	0.0005
no	19	4.2395	0.3142		

Source: own study.



**Figure 2. Box plot of the relationship between pH and the presence of a fruit ingredient**

Source: own study.

Beers containing fruit juice or fruit had a higher pH. The above means that in beers containing fruit or fruit juice, the environment is different, which may modify the development of selected microorganisms.

## Discussion

Despite the difficult conditions in the beer for the development of many microorganisms, the results of microbiological tests showed the presence of microorganisms that may determine the final quality (*Lactobacillus acidophilus*). The results of the analysis carried out on the possible contamination with lactic acid bacteria are confirmed by numerous reports from science and industry. Despite the fact that most common strain that infects beer is *Lactobacillus brevis*, there are many other species isolated from the brewing environment (Bohak, *et al.* 2006; Funahashi, *et al.* 2018; Riedl, *et al.* 2019; Wiśniewska, 2017).

Although many researchers (Hill, 2015; Satora & Tuszyński, 2004; Wiśniewska, 2007; Vriesekoop, *et al.* 2013) claimed that characteristics of beer provide effective protection against various pathogenic microorganisms or limit their growth, some that may pose a threat to consumers' health were found in 15 of 25 samples.

The possibility of growth and survival of *Salmonella* and *E. coli* under certain conditions was demonstrated by the studies of Menz, *et al.* (2011). Although in the study the growth of bacteria was observed only in non-alcoholic beers,



*Escherichia coli* O157: H7 and *Salmonella Typhimurium* could survive in beers for more than 30 days, while for Mid-strength beers (2.3–2.9 % ABV) the survival time was longer than for full strength beers (5% ABV). The survival possibility of these pathogens emphasizes the need for special care for hygiene, especially in the last stages of production, i.e. those closest to beer consumption. As Wiśniewska (2017) states, despite receiving a microbiologically stable beverage, contamination of product may occur at the final stages (such as beer bottling) because of inadequate hygiene of the installation (biofilm formed on dispensers through improper cleaning). Improper hygiene at last stages of production may be the reason of contamination found in tested samples, however, getting to know the exact cause would require more detailed research at the production site.

*Salmonella* found in tested samples pose a risk for consumer depending, among others, on amount of beer consumed. Outbreak investigations showed that the infectious dose of *Salmonella* ranges from  $<10^1$  to  $10^9$  cfu (Greig, 2010). Similarly, in the case of *E. coli*, samples tested present a risk of health consequences depending amount of beer consumed, but also on *E. coli* strain. The infectious dose, depending on the strain, may range from less than 100 and even less than 10 cfu (for O157: H7). Enteropathogenic, enterotoxic and enteroaggregative strains require a large number to cause diarrhea ( $10^6$ – $10^8$ ) (Greig, 2010). The contamination of beer by *Escherichia coli* deserves special attention due to many samples being contaminated by this pathogen.

## Conclusions

Although the ingredients and process of beer production hinder the development of unwanted microflora in this beverage, this is not an industry free of this type of contamination. Especially the unpreserved beers mentioned in this work may be to a large extent contaminated.

The results of microbiological tests have shown the presence in unpasteurized and unfiltered beers of both microorganisms that can determine the final quality of the product, as well as microorganisms able to pose a health risk to consumers.

An additional threat is the lack of legal limits on the number of harmful microflora in unpreserved beer in Polish and European Union law. The only Regulation containing limits for coliform bacteria in unpreserved beer is deemed to be revoked.

As no statistically significant relationships have been demonstrated between the presence of certain undesirable microorganisms and the alcohol content declared by the manufacturer, beer storage method in the point of sale or the content of selected additives, it would be appropriate to look for other variables that may

determine the presence of microbiological contamination in beers, such as for example the number of days remaining until the expiry date. Statistically significant relationships between pH and the presence of the fruit component indicate an indirect influence of these components on the development of microflora.

Obtained research results and changes on the beer market in recent years show the need to strengthen control of beers available to the consumer, with particular reference to unpreserved beers. It is also necessary to maintain high hygiene standards at breweries, especially in the last stages of production, due to the possibility of survival of pathogens for a certain time.

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## Legal Regulatory

PN-A-79098:1995 Piwo

Rozporządzenie Ministra Zdrowia z dnia 13 stycznia 2003 r. w sprawie maksymalnych poziomów zanieczyszczeń chemicznych i biologicznych, które mogą znajdować się w żywności, składnikach żywności, dozwolonych substancjach dodatkowych, substancjach pomagających w przetwarzaniu albo na powierzchni żywności (Dz.U. z 2003 r. Nr 37, poz. 326).

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## CONTROLLING THE PROCESS OF MASKING THE BITTER TASTE IN FOOD PRODUCTS BY USING PHARMACOPHORE METHODS

Dariusz Kikut-Ligaj<sup>1</sup>, Katarzyna Ruda<sup>1</sup>

### Abstract

The composition of many food products can be modified based on information on the activity of its components. This has a huge impact on the perception of the quality features of food products by a potential consumer. The connection of the structural features of the constituent food products with their organoleptic activity is extremely important in shaping the quality of the final product. Prediction organoleptic features, including bitter taste can be performed using pharmacophore models. It's very important questions, because bitter taste is the basic modulator of the undesirable qualities of the foods. The techniques used in the analysis of taste activity are SPM and MPM (Simple (Multipoint) Pharmacophore Model). This method consists in identifying the areas of the ligand molecule that are responsible for the taste stimulation. The recognized spatial characteristics are converted into taste activity by means of appropriate equations. Studies with the use of pharmacophore models were carried out in the group sweeteners such as sodium and potassium cyclamates, aspartame, acesulfame and saculose. The obtained results allowed to select the best sweeteners from this group, which does not cause a bitter aftertaste. This means that saculose is the best sweetener for application in food products.

### Keywords:

bitter taste, bitter aftertaste, pharmacophore model, sodium and potassium cyclamates, aspartame, acesulfame, saculose, food safety, food quality, acceptance of bitter taste in food products

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Natural Science and Quality Assurance, Poland; [dariusz.kikut@ue.poznan.pl](mailto:dariusz.kikut@ue.poznan.pl)

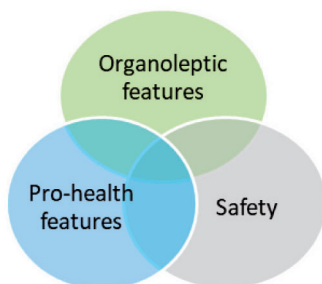
## Introduction

Safe and health-promoting foods are foods with appropriate sensory qualities, containing functional and nutritional ingredients, and free of toxic and potentially toxic ingredients. Ensuring the desired quality of food has always been a great technological challenge (Khoo & Knorr, 2014; Kikut-Ligaj, 2019a). This problem is extremely topical because food products are enriched with various substances from the group of food additives. The purpose of food enrichment is to give it the appropriate organoleptic characteristics. Such actions cause the food to contain excessive amounts of sugars, inorganic salts, sweeteners, dyes, flavor enhancers and aromas (Abdulgumeeen, Risikat & Sururah, 2012), which endanger the health of potential consumers. The expectations of consumers who are looking for food with a high saturation of bioactive ingredients are also an important element. Consumers expect the product to contain components from the group of antioxidants, anti-inflammatory substances, psycho- and metabo-stimulants, to prevent various diseases, and to maintain physical fitness, etc. (Kikut-Ligaj, 2019b; Tepper & Barbarossa, 2020). The effect of the increased content of bioactive additives in food is, among others, excessive bitterness. Other reasons for the occurrence of unfavorable organoleptic characteristics in traditional and unprocessed food are the original quality of the raw material, the technology of its processing, the type of packaging and the method of its storage (Azanedo, *et al.* 2020). The main factor determining the quality of a food product is the type of raw material. Another important factor influencing the quality of final products is the use of inadequate production technologies. The presented facts prove that there are many reasons for the sensory imperfection of products available on the market (Abdulgumeeen, Risikat & Sururah, 2012; Mihafu, Kamiyango & Issa, 2020). Taste quality of ingredients included in food products, can be predicted using appropriate pharmacophore techniques. The results obtained by the pharmacophore analysis make it possible to determine which ingredients of food products, are more safety for consumers. This research area covers issues related to bitter taste control as a qualitative differentiator for many food product ranges.

For the consumer, three areas of food quality, related to safety, health-promoting and organoleptic properties are crucial (Figure 1).

These quality areas are closely related to each other. For the consumer, the organoleptic quality is an indicator of the safety of a product and its pro-health features. Sensory feature associated with many food products is a bitter taste. The share of bitter compounds in food products affects their organoleptic, health-promoting and safety features (Mellentin & Crawford, 2008). By using appropriate tools to control the taste quality of food products (bitter taste control), we simultaneously shape their safety and health-promoting features. Taste quality of ingredients included in food products, can be predicted using appropriate

pharmacophore techniques (Kaserer, *et al.* 2015; Kikut-Ligaj, 2014). The results obtained by the pharmacophore analysis make it possible to determine which ingredients of food products, are more safety for consumers.



**Figure 1. Food products quality evaluation areas**

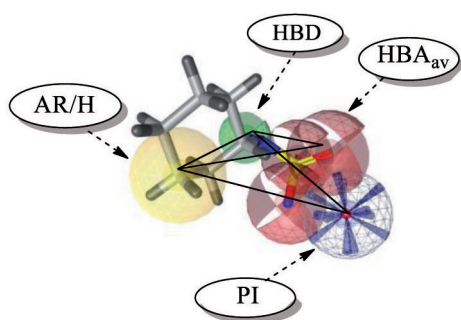
Predicting the processes of recognizing bitter taste and bitter taste activity requires the use of appropriate computer techniques known as virtual screening (VS – Virtual Screening) (Kaserer, *et al.* 2015). Among such methods, two basic screening methods are listed – the first is based on the known structure of the receptor (SBVS – Structure-Based or Target-Based Virtual Screening) and the second uses only the known ligand structures of a given receptor (LBVS – Ligand-Based Virtual Screening) (Kurczab, 2012; Maia, *et al.* 2020). Both techniques are based on the analysis of the physicochemical (steric-structural and electronic) properties of potential ligands. The interactions of a potential ligand with a molecular target (Wermuth, 2006) can be described using three-dimensional models (3D pharmacophore technique). The pharmacophore generated by this method makes it possible to specify and describe the spatial orientation of functional groups important for the interactions, necessary to generate the type of biological activity in question (Bielenica, & Kossakowski, 2010; Seidel, *et al.* 2020; Seidel, *et al.* 2010; Wolber, *et al.* 2008). The pharmacophore is, in a way, a general so-called “3D molecular form” displaying identical or similar features in a defined set of active ligands (Martin, 2007). The research used a proprietary technique called a Simple Pharmacophore Model (SPM) (Kikut-Ligaj, 2015), classified as one of the LBVS methods. The technique relies on three-center receptor recognition systems typical of many classes of chemical compounds.

## Prediction of bitter taste activity for studied sweeteners

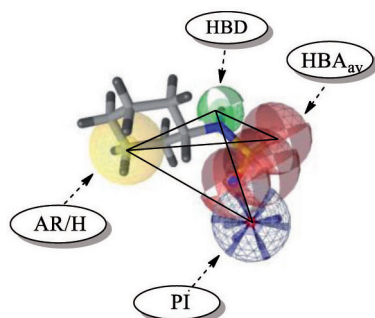
The structures of four popular sweeteners: aspartame, sacralose, sodium and potassium cyclamate have been optimized by the Density Functional Theory (DFT) method with B3LYP functional and 6-31G (2d, p)) base (Kim, *et al.* 2001).

The presence of water as a solvent was stimulated with the CPCM (Conducting Polarized Continuum Model) model (Chen, Baker, & Wei, 2010; Nunes, *et al.* 2008). The obtained representative conformations (RC) of sweeteners structures were used for pharmacophore analysis, the results of which are presented in Figure 2–6. Pharmacophore analysis was performed using the LigandScout program.

Prediction of the bitter taste by using the pharmacophoric method was aimed at selecting the best sweetener from the group of compounds tested to mask the bitter taste of caffeine. Caffeine is applied as a functional additive to many foods (Heckman, Weil, & Gonzalez De Mejia, 2010; Kikut-Ligaj, & Jasiczak, 2011). Due to demonstrate the bitter after-taste, not all sweeteners are suitable to mask the bitter taste. The pharmacophore analysis used was based on the identification of the regions of the ligand molecule that are responsible for the taste stimulation. The obtained spatial features (specified pharmacophore functions and representations) were converted into the value of the bitter taste intensity using appropriate equations (Kikut-Ligaj, 2015). This way, the predicted values of the bitter taste intensity of the tested sweeteners were obtained. The results of the conducted research are presented below.



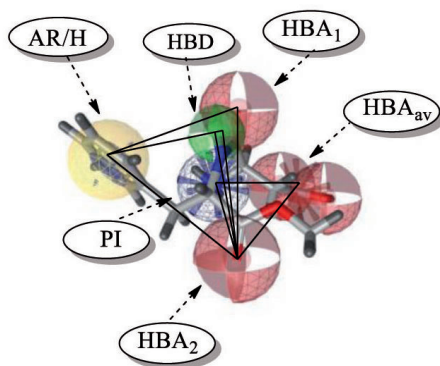
**Figure 2.** Distribution of pharmacophore functions (AR/H – yellow; HBA – red; HBD – green; PI – blue) and three-center pharmacophore representations for sodium cyclamate structure



**Figure 3.** Distribution of pharmacophore functions (AR/H – yellow; HBA – red; HBD – green; PI – blue) and three-center pharmacophore representations for potassium cyclamate structure

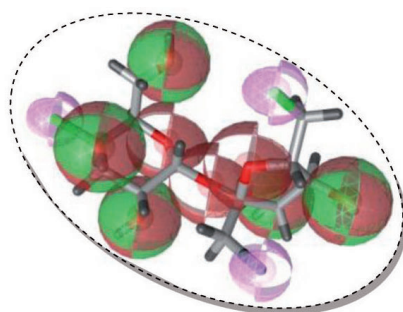
According to the pharmacophore analysis, sodium cyclamate contains four areas responsible for receptor stimulation (HBD, HBAav, PI and AR/H) and two three-center pharmacophore representations.

As a result of the conducted pharmacophore analysis, it was found that potassium cyclamate contains four areas involved in receptor activation (HBD, HBAav, PI and AR/H) and two three-center pharmacophore representations.



**Figure 4. Distribution of pharmacophore functions (AR/H – yellow; HBA – red; HBD – green; PI – blue) and three-center pharmacophore representations for aspartame structure**

The aspartame molecule has six regions involved in receptor activation (HBD, HBAav, HBA<sub>1</sub>, HBA<sub>2</sub>, PI and AR/H) and four three-center pharmacophore representations.

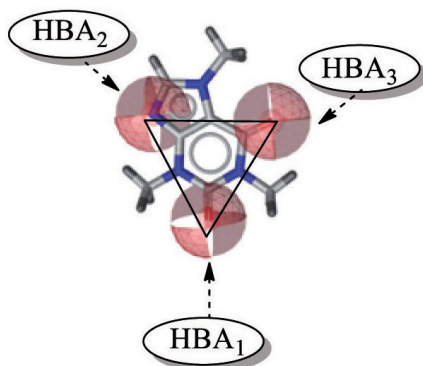


HBD-HBA-HALBD charge dispersion

**Figure 5. Distribution of pharmacophore functions (HBA – red; HBD – green) and three-center pharmacophore representations for sacralose structure**



According to the conducted pharmacophore analysis, saculose does not activate bitter taste receptors (TAS2R). This is due to the blurring of the donor-acceptor charge around the entire saculose molecule.



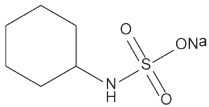
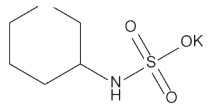
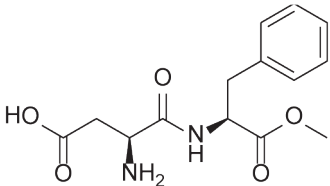
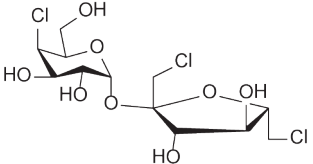
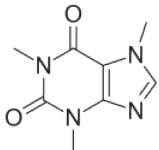
**Figure 6. Distribution of pharmacophore functions (HBA – red) and three-center pharmacophore representations for caffeine structure**

According to the conducted pharmacophore analysis, caffeine contains three areas responsible for receptor stimulation (HBA) and six three-center pharmacophore representations. Predicted  $IBT_p$  value based on pharmacophore modeling are listed in Table 1.

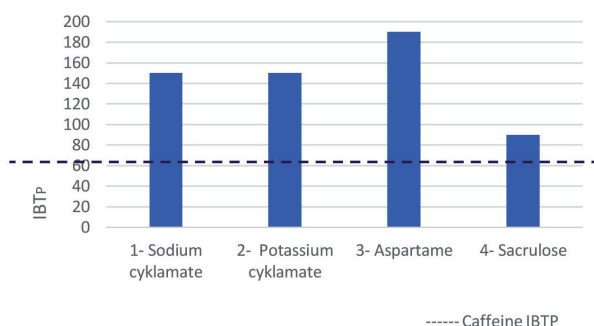
The conducted pharmacophore analysis shows that sodium and potassium cyclamates ( $IBT_p = 60$ ) have the mean predicted bitter taste intensity, while aspartame shows a much higher value ( $IBT_p = 100$ ). Saculose is unable to activate the bitter taste receptors (TAS2R) due to the blurring of pharmacophoreic functions around the molecule of this compound. This results in the inability to precisely define the donor or acceptor areas and thus disrupts the recognition process by receptor. Taking into account the effect of enhancing the intensity of the bitter taste by sweeteners showing bitter aftertaste, it should be stated that only saculose does not enhance the bitter feeling and will probably be the most suitable substance for masking the bitter taste of caffeine. This is confirmed by the total values of the bitter taste intensity ( $IBT_p$ ) of caffeine and the tested sweeteners, shown in Figure 7.

The result of the conducted pharmacophore analysis is the isolation of saculose as the optimal sweetener to mask the bitter taste of caffeine, which is an additive to food products.

**Table 1. Identification of pharmacophoric features, representations and intensity of the bitter taste for studied sweeteners**

Name of the ligand / No.	Ligand structure	Separated pharmacophore representations	Predicted values IBT <sub>p</sub> (IBT <sub>p</sub> = (∑NPI + ∑NIS) x k)
Sodium cyclamate /1		AR/H – HBD – PI AR/H – HBD – HBA <sub>av</sub>	(4+2) x 10 = 60
Potassium cyclamate/2		AR/H – HBD – PI AR/H – HBD – HBA <sub>av</sub>	(4+2) x 10 = 60
Aspartame/3		AR/H – HBA <sub>1</sub> – HBA <sub>2</sub> AR/H – HBD – HBA <sub>2</sub> PI – HBA <sub>av</sub> – HBA <sub>2</sub> AR/H – PI – HBA <sub>2</sub>	(6+4) x 10 = 100
Saculose/4		Type HBD/HBA/ HALBD charge dispersion	0
Caffeine/5		HBA <sub>1</sub> – HBA <sub>2</sub> – HBA <sub>3</sub> HBA <sub>3</sub> – HBA <sub>1</sub> – HBA <sub>2</sub> HBA <sub>2</sub> – HBA <sub>3</sub> – HBA <sub>1</sub> HBA <sub>1</sub> – HBA <sub>3</sub> – HBA <sub>2</sub> HBA <sub>2</sub> – HBA <sub>1</sub> – HBA <sub>3</sub> HBA <sub>3</sub> – HBA <sub>2</sub> – HBA <sub>1</sub>	(3+6) x 10 = 90

Source: own study.



**Figure 7. Summary values of the bitter taste intensity (IBTp) of caffeine and studied sweeteners**

Source: own study.

## Summary and discussion

Numerous reports on the bitter aftertaste of many sweeteners do not clearly specify which sweeteners available on the market have a bitter aftertaste. This is a very significant problem in the case of the application of sweeteners to food products, due to the often bitter aftertaste they exhibit. The accumulation of the bitter taste of the sweetener and the masked bitter substance leads to frequent food non-acceptance. The aim of the research was to determine the differences in the intensity of the bitter aftertaste using the pharmacophore analysis for the group of sweeteners such as: sodium and potassium cyclamate, aspartame and sacralose. The conducted pharmacophore analyzes showed that not all of the tested sweeteners showed a bitter aftertaste. According to the conducted research, these compounds have a different number of pharmacophoric features and interactive representations. This translates directly to the predict value of intensity of the bitter taste (IBTp). According to the pharmacophore analysis, the highest intensity of the bitter aftertaste is shown by aspartame (IBT = 100), and the other two sweeteners, sodium and potassium cyclamate, have an IBT value of 60. Cyclamates and aspartame should not be used as substances masking the bitter taste of caffeine, as they enhance they taste bitter. The bitter aftertaste of food products containing cyclamates and aspartame are confirmed by numerous scientific publications. The most appropriate substance to mask the bitter taste of caffeine is sacralose. According to the research, this substance does not have the ability to activate bitter taste receptors (TAS2R). The only taste characteristic of sacralose is sweet, which should compensate for the bitterness of caffeine. This means that sacralose does not enhance the bitter taste of the caffeine. This substance should therefore best mask the bitter taste of caffeine. On the other hand, due to the lack of a bitter aftertaste, sacralose does not show any toxic properties and is certainly safe for food consumers.

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## SELECTED INGREDIENTS AND PRODUCTS USED IN THE FORTIFICATION OF SELECTED CEREAL FOOD PRODUCTS – A SYSTEMATIC LITERATURE REVIEW

Paulina Kozik<sup>1</sup>

### Abstract

The purpose of this article is to review approaches to food fortification in the scientific literature from the previous two years. An SLR method was applied to assess the status of research on ingredients used for food fortification. Research papers in which cereal products were used as the carrier were searched for the analysis. Two electronic databases Web of Science and ScienceDirect were searched to identify relevant articles with the keywords: 'food fortification', 'food enrichment', 'fortified products', 'enriched products', 'cereal products' and 'flour'. For reporting, the scheme and guidelines of PRISMA were used. Based on the review of the available literature, a list of research articles was obtained on the use of various ingredients and finished products to enrich cereal products. It was found that research continues to be conducted on their enrichment with traditional ingredients. Nevertheless, significantly more research is focused on finding new products and testing whether their use in food fortification will provide positive results.

The main conclusions drawn from this research were that the fortification of cereal products can be conducted with a variety of additives, can lead to significant improvements in the physical properties of the carriers, and therefore help to reduce nutritional deficiencies.

**Keywords:** food fortification, food enrichment, additives, PRISMA, systematic literature review

<sup>1</sup> Cracow University of Economics; [kozik.paulina@gmail.com](mailto:kozik.paulina@gmail.com)

## Introduction

Cereals are a basic part of the daily diet of people all over the world. They are mainly consumed after processing (milling), by which they lose their valuable nutritional properties. They contain mainly carbohydrates, with small amounts of protein and other nutrients (vitamins, minerals, fibre and antioxidants) (Aktas-Akyildiz & Köksel, 2021; Desai, *et al.* 2021; Zięć, *et al.* 2021). As consumers' nutritional awareness increases, they are more often searching for products that have a positive impact on their health. Therefore, one of the recent trends and activities performed by food manufacturers is an attempt to improve the nutritional value of cereal products, especially flours and bread, while maintaining their technological and rheological properties. One possibility is to enrich cereal products with vitamins, minerals or other food products (Hussein, *et al.* 2021; Nogueira, *et al.* 2021).

Food fortification is a global public health programme that is used to help reduce and control micronutrient insufficiency. According to the definition proposed by the WHO and FAO, fortified food is created by the intentional addition of one or more nutrients (e.g. amino acids, minerals, vitamins) to food to increase its nutritional value, counteract deficiencies or provide a range of public health benefits. Food enrichment may be conducted whether the ingredient was naturally present in the product or not (Cardoso, *et al.* 2019; Kolanowski, 2018; Korbutowicz, 2018; Poniedziałek, Perkowska & Rzymiski, 2020). Depending on the target group to which fortified products are directed, the following types of fortification are distinguished:

- mass fortification – fortified foods are consumed by the entire population,
- targeted fortification – products are intended for specific groups, e.g. the elderly;
- market-driven fortification – where a voluntary business initiative is taken by food producers to add a specific nutrient to a product.

In addition, there is also household fortification and biofortification (i.e., enrichment of plants with specific elements during their cultivation) (Allen, de Bonoist & Hurrell, 2006; Cardoso, *et al.* 2019). Single ingredients – vitamins and minerals (e.g., vitamin D, A, zinc, iron), other food products (e.g., fruits, vegetables and cereals), innovative ingredients (e.g., insects, algae) or food waste and by-products from food production and processing (e.g., onion peels, grape or apple pomace) can be used as fortifiers. The most important objectives of fortification of food products are to correct the loss of nutrients occurring during the processing or storage of products, to counteract nutrient deficiencies both in entire populations and in individual groups, and finally to ensure a higher intake of vitamins and minerals that are important for maintaining health but are consumed in insufficient quantities or have a high health-promoting value. In this way, the

bioavailability of nutrients can be increased and the risk of many disorders or diseases such as anaemia, neural tube defects, pellagra, rickets, thyroid diseases can be reduced, as confirmed by many studies (Keats, *et al.* 2019; Mkambula, *et al.* 2020).

The aim of this article is to review the approaches to fortification of selected cereal products in the scientific literature over the last two years and to identify current trends and research conducted in this area.

## Research methods

A systematic literature review (SLR) method was used to assess the state of research on ingredients used for food fortification. The objective was to find articles presenting research results in which cereals, so-called pseudo-cereals, and products derived from them were used as carriers. Two electronic databases were checked: ScienceDirect and Web of Science. Five keywords were used: 'food fortification', 'food enrichment', 'fortified products', 'enriched products', 'cereal' and 'flour', using the logical operators 'OR', 'AND'. The search covered a period of two years: 2020-2021. The PRISMA 2020 (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines were used to present the results (Page, *et al.* 2020).

Only papers published in English and papers on the enrichment of cereal products, in which the effect of applied fortifiers on the nutritional value and sensory quality of products was examined, were analysed. Papers written in a language other than English and review articles, as well as papers assessing the effect of additives only on technical-functional and rheological properties were not analysed. The searches yielded a total of 981 scientific publications (320 indexed in the ScienceDirect database and 761 indexed in the Web of Science database). However, 36 articles were duplicates. Therefore, the titles and abstracts of 945 articles were analysed to select papers that would fulfill the set assumptions. Following the above analysis, 239 articles were retrieved and their full-text versions were downloaded. Full-text versions of 49 articles were not found. One article was excluded because it was only available in French, three papers presented the results of the same study, and 39 papers did not fulfil the objectives of the analysis. Therefore, in the end, 151 articles were included in the review. The literature selection process is shown in Figure 1.



# Results

On the basis of a systematic literature review procedure, research papers were found on the enrichment of selected cereal products and the assessment of the influence of the applied fortifiers on the nutritional value and sensory quality of the obtained products. The assumptions mentioned above were fulfilled by 151 publications. After analysing the full texts of the articles, it was found that fortificants which are currently used to enrich cereal products, can be classified into 4 main groups:

1. waste products and by-products from food processing – 46 articles,
2. innovative ingredients (e.g. seaweed, insects, meat and fish products) – 20 articles,
3. other food products (e.g. fruit, vegetables, plants, other cereal products, mushrooms, spices) – 73 articles,
4. individual components (e.g. vitamins and minerals, protein isolates, bioactive compounds) – 12 articles.

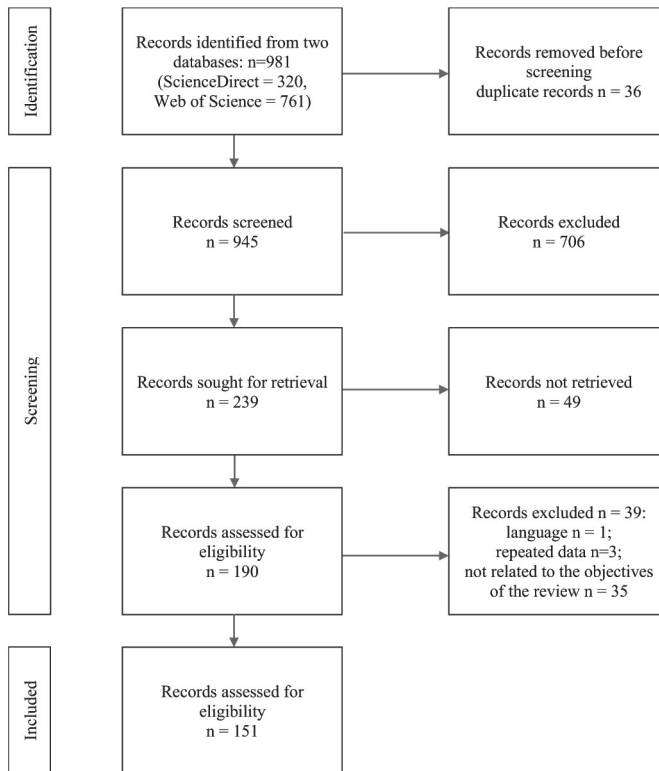


Figure 1. The PRISMA diagram

Source: own elaboration.

## Waste products and by-products from food processing

A relatively large number of research focuses on improving the nutritional value of cereals and products derived from them through the addition of waste products and by-products that are produced during the processing and production of other foods. There were 46 articles found in the databases, representing 30% of all papers analyzed. The food industry produces vast amounts of waste every year, which could be reused as a source of fibre, protein, etc. The effort to reuse them has been one of the most important global issues in recent years, which is in accordance with the growing popularity of the zero waste trend and the principles of circular economy (Nakov, *et al.* 2020; Szydłowska-Czerniak, Poliński & Momot, 2021).

Frequently used fruit and vegetable leftovers are their peels. Mayo-Mayo, *et al.* (2020) evaluated the addition of mango peel powder in tortilla chips, Gaita (2020) enhanced pasta with the supplementation of grape pomace peels, and Yaqoob, *et al.* (2021) used the kinnow peels and pomace to enrich cookies. Sagar & Parek (2020; 2021), Michalak-Maciejewska, *et al.* (2020), Masood, Bashir and Imran (2020) studied the effect of onion peel addition on cereal products. On the other hand, Shiau, *et al.* (2020) examined how the addition of pitaya peel powder affects the nutritional value of pasta, and Muniz, *et al.* (2020) added guava peel to cereal bars. Potato peel as an additive for pasta was used by Fradinho, *et al.* (2020) and as an additive for deep fried wheat chips was used by Durmaz & Yuksel (2021). The albedo, the inner part of the citrus peel, can also be used as an additive in cereal products – research in this area was conducted by Teglieri, *et al.* (2021). Many current studies concentrate on the use of seeds or grains of fruits and vegetables as fortifiers. Among others, defatted apple seeds (Purić, *et al.* 2020), Carica papaya seeds (Ávila, *et al.* 2020), pumpkin seeds (Apostol, *et al.* 2020), olive stones (Bölek, 2020; Jahanbakhshi & Ansari, 2020); almonds from Pequi (Ramos, *et al.* 2021), defatted sunflower seeds (Grasso & Methven, 2020) Sacha inchi seeds (Jiapong & Ruttarattanamongkol, 2021), fig seeds (Bölek, 2021), pomegranate seeds (Ayoubi, *et al.* 2021) or ground Ajwa seeds are added to cereal products. Another common group of waste from fruits and vegetable processing used as fortificants is pomace. Nakov, *et al.* (2020), Antonic, *et al.* (2021), Rocchetti, *et al.* (2021) and Tolve, *et al.* (2021) used grape pomace, Gumul, *et al.* (2021) and Curutchet, *et al.* (2021) – apple pomace, Saad, *et al.* (2021) – cucumber pomace and de Castro, *et al.* (2020) – orange pomace.

In addition, the following ingredients can be used to enrich cereal products: overripe bananas (Ng, Tengku Ismail & Wan Ishak, 2020), coconut testa (Marikkar, *et al.* 2020), all parts of pomegranate as juice and by-products (Incoronato, *et al.* 2021), olive mill waste olive paste and leaves (Cedola, *et al.* 2020a; Cedola, *et al.* 2020b), opuntia cladodes (Sciacca, *et al.* 2021), onion residue (Jiang,

*et al.* 2021), brewers' spent grains (Amoriello, *et al.* 2020; Schettino, *et al.* 2021; Sahin, *et al.* 2021), wheat bran (Alzuwaid, *et al.* 2021b), rapeseed press-cake (Szydłowska-Czerniak, Poliński & Momot, 2021), gill and viscera by-products of sardine (Benkhoud, *et al.* 2021), a by-product from the filleting of sea bass (Ainsa, *et al.* 2021).

The use of the above-mentioned additives contributed in most cases to the improvement of the nutritional value of cereal products, increasing the content of e.g. minerals, polyphenols, fiber, protein and unsaturated fatty acids.

## Innovative ingredients

Another group of fortifiers are innovative additives – such as algae or insects. They are innovative sources containing valuable nutritional compounds (polyunsaturated fatty acids, polyphenols, essential amino acids, fibre and vitamins). As a result, they are a popular addition to other foods (Contreras Jiménez, *et al.* 2020; Hernández-López, *et al.* 2021). An attempt to enrich maize flour with flour obtained from *Sphenarium purpurascens* (grasshopper) was conducted by Contreras Jiménez, *et al.* (2020), also Çabuk & Yılmaz (2020) added grasshopper and mealworm flour to cereal products. Jakab, *et al.* (2020) enriched flour with *Gryllus bimaculatus* (cricket) and Igual, García-Segovia & Martínez-Monzó (2020) applied *Acheta domesticus* (house cricket) to enrich extruded corn snacks. Montevicchi, *et al.* (2021) used black soldier fly prepupae to improve the nutritional value of wheat flour, while Roncolini, *et al.* (2020) used *Alphitobius diaperinus* (lesser mealworm) for this purpose. The influence of the addition of various insects on the nutritional value of cereal products was also investigated by: García-Segovia, Igual and Martínez-Monzó (2020), Akande, *et al.* (2020) and Althwab, *et al.* (2021). The main objective of enriching food with insects was to obtain protein-rich products, which was achieved according to the presented results.

Algae and seaweed can also be used as innovative ingredients in cereal products. The effect of adding spirulina to wheat bread was tested by Zalteva, *et al.* (2020). The obtained product was characterised by an increased content of phenolic compounds and antioxidant activity. While Fradinho, *et al.* (2020a) used spirulina to enrich gluten-free pasta made from rice flour. Also, in this case, the addition of the algae contributed to improving the nutritional value of the product and did not adversely affect its technological properties. The influence of spirulina was also examined by Mostolizadeh, *et al.* (2020) and El-Said, *et al.* (2021).

Furthermore, innovative fortifiers are edible seaweeds and algae (Corsetto, *et al.* 2020; Qazi, *et al.* 2021; Hernández-López, *et al.* 2021), rare species of mushrooms

(Chen, *et al.* 2021), fish: anchovy (Yilmaz & Koca, 2020), catfish (Bakare, *et al.* 2020) and cod (Desai, *et al.* 2021).

## Other food products

According to the analysis of literature studies, the most popular group of additives to cereal products are other food products (fruits, vegetables, cereals, mushrooms, plants, spices, dairy products, legumes or nuts). They represent almost half of all searched publications (48%). Most often, these additives are introduced into cereal products in powder form. Researchers are using a variety of plants to fortify cereal products. In the last two years, 17 plants were used as fortificants: *Moringa oleifera* leaves (Zungu, *et al.* 2020) and moringa sprouts (Coello, *et al.* 2021), cistus extract (Mikulec, *et al.* 2020), carob (Arribas, *et al.* 2020; Babiker, *et al.* 2020), fenugreek (Negu, Zegeye & Astatkie, 2020), sage (Yuksel, Ilyasoglu & Baltaci, 2020), elephant foot yam (Saklani & Kaushik, 2020), chia seeds (Miranda-Ramos, Millán-Linares & Haros, 2020), flaxseed (Belc, *et al.* 2020; Zarzycki, *et al.* 2020), kenaf leaves (Lim, Sim & Nyam, 2020), peach palm (Santos, *et al.* 2020), date palm flower (Karra, *et al.* 2020), safflower seeds (Kutsenkova, Nepovinnikh & Guo, 2020), hemp seeds (Teterycz, *et al.* 2021), baobab (Barakat, 2021), sunflower (Hussein, *et al.* 2021), amaranth (Piga, *et al.* 2021) and stevia (Yildiz & Gocmen, 2021).

Mushrooms are another interesting group of fortifiers. Studies on the fortification of cereal products with mushrooms were conducted by Baltacioğlu, *et al.* (2021), Bamidele and Fasogobon (2020) and Wang, *et al.* (2021) who used *Pleurotus ostreatus* as additives. As a result, they obtained products with a higher content of protein, fibre, phenols or minerals. Rosli, *et al.* (2021) used *Pleurotus sajorcaju* – also this research showed a positive effect of the addition of oyster mushrooms on the nutritional value of fortified products. Researchers have also examined the enrichment potential of white button mushrooms, shiitake and porcini mushrooms (Lu, *et al.* 2020) and edible truffle *Terfezia boudieri* (Najjaa, *et al.* 2020a).

Another frequently chosen group of finished products are fruits. Pawde, Talib and Parate (2020) enriched the biscuits with dragon fruit, Najja, *et al.* (2020b) used jujube fruit and Eyenga, *et al.* (2020) – safou fruit. Furthermore, the effect of the addition of the following fruits was tested: Saskatoon berry fruit (Lachowicz, Świeca & Pejcz, 2020; Lachowicz, Świeca & Pejcz, 2021), persimmon (Lucas-González, *et al.* 2020), sea buckthorn berries (Ghendov-Mosanu, *et al.* 2020), green banana (Khoozani, *et al.* 2020), black chokeberry (Petković, *et al.* 2021), passion fruit (Ning, *et al.* 2021), barberry fruits (Bakmohamadpor, *et al.* 2021), apricot (Nisar, *et al.* 2021), raspberry, red currants and strawberry (Tarasevičienė, *et al.* 2021). Of the vegetables, the following were used as fortificants: purple sweet potato

(Liu, *et al.* 2020), red bell pepper (Kaur, *et al.* 2020), yellow-fleshed cassava (Ajani, *et al.* 2020), asparagus (Vital, *et al.* 2020) or vegetable paste (Dhillon, *et al.* 2021).

Researchers also search for fortifiers among legumes. Da Costa, *et al.* (2020) studied the effect of whole chickpea flour on, among other things, the nutritional characteristics of bread, de Pasquale, *et al.* (2021) added chickpea flour to the pasta, and Xing, *et al.* (2021) used it to enrich wheat bread. Benayad, *et al.* (2021b) examined how the characteristics of durum wheat couscous would change when lentil semolina was added and Salazar, Rodas and Arancibia (2020) studied the effects of faba-bean and white-bean on the characteristics of corn tortillas. The conducted studies have confirmed the usefulness of the use and the positive effect of the addition of legumes on the nutritional value of these products. In addition to those listed above, the following have also been used as fortifiers: faba bean (Benayad, *et al.* 2021a; Gangola, *et al.* 2021; Hoehnel, *et al.* 2020) soybean (Bolarinwa & Oyesiji, 2021; Filipini, *et al.* 2021) and green gram (Bhavya & Prakash, 2021).

Fairly popular additives to enrich cereal products are other cereals – both traditional and so-called pseudo-cereals. In research, mainly bran is used, for example, corn, rice and sorghum (Mounjouenpou, *et al.* 2020), black rice (Sethi, Nanda & Bala, 2020), maize (Hussain, *et al.* 2021), triticale (Kaszuba, *et al.* 2021), wheat (Alzuwaid, *et al.* 2021a). Another way to use cereals as food fortifying products is their milling and adding in the form of flour. Research in this field has been conducted with the use of buckwheat flour (Wang, *et al.* 2020), teff flour (Zięć, *et al.* 2021), whole grain quinoa flour (El Sohaimy, *et al.* 2021). Cereals are also added in freeze-dried form – Kraska, *et al.* (2020) added freeze-dried spelt grains in various proportions to spelt wheat flour. This addition helped to improve the nutritional value of bread obtained from this mixture.

Research has also been conducted on the use of nuts (Gaglio, *et al.* 2020; Mashau, *et al.* 2020; Oyeyinka, *et al.* 2021; Pycia & Ivanišová, 2020; Pycia, Kapusta, & Jaworska, 2020; Rogaska, *et al.* 2021), spices (Issaoui, *et al.* 2020) or dairy products (Graça, Raymundo, & Sousa, 2020; Ibrahim, Bahgaat, & Hussein, 2021) to improve the nutritional value of cereal products.

## Individual components

Experiments to increase the content of single ingredients, bioactive components, in a given product also continue, although they represent only 8% of the analysed publications. Nearly two billion people worldwide suffer from macronutrient and micronutrient deficiencies. This contributes to the development of many diseases, reduced growth, etc. In order to solve this problem, attempts are being made to add specific ingredients, e.g. iron, iodine to commonly consumed products

(Bonto, *et al.* 2020). Rossi, *et al.* (2020) enriched wheat bread in calcium and then tested the effect of the applied fortificant. Research has confirmed an increase in the content of this element and an improvement in its bioavailability. Studies on the addition of calcium to wheat flour were conducted by Khan, *et al.* (2021), who extracted calcium from chicken eggshells. Research has also been performed on enriching rice with iron, which is lost during the milling process. Bonto, *et al.* (2020) showed that soaking rice in an aqueous iron solution resulted in a 28-fold increase in this element compared to unenriched rice. The beneficial effect of the iron addition on the nutritional value of extruded barley snacks was confirmed also by Reshi, *et al.* (2020).

Other individual components added to cereal products and snacks are vitamins (Aktas-Akyildiz & Köksel, 2021), resveratrol (Ahmad & Gani, 2021), protein isolates (Nogueira, *et al.* 2021; Sánchez-Villa, *et al.* 2020; Sofi, *et al.* 2020) or fiber and  $\beta$ -glukans (Ballester-Sánchez, Fernández-Espinar, & Haros, 2020; Krawecka, Sobota, & Sykut-Domańska, 2020). On the other hand, the study by Bhat, Wani and Hamdani (2020) examined the supplementation of lycopene and tomato powder to cookies obtained from wheat flour. The addition of these ingredients mainly improved the antioxidant properties of the cookies without negatively affecting their organoleptic and physical properties.

## Conclusions

A systematic literature review was conducted to identify current trends in the fortification of cereal products. It has become apparent that the enrichment of these products is a very popular research topic. A very popular group of fortifiers are waste and by-products of food processing, which is related to the recently more and more popular idea of zero waste. A growing number of efforts are being directed towards the re-use of waste to, among other things, protect the environment and conserve bio-resources. Also extremely popular is so-called food-to-food fortification and improving the nutritional value of products by adding nutrient-rich fruits, vegetables, but also other cereal products or even plants that are not consumed daily by the general public.

The analysis confirms the interest in enriching cereal products, which are poor in valuable nutrients. This is a response to the changing demands and needs of consumers, who are increasingly aware of nutrition and the choice of wholesome nutrients. And research confirms that the addition of a variety of ingredients can significantly improve the nutritional value of cereal products.

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Part 2

DESIGN,  
QUALITY  
AND SAFETY  
OF NON-  
-FOOD  
PRODUCTS

## NATURAL SUNSCREEN PRODUCTS – A NEW QUALITY ON THE COSMETICS MARKET

Monika Engler-Jastrzębska<sup>1</sup>, Aleksandra Wilczyńska<sup>1</sup>

### Abstract

Growing ecological awareness of consumers and increased interest in natural products is causing the market of natural cosmetics to develop very dynamically. The trend associated with a healthy lifestyle and responsible use of natural resources, supports the development of the natural cosmetics market. This category of products has recently recorded enormous growth. The reports on research and analysis of the cosmetics market as well as the results of scientific studies show the increasing popularity of natural cosmetics and the growing interest in this type of products.

In response to the changing needs of consumers on the cosmetics market, sunscreen products called “natural”, have started to appear. These cosmetics are based on substances of natural origin, contain mineral sunscreens and are often enriched with antioxidants and ingredients of natural origin with proven photoprotective properties. The quality of natural sunscreen products is confirmed by certification organizations, which also guarantee: environmentally friendly production and processing processes, responsible use of natural resources, respect for biodiversity.

The aim of this study is to draw attention to the new category of sunscreen cosmetics and to present selected raw materials and substances with proven radioprotective properties, used in natural sunscreen products.

**Keywords:** cosmetics market, natural sunscreen products, photoprotective substances

<sup>1</sup> Gdynia Maritime University, Department of Quality Management, Faculty of Management and Quality Science, Poland; [m.engler-jastrzebska@sd.umg.edu.pl](mailto:m.engler-jastrzebska@sd.umg.edu.pl); [a.wilczynska@wznj.umg.edu.pl](mailto:a.wilczynska@wznj.umg.edu.pl)

## Introduction

The increase in the incidence of skin cancer has led to greater consumer interest in sunscreens. Over the years, public awareness of the negative effects of solar radiation has also increased. The cosmetics market offers a large selection of sunscreens, unfortunately, the safety and efficacy of many compounds used in formulations are still questioned (Diffey, 2019; Latha, *et al.* 2013).

Sunscreen products are an important element in the prevention of skin damage caused by ultraviolet radiation emitted by the sun. They protect the skin against: erythema, sunburn, hyperpigmentation, photoaging, immunosuppression, phototoxic and photoallergic effects, reactive oxygen species formation, precancerous conditions and skin cancers such as basal cell carcinoma, squamous cell carcinoma and melanoma (DeBuys, *et al.* 2000; Egambaram, *et al.* 2019; Lucas, *et al.* 2018; Narbutt, *et al.* 2018). In addition to the natural protective mechanisms present in the skin, such as melanin, stratum corneum cells, lipid coat, and transurocapnic acid, there are three ways to protect the skin from solar radiation:

1. protecting the skin by wearing clothing, head coverings, sunglasses,
2. avoiding exposure to the sun, especially during the strongest insolation periods,
3. use of sunscreen products containing UV (ultraviolet) filters (Placek, 2008; Pop, 2015; Wołowiec & Dadej, 2003).

Radioprotective products, despite being classified as cosmetic products in many countries, do not serve only skin care purposes and their action is not limited to moisturising, nourishing or firming the skin. The main function of sunscreen cosmetics is to protect the skin from the biological effects of solar radiation (Egambaram, *et al.* 2019; Lucas, *et al.* 2018; Narbutt, *et al.* 2018).

In the European Union, sunscreen products are cosmetic products within the meaning of Article 2(1) of Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 concerning cosmetic products. According to the definition, a “cosmetic product” is: “any substance or mixture intended to come into contact with the external parts of the human body (epidermis, hair, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity, exclusively or mainly for the purpose of cleaning, perfuming, changing the appearance, protecting, keeping in good condition or correcting body odours” (Regulation (EC) No 1223/2009).

In the United States, Canada and Australia, most photoprotective products are registered as OTC (Over the Counter), i.e. over-the-counter medicines. These products are subject to more extensive and restrictive efficacy and safety testing (Baron & Stevens, 2002; Krzyżostan, 2018).

The photoprotective effect of sunscreen cosmetics is provided by radiolucent substances, i.e. UV filters. According to the Regulation (EC) No 1223/2009, these are substances that protect the skin against the harmful effects of ultraviolet radiation. They work by absorbing, reflecting or scattering UV radiation. Sunscreens are divided into physical and chemical filters. They form the basis of traditional sunscreen cosmetics commonly available on the market, they are also added to personal care cosmetics: creams for everyday use, protection creams, lipsticks, colour cosmetics (Bojarowicz & Bartnikowska, 2014; Regulation (EC) No 1223/2009). In Europe, sunscreen products may contain one or more of the 28 substances allowed in cosmetic products to protect the skin against ultraviolet radiation (Regulation (EC) No 1223/2009). Ultraviolet radiation emitted by the sun is divided into three wavelength ranges: UVA (320–400nm), UVB (290–320 nm) and UVC (220–290 nm). In addition, UVA radiation is divided into short-wave (320–340 nm) and long-wave (340–400 nm). The ozone layer of the earth absorbs 100% of UVC radiation and about 90% of UVB radiation, but it does not stop UVA radiation and visible light reaching the earth's surface. UVA radiation affects the skin all year round and gets through window glass and car windows. Due to daily exposure to the sun, it is important that sunscreen products are widely used and applied on daily basis. It is therefore important that photoprotective cosmetics are safe yet effective (Almutawa, *et al.* 2013; DeBuys, *et al.* 2000; Narbutt, *et al.* 2018).

Recently, there has been a growing consumer interest in natural cosmetics. Growing ecological awareness of consumers and increased interest in sustainable consumption is causing the market of natural and ecological cosmetics to develop very dynamically. The trend associated with a healthy lifestyle, responsible use of natural resources and respect for the environment supports the development of the natural cosmetics market. Already in 2013, the European trade organisation, Cosmetics Europe, became actively involved in this topic, and in 2015, the Sustainable Development Working Group of the Polish Cosmetics Industry Association was established. Forecasts presented in the report “Bio food and natural cosmetics market in Poland 2019. Market analysis and development forecasts for 2019–2024”, indicate that the natural cosmetics market in Poland will grow rapidly in the coming years. The report shows that natural cosmetics already account for almost 4% of the value of the entire cosmetics market, and consumers in Poland are showing increasing interest in cosmetics made from natural raw materials (PMR Report, 2021). According to research conducted by the SW Research Institute on behalf of the Ekocuda fair organisers, as many as 93% of Polish women from large cities declare that they use natural cosmetics, with one in ten of them buying such products exclusively (SW Research Institute, 2019).

Researching key business trends within a product, category or market, using scanning data as well as information collected through traditional channels from



thousands of shops, Nielsen specialists have examined in detail the presence of the eco/natural trend in the cosmetics and home care industry. The report “Home & beauty loves eco” shows that almost 40% of Poles pay attention to ecology and naturalness issues when shopping and these are not only young residents of large cities, but also people from rural areas and those who are middle-aged (Nielsen report “Home & beauty loves eco”, 2019). Research findings also indicate the increasing popularity of natural cosmetics and the growing interest in such products (Kantor & Hübner, 2019; Platta & Żyngiel, 2015).

The natural radioprotectants market, like other segments of the cosmetics market, is dependent on current social needs and preferences. Among the many goods available on the market, consumers choose those that meet their expectations. These choices are an important factor influencing the demand for natural radioprotective cosmetics. This results in the appearance of new or improved products on the market, changes in components, implementation of solutions related to responsible use of natural resources.

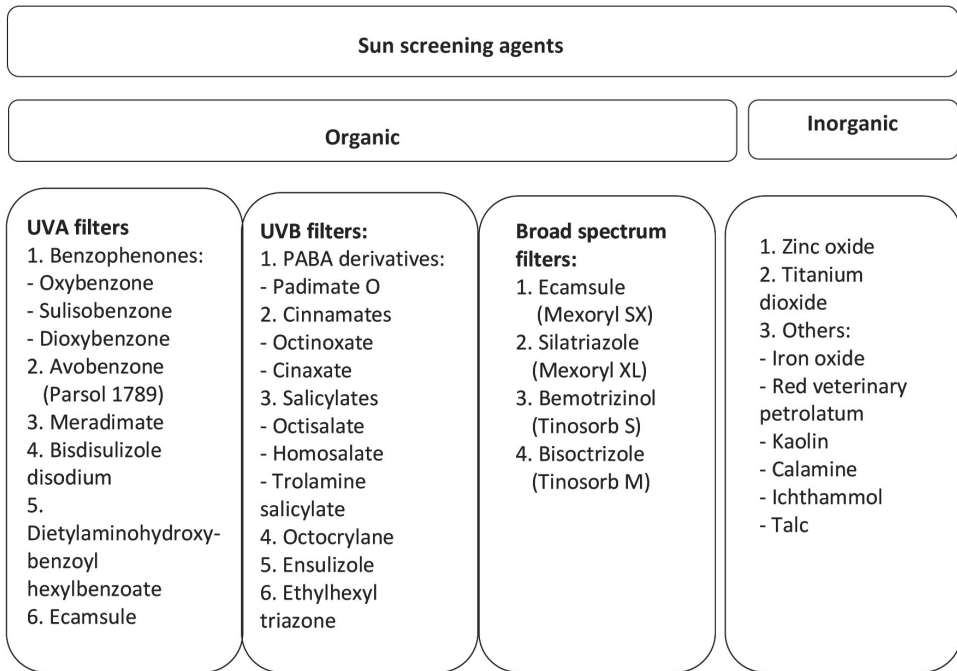
The aim of the study is to draw attention to the new, developing category of sunscreen cosmetics, to indicate new product solutions, as well as to present selected natural ingredients with proven radioprotective properties that are used in sunscreen products and to compare their effectiveness.

## Traditional sunscreen products

Traditional sunscreen products on the market contain a combination of substances to provide broad-spectrum sun protection. They contain one or several sunscreen substances and include both chemical (organic) and physical (inorganic) filters (Fig. 1).

Some substances used as chemical filters in sunscreen products are controversial. Researchers point to the estrogenic effects of 4-methylbenzylidene-camphor and oxybenzone. Allergenic, irritant, photosensitising and phototoxic effects induced by some chemical filters, in particular octocrylene, benzophenone 3 and avobenzone, have also been reported in the literature (Narbutt, *et al.* 2018). UV filters are also of increasing concern due to their environmental impact, many of them exhibiting hormonal activity towards aquatic as well as terrestrial organisms. In vitro and in vivo studies in selected fish and rodent species have demonstrated estrogenic and androgenic effects of some substances used as chemical filters, including: 2-ethylhexyl 4-methoxycinnamate (EHMC), 2-ethylhexyl 4-(dimethylamino) benzoate (OD-PABA), homosalate, benzophenone 3 (BP3), 3-(4-methylbenzylidene) camphor (4MBC), acetocrylene (OC) and butyl methoxydibenzoylmethane (BM-DBM) (Díaz-Cruz, *et al.* 2012). Many researchers point to the widespread occurrence of UV filters in aquatic systems. They enter

the aquatic environment directly through washings from skin, from fabrics or indirectly through wastewater or pool water (Calafat, *et al.* 2008; Fent, Zenker & Rapp, 2010; Zwiener, *et al.* 2007).



**Figure 1. Sunscreen substances**

Source: compiled from: (Latha, *et al.* 2013).

Organic filters contained in conventional sunscreen products work by absorbing harmful ultraviolet radiation. This group of substances includes UVA- and UVB-absorbing filters as well as broad-spectrum filters that absorb both wavelengths. These filters can undergo photochemical transformations resulting in the formation of unstable, reactive products, sometimes toxic in nature, often superoxide and hydroxyl radicals (Serpone, Dondi & Albini, 2007). Reports on the adverse effects of some compounds used as chemical filters determine the search for new solutions and raise the question whether it is possible to replace the controversial chemical filters with other radiolabelling substances and still provide effective sun protection.

## Natural sun protection products

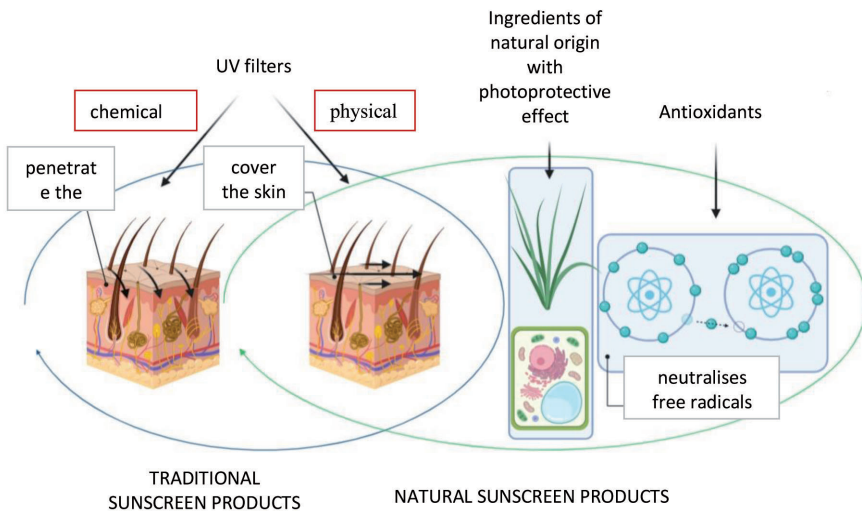
Increasingly, products described as “natural” are appearing on the cosmetics market. This trend is also being observed in the sunscreen market. Photoprotective products based on substances of natural origin are emerging. Unfortunately, until now there are no legal regulations defining natural and ecological cosmetics. These terms are sometimes used interchangeably or other expressions are used, such as organic or biological cosmetics.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 concerning cosmetic products does not differentiate cosmetics in this respect. The Act of 4 October 2018 on cosmetic products also does not introduce a definition of “natural product” or “ecological product” and does not provide for separate regulations for natural cosmetics. Commission Regulation (EU) No 655/2013 of 10 July 2013 establishing common criteria for the substantiation of claims used in connection with cosmetic products refers to marketing claims on packaging and any promotional material for products and aims to ensure the protection of end users, in particular against misleading claims. However, it does not describe the conditions that must be met by cosmetic manufacturers in order to use claims such as “natural cosmetic” or “ecological cosmetic” in relation to a given product (Regulation (EC) No 1223/2009; Commission Regulation (EU) No 655/2013; Act of 4 October 2018 on cosmetic products).

At the request of the European Commission, an independent, non-governmental international organisation of 165 national standardisation bodies- ISO (International Organization for Standardization) has established standard 16128-1:2016 and 16128-2:2017, which defines natural and organic ingredients and presents a methodology for calculating the following indices: naturalness, natural origin, organicness and organic origin. The ISO standard relates only to the presence, the way of obtaining and the level of processing of the ingredients present in a cosmetic product. It informs about the percentage of natural or natural origin ingredients in a given cosmetic product. Like other ISO standards, standard 16128 is not mandatory.

In addition to the ISO standard, there are many organisations certifying natural and organic cosmetics, each with its own requirements relating to these two categories of cosmetics. In Europe, an attempt has been made to unify the standards and therefore the following organisations: BDIH (Germany), Cosmebio/EcoCert (France), ICEA (Italy) and Soil Association (UK), created a common standard for natural and organic cosmetics- Cosmos Standard AISBL (Association Internationale Sans but Lucratif) (COSMOS-standard, Version 3.1 – 1, 2020). World-leading organisations include Ecocert and Natrue, which have established rigorous and transparent labelling criteria for natural and organic cosmetics. Ecocert is the world’s leading specialist in the certification of sustainable practices. It has almost

30 years of experience in auditing and certifying organic products in France and in over 130 countries worldwide. Natrue is an international non-profit association dedicated to the promotion and protection of natural and organic cosmetics worldwide (Ecocert, 2021; Natrue, 2021). In Poland, the certification body for natural and organic cosmetics is the Polish Centre for Testing and Certification, which grants a certificate and the right to label products with the EU Ecolabel, EKO Certified Natural Cosmetic and EKO. The EKO – Certified Natural Cosmetic label is awarded to cosmetic products consisting of more than 90% natural ingredients or of natural origin, while EKO – to organic cosmetics (Polish Centre for Testing and Certification, 2021).



**Figure 2. Radioprotective substances in sunscreen products**

Source: own study.

Sunscreen products labelled as “natural” often combine mineral filters, substances of natural origin with photoprotective effect and antioxidants to complement the sun protection and are based on substances of natural origin or natural substances. Recently, natural sunscreen products labelled COSMOS NATURAL have appeared on the cosmetics market, meeting the requirements of the natural cosmetics world’s leading certifying organisations. They do not contain chemical sunscreens or nanomaterials, but use raw materials and natural substances of natural origin, sometimes even obtained from organic farming. These cosmetics often also contain antioxidants to prevent oxidation. Sunscreens with the logo of the certifying body or association and the COSMOS NATURAL label on their packaging have to meet a number of requirements in order to be certified by individual institutions. The main principles of the COSMOS standard are:

prohibition of nanomaterials, genetically modified raw materials or ingredients, gamma rays and X-rays. Cosmetic products must not be tested on animals, and palm oil and palm kernel oil must come from certified organic sources. A certified natural cosmetic may contain a maximum of 5% synthetic substances (COSMOS-standard, Version 3.1 – 1, 2020).

Mineral filters used in natural sunscreen products are mainly recommended for the protection of sensitive skin, with a tendency to allergies and atopic lesions and skin hypersensitive to sunlight. They are also recommended for use after aesthetic medicine procedures and for delicate children's skin (Stanisz, 2009). Mineral filters work by reflecting and scattering UV radiation. They include two types of products: coloured pigments with a particle size of 200–300  $\mu\text{m}$ : titanium dioxide ( $\text{TiO}_2$ ), zinc oxide ( $\text{ZnO}$ ), iron oxide ( $\text{Fe}_2\text{O}_3$ ,  $\text{Fe}_3\text{O}_4$ ), mica-titanium oxide system (mica sheets covered with titanium oxide) and micronised pigments with a particle size of 20–80 nm: zinc oxide, titanium oxide. Large particle filters leave a white film on the skin and are difficult to spread. By using a micronisation process for physical filters, this phenomenon has been overcome (Bojarowicz & Bartnikowska, 2014; Placek, 2008). Additionally, in order to facilitate their spreading on the skin surface, they are often coated with silicones, aluminium oxide or fatty acids (Hongbo, *et al.* 2013). Substances used as physical filters are: zinc oxide and titanium dioxide, they are considered safe and often described in the literature as natural, they form a barrier on the skin surface without penetrating the epidermis. Their photoprotective effect, unlike chemical filters, is not associated with any chemical reactions (Kryczyk, *et al.* 2018; Stanisz, 2009). The significant advantages of mineral filters over chemical filters are often pointed out, including greater photostability, a broader spectrum of protection, and less sensitisation (Smyk, *et al.* 2016). Physical filters have a broad spectrum of action, protecting the skin against both UVB and UVA radiation. Zinc oxide provides protection against UVA radiation, while titanium dioxide shows protective effects against UVB and short-wave UVA radiation (Kryczyk, *et al.* 2018).

The role of antioxidants in photoprotective products is widely reported in the literature. Topical application of antioxidants has been proven to be an effective strategy to protect the skin from oxidative damage caused by UV radiation. Exposure to sunlight induces oxidative processes that result in the formation of free radicals and reactive oxygen species (ROS). These can cause a lot of damage, among others to biological membranes and the genetic material of the cell. It has been proven that UVA radiation, through the formation of reactive oxygen species, damages the DNA of cells, which can consequently lead to carcinogenesis (Ebisz & Brokowska, 2015; Nowak, *et al.* 2014). Antioxidants are compounds that eliminate free radicals from the human body. Their antioxidant activity involves different mechanisms of action. Antioxidant substances have the ability to capture and neutralize free radicals, protect epidermal lipids from oxidation, seal the walls

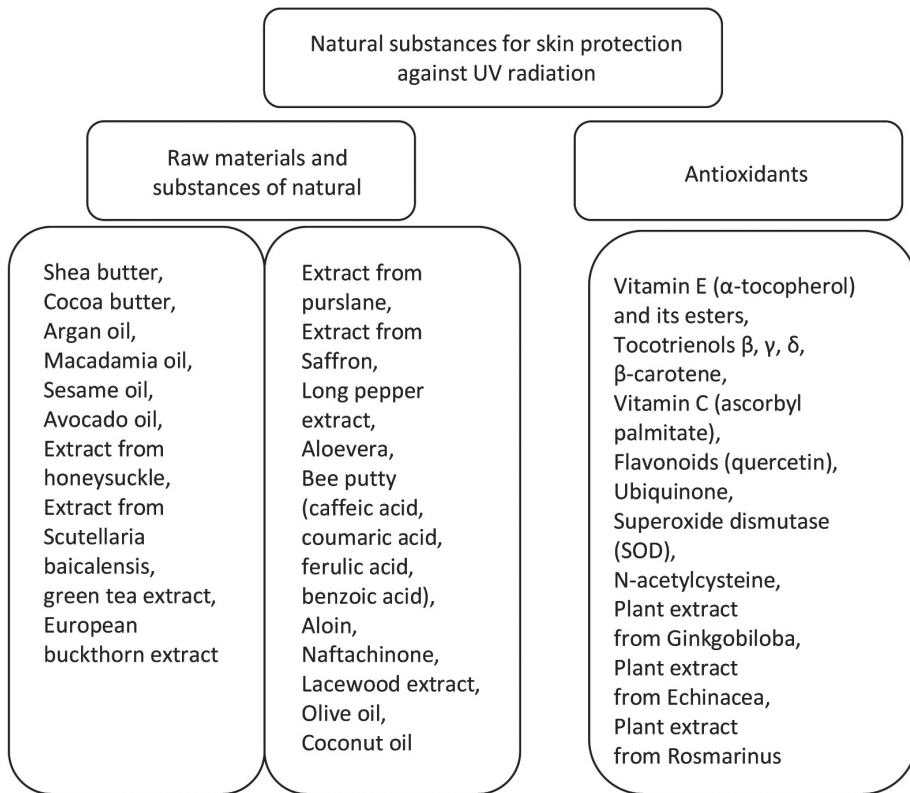
of blood vessels, and also have anti-inflammatory and anti-mutagenic effects (Puzanowska-Tarasiewicz, Kuźmicka & Tarasiewicz, 2010).

Many substances of natural origin show a protective effect against UV radiation, but it is too low for them to be used as the only protection in sunscreen products. Research indicates that sunscreen products combining natural substances with UV filters have a safe and effective photoprotective effect. Due to this fact, the UV-protection content in sunscreen products, especially of chemical filters, can be reduced. Although raw materials and substances of natural origin should not be used as the only protection against UV radiation, due to insufficient sun protection, studies have indicated that they are able to increase the protective effect of products containing physical filters (Seok, *et al.* 2016).

## Substances of natural origin with photoprotective effect

The photoprotective effect of many compounds, raw materials and substances of natural origin has been reported in the literature, their efficacy varies widely, but most of them show a weak protective effect against UV radiation, so it is recommended to combine them with physical and/or chemical filters (Smyk, *et al.* 2016; Yarnell & Abascal, 2012). Many of them have antioxidant and skincare functions; in addition to their protective effect against UV radiation, they can improve the condition of the skin, moisturise it, and counteract the ageing process. As sunscreens should be used as part of your daily skincare routine, it's worth making sure that the ingredients they contain not only protect your skin from the sun radiation, but also care for it.

Propolis is a substance also known as bee putty or bee glue. It is produced by bees from tree resin combined with beeswax and secretions of the bees' salivary glands. The history of the use of propolis dates back to ancient Egypt, where it was used to embalm bodies and protect the mummy from fungi, bacteria and viruses. The composition of propolis includes: fatty and aliphatic acids (24–26%), flavonoids (18–20%), trace elements (0.5–2.0%), sugars (15–18%), aromatic acids (5–10%), esters (2–6%), alcohol and terpenes (2–3.3%), vitamins (2–4%), others (21–27%) (Sawicka, *et al.* 2012). The antifungal, antibacterial, antiseptic, immunomodulatory and antioxidant effects of propolis have been demonstrated (Bolfá, *et al.* 2013). The antibacterial action of propolis can be used in acne skin care preparations (Kopczyńska, Klasik-Ciszewska & Duda-Grychtoł, 2018). Propolis, due to polyphenols contained in its composition, has UV-absorbing properties (Saewan & Jimtaisong, 2015). Studies on the efficacy of selected propolis components have shown that caffeic acid provides protection of SPF=20 already at a concentration of 4% (Gregoris, *et al.* 2011; Kryczyk, *et al.* 2018).



**Figure 3. Natural substances protecting the skin against UV radiation**

Source: own elaboration based on (Bojarowicz & Bartnikowska, 2014; Placek, 2008; Pop, 2015; Stanisz, 2009; Yarnell & Abascal, 2012).

Common aloe vera (*Aloe vera*) also called Barbados aloe (*Aloe barbadensis*) is a succulent, i.e. a plant adapted to life in conditions of limited water availability. It is an evergreen, perennial herb (Radosz, Klasik-Ciszewska & Duda-Grychtoł, 2018). The main components of aloe vera include amino acids, anthraquinones, enzymes, lignin, minerals, monosaccharides and polysaccharides, salicylic acid, saponins, sterols and vitamins. Aloe vera accelerates collagen production and is an extremely effective moisturiser and skin healing agent (Korać & Khambholja, 2011). Aloe vera is a rich source of bioactive substances; the extract from aloe leaves, called biostimine, contains biological stimulators that are responsible for the body's immunity (Lamer-Zarawska, Chwała & Gwardys, 2012). Aloe vera has the following properties: regenerative, soothing, astringent, anti-inflammatory, moisturising, firming, it can also be used in some skin disorders such as eczema, acne or psoriasis (Radosz, Klasik-Ciszewska & Duda-Grychtoł, 2018).



Aloe leaf extract in a study of photoprotective properties showed SPF = 28.86 ± 0.11, and was also found to be photostable. A study conducted by Napagoda, *et al.* also included other raw materials and showed photoprotective potential of plants such as: *Leucas zeylanica* SPF = 39.8 ± 0.35, *Ophiorrhiza mungos* SPF = 39.2 ± 0.92, *Mollugo cerviana* SPF = 29.5 ± 0.30, *Hibiscus furcatus* SPF = 29.4 ± 0.40, *Atalantia ceylanica* SPF = 26.8 ± 0.16, *Olax zeylanica* SPF = 24.5 ± 0.47, *Aporosa lindleyana* SPF = 21.4 ± 0.90 (Napagoda, *et al.* 2016).

**Table 1. Photoprotective potential of plant raw materials**

Plant-based substances	Sun Protection Factor (SPF)
<i>Aloe vera</i>	28.86 ± 0.11
<i>Leucas zeylanica</i>	39.8 ± 0.35
<i>Ophiorrhiza mungos</i>	39.2 ± 0.92
<i>Mollugo cerviana</i>	29.5 ± 0.30
<i>Hibiscus furcatus</i>	29.4 ± 0.40
<i>Atalantia ceylanica</i>	26.8 ± 0.16
<i>Olax zeylanica</i>	24.5 ± 0.47
<i>Aporosa lindleyana</i>	21.4 ± 0.90

Source: based on: (Napagoda, *et al.* 2016).

The photoprotective effect of some plant oils has also been reported in the literature. Kauri & Saraf (2010) evaluated the ultraviolet absorption capacity of selected vegetable oils used in cosmetics and determined in vitro by spectrophotometric method their sun protection factor (SPF). The most promising SPF values have been shown by: olive oil (*Olive oil*) SPF = 7.549; coconut oil (*Coconut oil*) SPF = 7.119; peppermint oil (*Peppermint oil*) SPF = 6.668; Tulsi oil (*Ocimum sanctum*) SPF = 6.571; lemon grass oil (*Lemon grass oil*) SPF = 6.282 (Tab. 2). It is worth noting that these oils also have many other properties, including skin care. Coconut oil is used in cosmetics in many ways, it has moisturizing, emollient, anti-inflammatory, and antioxidant properties due to the content of vitamin E and polyphenols. It can be safely used in newborns, patients with atopic dermatitis and keratinisation disorders (Piotrowska, Totko-Borkusiewicz & Klucznik, 2019). Olive oil is rich in active substances with multidirectional healing and beautifying effects. In the context of skin protection against UV radiation, its antioxidant properties are the most important, vitamin E contained in it prevents oxidative damage to the skin caused by UV radiation, while squalene rebuilds



the skin's lipid mantle and prevents lipid peroxidation. In addition, it has anti-aging, soothing, antimicrobial effects, lubricates, softens and makes the skin more elastic, and protects against excessive water loss from the epidermis (Marwicka, Makuch & Niemyska, 2015).

**Table 2. Sun protection factor of vegetable oils**

Vegetable oils	Sun Protection Factor (SPF)
olive oil ( <i>Olive oil</i> )	7.549
coconut oil ( <i>Coconut oil</i> )	7.119
mint oil ( <i>Peppermint oil</i> )	6.668
Tulsi oil ( <i>Ocimum sanctum</i> )	6.571
lemongrass oil ( <i>Lemon grass oil</i> )	6.282

Source: based on: (Kaur & Saraf, 2010).

Raspberry seed oil (*Rubus Idaeus L.*) is quite commonly used in the cosmetics industry. It contains high amounts of tocopherol and carotenoids, which show strong antioxidant properties. Raspberry seed oil can act as a broad-spectrum UV protection agent and provide protection against both UVA, an exogenous source of oxidative stress, and UVB. Its sun protection factor is determined between SPF 20-50 (Oomaha, *et al.* 2000).

Spotted thistle (*Silybum marianum* (L.) Gaertner) has been used in medicine since ancient times and is now cultivated for the pharmacological industry. The fruits (*Fructus sylibi mariani*) are the herbal raw material; they contain silymarin, flavonoids, organic acids, biogenic amines, mucilage, vitamin C and K, phytosterols, proteins, sugars, tannins, linoleic acid and mineral salts. The most important ingredient, which protects the skin against the harmful effects of UV radiation, is silymarin with a strong antioxidant effect. Silymarin is a mixture of flavonolignans, among which the following are distinguished: silybin, silydianin and silycristin (Szczenińska, *et al.* 2003). Svobodova, *et al.* (2007) evaluated the ability of selected flavonolignans (silibinin and 2,3-dehydrosilibin) to mitigate UV-induced damage. The results indicate that they inhibit oxidative stress induced by UVA and may be useful in the treatment of skin damage caused by this type of solar radiation (Svobodová, *et al.* 2007). It is also worth noting the results of a study on the dietary use of silybin, which prevents early biomarkers of UVB-induced carcinogenesis. The study was conducted on the hairless epidermis of female SKH-1 mice. It has been shown that a diet enriched with silibin significantly reduces UVB-induced thymine dimer-positive cells in the skin of mice. Thymine dimers are formed in DNA immediately after UVB irradiation and

are considered an early and important biomarker of DNA damage after UVB exposure. They are also used as a biomarker to test the photoprotective effect of sunscreen formulations in the UVB range (Gu, *et al.* 2005).

Although raw materials and substances of natural origin should not be used as the only protection against UV radiation, due to insufficient sun protection, studies indicate that they are able to enhance the protective effect of physical sunscreen products. *Scutellaria radix* root extract and its butanol fraction (BuOH) were shown to strongly absorb UV radiation and exhibit antioxidant activity. A sunscreen containing a mineral filter (24% ZnO) showed an SPF=17.8, and when supplemented with the 5% BuOH fraction of *Scutellaria radix* extract, this value increased to SPF= 22.7. It can therefore be concluded that the ingredients in *Scutellaria radix* can be used as additives to sunscreen cosmetics, increasing their protective effect (Seok, *et al.* 2016).

## Summary

Recently, there has been a growing interest in natural products. The slogan “back to nature” has lately become one of the driving elements for the FMCG (Fast Moving Consumer Goods) market, to which the cosmetics industry belongs. This trend is conditioned by a number of factors, primarily resulting from new consumer needs and interests, but is also related to society’s concerns about the natural environment. Growing popularity of natural cosmetics is a manifestation of the developing eco-consumption. New product solutions tailored to the preferences and expectations of modern consumers are appearing on the market. One of the new product categories are natural radioprotective cosmetics.

Increasingly, cosmetics manufacturers are trying to create a product containing the maximum amount of natural substances or substances of natural origin. As it concerns radioprotective products, they also have the possibility to use raw materials and substances of plant origin showing photoprotective effects. Many sources indicate the photoprotective effect of selected substances of natural origin, but due to their relatively low sun protection factor it is suggested that they are combined with UV filters. This can reduce the UV filter content in sunscreen products, especially organic substances, some of which are still controversial. Based on literature data, substances of natural origin used in this type of cosmetics include: shea butter, cocoa butter, argan oil, macadamia oil, sesame oil, avocado oil, extract from honeysuckle, extract from *Scutellaria baicalensis*, green tea extract, european buckthorn extract, extract from purslane, extract from saffron, long pepper extract, aloe vera, bee putty (caffeic acid, coumaric acid, ferulic acid, benzoic acid), aloin, naftachinone, lacewood extract, olive oil, coconut oil, petroleum jelly, *Leucas zeylanica*, *Ophiorrhiza mungos*, *Mollugo cerviana*, *Hibiscus*

*furcatus*, *Atalantia ceylanica*, *Olax zeylanica*, *Aporosa lindleyana*, mint oil, Tulsi oil, lemongrass oil, raspberry seed oil, spotted thistle, *Scutellaria radix extract*.

Exposure to the sun's rays causes so-called oxidative stress, which results in the increased formation of free radicals. They cause a number of negative effects, affect the degenerative processes of skin cells and accelerate its ageing. This process can be reduced by using antioxidants (Nowak, *et al.* 2014). Valuable sources for obtaining them are plant raw materials. Antioxidants of natural origin can be found in many plants and can be an excellent complement to sunscreen products. Natural substances with photoprotective effects can also be a valuable ingredient in daily skin care cosmetics. The current trend associated with the popularity of natural cosmetics, as well as the increased consumers' concern for the environment, are supporting the development of a new product category of natural sunscreen cosmetics. The growing interest of society in sustainable development, care for the environment and at the same time the quality of life determines the change of consumers' behaviour and preferences and their increased interest in natural products.

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## THE ANALYSIS OF MARKET OFFER OF CLEANSING WET WIPES FOR BABY SKIN CARE AVAILABLE IN THE POLISH MARKET

Paulina Malinowska<sup>1</sup>

### Abstract

In this work the analysis of market offer of cleansing wet wipes for baby skin care was conducted. 135 products available in drugstores, discount stores, hyper- and supermarkets in the Polish market were analyzed in the fourth quarter of 2020. The aim of work was to analyse the pricing strategy and marketing communication used by wet wipes producers and to estimate the kinds of active ingredients used in wipes formulations. The analysis of pricing strategies showed that the producers use mostly penetration pricing strategy, price leadership strategy and psychological pricing strategy. The analysis of marketing communication of cosmetic properties of baby wet wipes showed that the key cosmetic claims used by producers concern naturalness, protection of baby skin, prevention against the irritations, conducted tests, safety and recommendations. The claims concerning properties of analysed wet wipes do not go beyond the definition of a cosmetic. A detailed analysis of the chemical composition of baby wet wipes identified 30 active ingredients. The most popular actives turned out to be glycerin, allantoin, aloe juice, panthenol, camomile extract, vitamin E and olus oil. These all active ingredients soothe irritation, moisturize and calm the baby skin.

### Keywords:

baby cleansing wet wipes, active ingredients, pricing strategy, marketing communication, cosmetics market

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Technology and Instrumental Analysis; [paulina.malinowska@ue.poznan.pl](mailto:paulina.malinowska@ue.poznan.pl)



## Introduction

Wet wipe is a drenched piece of non-woven cloth or plastic. On the basis of technology, the global wet wipes market has been divided into spunlace, airlaid, wetlaid, and others. These wipes are used for cleaning purposes including personal hygiene, home care and even cleaning equipment in the industrial sector. Consumers use wet wipes for cleaning purposes such as cleaning kitchen counters, appliances, floors, and toilets, among others. The demand for disinfectant wet wipes in the homecare segment is increasing as they are easy to use and keep the surfaces free from bacteria and germs. Besides, wet wipes are used for cleaning the sensitive skin of babies and highly contribute to maintain hygiene and well-being. Also, the increasing average global birth rate is expected to fuel the demand for baby wet wipes. The increasing preference for wet wipes has influenced the manufacturers to focus on product development. Through product development, the manufacturers are increasing the base of the target audience ([www.factmr.com](http://www.factmr.com)).

Baby wipes are used to clean the sensitive baby skin. These industrially manufactured wipes are mostly disposable or meant for one-time use. Similar to the ones used in the manufacturing of dryer sheets, non-woven fabrics are a primary raw material required to produce baby wipes. Considering their easy disposing and moisture maintenance features, plastic tubs are commonly used to pack baby wipes. Manufacturers recommend gentle cleaning solutions to treat baby wipes ([www.factmr.com](http://www.factmr.com)).

## Baby skin care

The baby's skin is characterized by being sensitive, thin and fragile (Fernandes, Machado & de Oliveira, 2011). The skin barrier, a protective shield against environmental factors and pathogens, is primarily formed by the outermost skin layer. Although full-term infants are born with a competent skin barrier, this layer matures further over a child's first years of life. Moreover, the epidermis and the Stratum corneum are thinner, the corneocytes are smaller, the content of natural moisturizing factor (NMF) is lower, and the skin is more prone to pH imbalances. Furthermore, sebaceous and sweat glands are less developed and regulated, and the melanin content is lower (Stork, Mehling & Schulte, 2019). As a consequence, there is greater transepidermal water loss, increased percutaneous absorption of chemicals and easily induced skin trauma, even with the removal of any adhesive bandage. This leads to tendency to infections, toxicity and difficulties in fluid homeostasis. Since the epidermal barrier is immature in infants, skin permeability is very high, especially during the first fortnight of life. This causes a substantial

risk of toxicity from the percutaneous absorption of drugs. Moreover, this skin is also more easily attacked mechanically, as in the area of contact with diapers or with the use of wipes, which cause repeated and localized removal of cells of the stratum corneum and, consequently, increase the permeability of the skin. Over time, the child's skin becomes more and more impermeable (Fernandes, Machado & de Oliveira, 2011).

All these characteristics give baby skin less protection against moisture loss, cold and hot temperatures, but also against environmental aggressors such as pathogens, irritants and UV radiation. Due to these and other factors, baby skin requires specific care, e.g. through products that provide extra mild cleansing, protect or maintain the healthy skin barrier, and ensure sufficient moisturization (Stork, Mehling & Schulte, 2019). Cosmetic products intended for their hygiene and protection require special attention in their formulation. One of the paramount conditions is that all the ingredients that may be potentially aggressive to the skin be excluded. The percutaneous absorption of drugs and topical agents is influenced by the physical and chemical characteristics of the drug and also by the properties of the skin barrier. The higher the body surface area: body weight ratio, the greater the risk of percutaneous toxicity. Other factors involved are immature systems of drug metabolism and, in newborn babies, especially premature infants, immaturity of the epidermal barrier. In the hygiene of babies and children, products containing perfumes and dyes should also be avoided (due to the risk of contact dermatitis), as well as additives that mimic the scents and colors of fruit and sweets, since they stimulate the ingestion of cosmetics (Fernandes, Machado & de Oliveira, 2011). Synthetic bathing products commonly used for neonatal skincare contain chemicals which may affect skin pH and be potential irritants to the skin. Researchers advise against the use of cleansing products, including baby wipes, for all babies during the first month of life (Jackson, 2008).

There are many products for caring the babies available in the market: lotions, creams and ointments, powders and oils, body cleansing products and shampoos, bath essences, wet wipes, and sunscreens. In 2018 alone, the Mintel Global New Products Database (GNPD) listed more than 1000 baby care launches in the European Union – with skin care, bath and soap, and wipes each accounting for around 30% of total launches, and the remainder made up by hair care products and fragrances. Suitability for sensitive skin is the most prominent claim among new baby care product launches with particular emphasis globally being placed on related function all claims such as mildness, extra protection and moisturization. As in other personal care segments, the trend towards green and natural products is on the rise, with a focus on the use of natural and organic ingredients, vegan formulations, environmentally-friendly products and packaging, as well as “free-from” claims (Stork, Mehling & Schulte, 2019).

## Cleansing baby wet wipes market

Wet wipes are often used in as part of a baby's hygiene routine as substitutes for water and soap when changing nappies as well as for cleaning the baby's face and hands. Wet wipes are defined as products sold in the market as single-use wipes which are pre-moistened with lotion, and do not pass strict legally defined standards for 'flushables'. Wet wipes are made from non-woven fabrics and are saturated with a cleansing solution. Disposable baby wipe consists of three main components – the basesheet, the formulation, and package. The packaging and the basesheet are the most physically obvious components of a wipe. There are three types of basesheets with differences in composition which translates into differences in thickness, absorbency, and softness to touch. These differences can impact cleaning performance but the materials themselves are quite common – wood pulp, polypropylene, polyester, or combinations thereof (Rodriguez, *et al.* 2020).

Over the last two decades, significant advances have been made to baby wipes. More recently, efforts have been centered on the removal of ingredients with irritation or skin-sensitizing potential. The clinical studies have demonstrated that the use of modern baby wipes is superior to using water and cloth to clean diapered skin. In 2016, a recommendation was made by the European Roundtable Meeting on Best Practice Healthy Infant Skin Care stating that a wet wipe for infant skin should contain pH buffers to maintain the slightly acidic pH of the skin, should be free of potential irritants, and should contain well-tolerated preservatives. Formulating a hypoallergenic, safe, gentle, and effective baby wipe can be challenging as the wipe must meet regulatory, safety, and performance measures while remaining aesthetically pleasing. It is preferred that baby wipes are formulated with a very large percentage of water. However, water alone is not enough to effectively remove water-insoluble residues from feces and prevent the growth of microorganisms or maintain a healthy skin pH. Thus, it is important that baby wipes also contain an extremely mild surfactant (detergent or cleanser) to lower surface tension for better cleaning, a preservation system to ensure product freshness before and during use, a pH adjusting (buffering) system to maintain a solution pH similar to infant skin, and, optionally, skin-benefiting ingredients that reduce frictional damage, replenish the skin lipids, etc. A common misconception about baby wipes is that they contain drying alcohols such as ethanol and isopropanol. While ethanol and isopropanol can be found in some sanitizing wipes, these ingredients have not been used in branded baby wipes (Rodriguez, *et al.* 2020).

The European wipes market is steadily increasing and is driven by baby wipes, which stands for almost 70% of total sales with price as a major competitive factor. Based on volume, baby wipes represent over 80% of all personal care wipes in

Europe with higher usage in Western Europe compared to Eastern Europe and variations between countries in the north and the south. There are many reasons why consumers like baby wipes; they are soft, smooth and skin friendly, they clean well and not the least, they are environmentally friendly and chlorine free. Interestingly baby wipes are being used for much more than just baby care. Adults use them for themselves, make-up removal, household, pets, when doing sports, removing stains (Engqvist, 2014).

The wet wipes market in Poland is also developing very intensively. Each manufacturer tries to respond to customer needs as quickly as possible, both in terms of packaging and composition. The consumer can buy an economic package or a multipack. Consumer also pays more attention to the chemical composition of the products, choosing these with a simple list of ingredients. The development of the wet wipes segment is influenced by ecological trends that stimulate the emergence of biodegradable or rinse-off products, the use of organic cotton non-woven fabric or sustainable cellulose in the production process. Products in recyclable packaging are also recording an upward trend. The baby wipes market belongs to three main brands: Bambino (Beiersdorf Group), Pampers (Procter & Gamble) and Bobini (Global Cosmed Group) (Pierzchała, 2020).

In this work the analysis of Polish market offer of cleansing wet wipes was conducted. 135 products available in the Polish cosmetic market were analyzed in Auchan, Biedronka, Carrefour, Kaufland, Lidl, Netto, Rossmann, Superpharm, Stokrotka and Tesco. The aim of work was to analyse the pricing strategy and marketing communication used by wet wipes producers and to estimate the kinds of active ingredients used in wipes formulations.

## The methodology of analysis

The analysis of the market offer of cleansing wet wipes for babies skin care was carried out in 10 points of sales: Auchan, Biedronka, Carrefour, Kaufland, Lidl, Netto, Rossmann, Superpharm, Stokrotka and Tesco in 2020. Analysed products came from 33 brands: Babydream, Bambiboo, Bambino, Bebetto, Bella Happy, Bevola, Bobini, Cosmia, Dada, Derma Baby, Dove, Fred&Flo, Gaga, Hipp, HomeCare, Huggies, Isana, Jelp, Johnson's, Kindii, Linteo, Life Baby, Lula, Lupilu, Nello, Nivea, Pampers, Tami, Tibelly, Water Wipes, Velvet.

The analysis proceeded in five stages:

Stage 1: Reviewing the market offer of leading brands of cleansing wet wipes available in the Polish market:

- marking out the leading brands of wet wipes available in the Polish market,
- getting familiar with the market offer of wet wipes in points of sales.

Stage 2: Selecting cleansing wet wipes designed for baby skin care from the market offer:

- identifying wet wipes designed for baby skin care,

Stage 3: Analysis of the pricing strategy of wet wipes available in the Polish market:

- dividing all wet wipes into 4 categories: comfort, sensitive, water and biodegradable,
- analysing the prices of wet wipes in 10 points of sales: Auchan, Biedronka, Carrefour, Kaufland, Lidl, Netto, Rossmann, Superpharm, Stokrotka and Tesco.

The average prices and number of wet wipes in packaging were taken under consideration during analysis. With the aim of conducting of pricing analysis, the unit of measurement was average price (in PLN) of one wet wipe from packaging. It was used the tools of descriptive statistics and it was conducted the descriptive and comparative analysis. The results of this study have cognitive and diagnostic character.

Stage 4: Analysis of main marketing communication concerning the cosmetic properties of wet wipes.

Stage 5: Carrying out a detailed analysis of the chemical composition of wet wipes designed for baby skin care:

- identifying active ingredients in the analysed products,
- marking out the most often used active ingredients.

The most important active ingredients were identified based on their Latin and English names present in the INCI (the International Nomenclature of Cosmetic Ingredients) composition indicated on the packages.

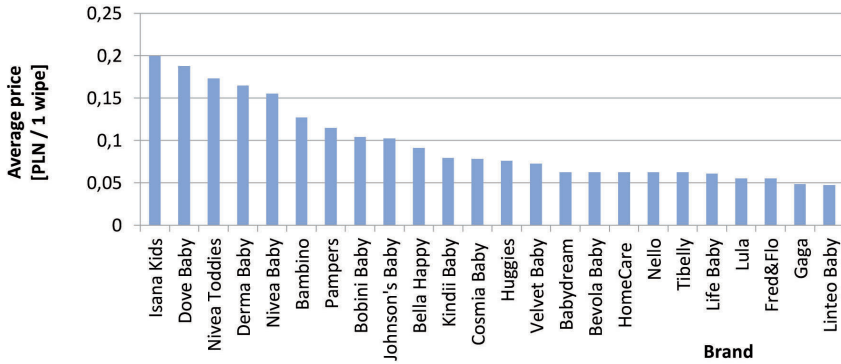
## Results and discussion

### The pricing strategy of baby wet wipes

Pricing strategies play a very significant role in each organization's strategy. A process of establishing a pricing strategy consists both of economic and non-economic conditions. According to the traditional marketing mix includes products, promotion, price, place, people, processes and physical evidence. Another approach is that price is determined by measures such as costs, revenues and profits. Pricing is definitely a dynamic process, as nothing will remain constant: the economy, taste, innovations, as well as competitors actions and reactions. There are many ways in which price can be used for strategic purpose (Kent, 2003; Kotler & Armstrong, 2010; Nagle & Holden, 2002): price skimming, penetration pricing, product-line pricing, related product pricing, demand manipulation, price discrimination, price leadership, loss leader, psychological pricing, dynamic pricing, target pricing and bundling and quantity discounts.

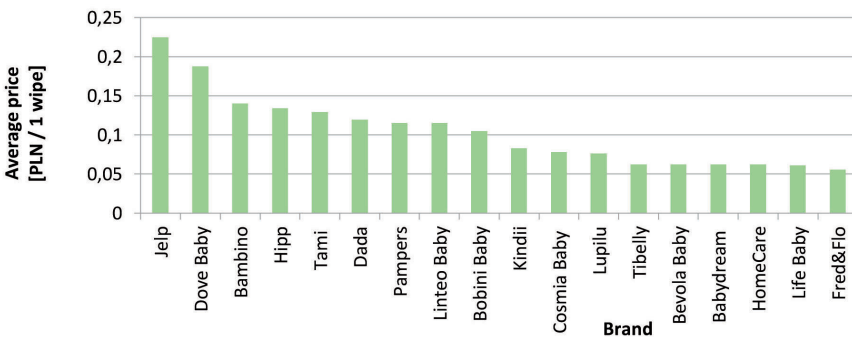
Technological progress made in recent years altered the wet wipes segment into one that offers additional benefits such as purpose for sensitive and atopic skin, water content or biodegradable. What is why, the baby wet wipes are divided into four categories depending on their application, such as: comfort (standard), sensitive, water and biodegradable. Going through the counters, one can notice the considerable price differences that exist between the various wet wipes varieties. Some of price differences we see can be very substantial, so that one brand of wet wipes costs as much as few times more than wet wipe from another brand. There are few factors, which determine the price of wet wipes: the place of their production, the cost of non-woven and ingredients.

According to the analysis the most expensive comfort baby wet wipes were from brands: Isana Kids, Dove Baby, Nivea Toddies and Derma Baby and cost around 0.16–0.20 PLN/1 wipe. The cheapest comfort wet wipes were from brands: Linteo Baby, Gaga, Fred&Flo, Lula, Life Baby, Tibelly, Nello, HomeCare, Bevola Baby and Babydream which price oscillated from 0.05–0.06 PLN/1 wipe (Fig. 1).



**Figure 1. Average prices of comfort baby wet wipes available in Polish cosmetics market**

Source: own study.

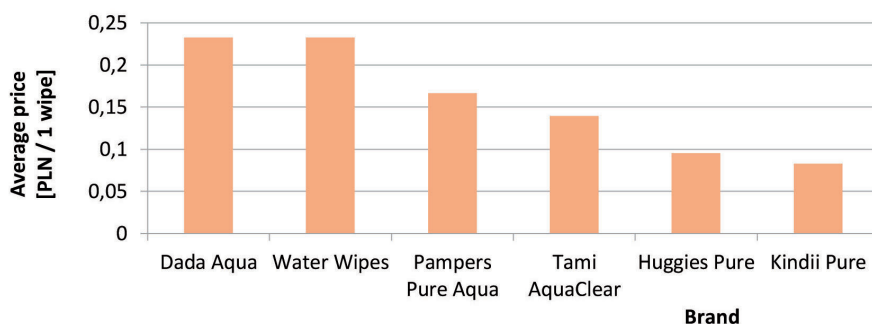


**Figure 2. Average prices of sensitive baby wet wipes available in Polish cosmetics market**

Source: own study.

The next step of the analysis was to assess the average price of sensitive baby wet wipes available in Polish cosmetics market. The prices differed a lot. In the Figure 2 one can noticed that the most expensive is Jelp (0.22 PLN/1 wipe) and Dove Baby (0.19 PLN/1 wipe). The cheapest were Fred&Flo, Life Baby, HomeCare, Babydream, Bevola Baby and Tibelly (0.05–0.06 PLN/1 wipe).

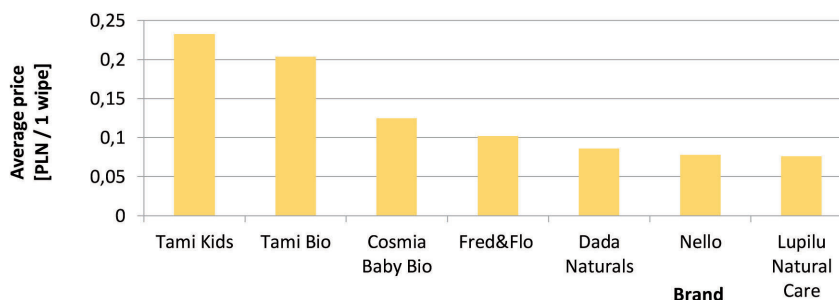
In the Figure 3 were presented the results of the average price of baby water wipes available in cosmetics market. The most expensive was Dada Aqua and Water Wipes (0.23 PLN/1 wipe), then Kindii Pure and Huggies Pure could be purchased for 0.08–0.09 PLN/1 wipe.



**Figure 3. Average prices of water baby wet wipes available in Polish cosmetics market**

Source: own study.

In the Figure 4 were presented the results of the average price of biodegradable baby wet wipes available on Polish cosmetics market. The most expensive is Tami Kids and Tami Bio (0.20–0.23 PLN/1 wipe) and the cheapest are Dada Naturals, Nello and Lupilu Natural Care (0,08-0,09 PLN/1 wipe).



**Figure 4. Average prices of biodegradable baby wet wipes available in Polish cosmetics market**

Source: own study.

Pricing strategies can have a large impact on profit. A higher or lower price can dramatically change both gross margins and sales volume. Price a premium item too low may cause that customers will not believe the quality is good enough. Conversely, put too high a selling price on value lines and customers will purchase competitors' lower-price items. The results of the analysis proved that some typical pricing strategies are commonly used by wet wipes producers on Polish market. Brands such as Babydream, Bevola Baby, Dada, Fred&Flo, Gaga, HomeCare, Life Baby, Linteo Baby, Lula, Lupilu, Nello, Tibelly use penetration pricing strategy. Bambino, Dove Baby, Isana Kids, Jelp, Nivea, Tami and Water Wipes profit from price leadership strategy. On a daily basis psychological pricing strategy is being used by most brands: Babydream, Bambino, Bella Happy, Bevola, Bobini, Cosmia, Dada, Dove, Fred&Flo, Hipp, Huggies, Isana, Kindii, Lula, Nello, Pampers, Tami, Tibelly, Velvet. Many of them take advantage of bundling and quantity discounts like one wet wipes packaging in a regular price plus second one for free or two, three, four or five wet wipes packagings in better price than bought as single product.

## Marketing communication of cleansing baby wet wipes

The success in the sale of cosmetics is to convince consumers of their benefits and to make consumers believe that only this product will satisfy their needs. Properly prepared and transmitted to the consumer information about the product contributes to the creation of simple direct associations, which connect the brand with specific benefits of used cosmetic. The areas of communication are labels, leaflets, articles, press information, trainings and lectures. The significant elements, used in marketing communication, are claims.

The analysis of marketing communication of cosmetic properties of baby wet wipes showed that the key cosmetic claims used by producers concern naturalness, protection of baby skin, prevention against the irritations, conducted tests, safety and recommendations. The most popular declarations are:

- Natural; raw materials of natural origin; natural ingredients;
- Organic; bio; vegan friendly; gluten free;
- Based on natural emollients;
- "Pure water"; as gentle as pure water;
- Care properties; protect against dryness; protect against irritations; create a protective barrier; support the natural lipid barrier; prevent the skin from drying out; the skin microbiome friendly;
- Ultra-safe formulation; safe and gentle for the baby skin; safe source;



- From the first day of life;
- Eye-friendly – ophthalmologically tested; intended for neonatal skin confirmed in clinical trials; dermatological tests conducted on sensitive and allergy-prone skin; hypoallergenic tested by a dermatologist; clinically tested; pediatrician approved; recommended by (...);
- Out of concern for sustainable development.

With reference to certain claims and active ingredients, baby wipes manufacturers are required to meet the expected regulatory conditions. According to Regulation 655/2013 (Commission Regulation (EU) No 655/2013) product claims concerning cosmetics should meet six common criteria: legal compliance, truthfulness, evidential support, honesty, fairness, informed decision-making. With reference to claims and active ingredients, cosmetics manufacturers are required to meet the expected regulatory conditions. The analysis confirmed that the claims concerning properties of cosmetics do not go beyond the definition of a cosmetic.

## The chemical composition of baby wet wipes

A detailed analysis of the chemical composition of baby wet wipes was carried out. Particular attention was paid to active ingredients that have a fundamental influence on the caring properties of the products. The analysis took into account not only the kind but also the frequency of using individual active ingredients.

In baby wet wipes, 30 active ingredients were identified. The most popular ones are presented in Table 1. The analysed active ingredients most often show anti-inflammatory, antioxidative, anti-swelling, emollient, soothing and moisturizing effects. Depending on the brand of wet wipes, the content of active ingredients ranges from one to several. There are some products which contain only basic ingredients, not any active ones.

The most popular ingredient is glycerin – basic humectant of many cosmetics. It keeps the right moisture level of the baby skin. Other important ones are soothing ingredients as allantoin, aloe, panthenol, camomile and E vitamin. These all active ingredients soothe irritation, moisturize and calm the baby skin (Arct, *et al.* 2010).

The analysis of the chemical composition of wet wipes showed that in most cases active ingredients are precisely selected and correspond to the actual needs of the target group of users for whom these products are designed, i.e. babies with delicate skin. The products contain natural extracts and oils and most often show soothing and moisturizing effects.

**Table 1. The most popular active ingredients in cleansing wet wipes for baby skin care**

No.	INCI name	Presence in products [%] <sup>a</sup>
1.	Glycerin	30.4
2.	Allantoin	23.7
3.	Aloe Barbadensis Leaf Juice	23.7
4.	Panthenol	12.6
5.	Chamomilla Recutita Flower Extract	11.9
6.	Tocopheryl Acetate (vitamin E)	9.6
7.	Olus Oil	6.7
8.	Prunus Amygdalus Dulcis Oil	5.2
9.	Bisabolol	4.4
10.	Glucose	4.4
11.	Levulinic Acid/ Sodium Levulinate	3.7
12.	Gossypium Herbaceum (Cotton) Seed Oil	3.7
13.	Olea Europea (Olive) Fruit Oil	2.2
14.	Betaine	2.2
15.	Citrus Grandis (Grapefruit) Seed extract	2.2
16.	Calendula Officinalis Flower Extract	2.2
17.	p-Anisic Acid	1.5
18.	Avena Sativa (Oat) Kernel Extract	1.5
19.	Helianthus Annuus Seed Oil	1.5
20.	Linum Usitatissimum Seed Oil	1.5
21.	Alpha-Glucan Oligosaccharide	1.5
22.	Malic Acid	1.5

Explanation: <sup>a</sup> – percentage of the products containing (according to producers' declarations) the ingredients per total 135 analysed.

Source: own study.

## Conclusion

Baby wipes are one of the impressively expanding consumer goods markets having strong potential for growth in emerging as well as developed regions. The conducted analysis of pricing strategies showed that the wipes' producers use mostly penetration pricing strategy, price leadership strategy and psychological pricing strategy. The wipes' producers use glycerin, allantoin, aloe juice, panthenol, camomile extract, vitamin E and olus oil as active ingredients and

declare naturalness, protection of baby skin, prevention against the irritations, conducted tests and safety.

In the coming years, there could be a telling consumer preference for herbal, natural, biodegradable and water baby wipes. However, the global baby wipes market is expected to find its growth derailed because of the high price factor, as biodegradable and water wipes are definitely more expensive than comfort or sensitive ones. In addition, it is a product that is easy to replace, for example with a cotton pad with boiled water – that may seem safer, more natural and gentler for baby's skin.

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## APPLICATION OF INNOVATIVE ACTIVE INGREDIENTS IN TOPICAL ANTI-SCARRING AND SCARS REDUCING PRODUCTS AVAILABLE IN POLISH PHARMACEUTICAL MARKET

Paulina Malinowska<sup>1</sup>

### Abstract

In this work the analysis of Polish pharmaceutical market offer of topical anti-scarring and scar reducing products was conducted. The aim of work was to analyse of marketing communication used by producers and to estimate the kinds of physicochemical forms and active ingredients used in products. The analysis showed that the biggest group of topical anti-scarring products are medical devices and cosmetics, occurring mainly in form of gels. The most popular active ingredients turned out to be polysiloxanes in medical devices, and plant derivatives in cosmetics and medicines. Plant extracts, such as onion extract, chamomile, calendula, Asian pennywort, sea buckthorn, aloe, tamarind, red clover and paper mulberry also turned out to be very popular ingredients in anti-scarring cosmetics and medicines. Another commonly used actives are vitamin E, heparin, plant oils, essential oils, nacre and snail secretion filtrate. The analysis of marketing communication showed that the key cosmetic claims used differ depending on the legal status of products. The analysis confirmed that the claims concerning properties of cosmetics do not go beyond the definition of a cosmetic. In turn the claims concerning properties of medical devices and medicines in accordance with legal requirements say about preventing, treating or alleviating the scars.

### Keywords:

topical anti-scarring and scar reducing products, active ingredients, marketing communication, pharmaceutical market

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Technology and Instrumental Analysis; [paulina.malinowska@ue.poznan.pl](mailto:paulina.malinowska@ue.poznan.pl)

## Introduction

Scarring is a very common skin problem. Most skin injury types can contribute to scarring. This includes cuts, burns, acne scars, chickenpox scars, ear piercing, scratches, surgical cuts, and vaccination sites. Most skin scars are flat and leave a trace of the original injury that caused them. Hypertrophic scars are raised above the surrounding skin. Keloid scars have the similar appearance of hypertrophic scar but can grow outside of the original wound area. Atrophic scars take form of sunken recess in the skin, which has a pitted appearance. Scars result from the healing process of wound repair in the skin. Scar tissue is composed of the same protein (collagen) as the tissue that it replaces, however the fiber composition of the protein is different from the uniform and orderly orientation of the collagen fibers found in normal tissue. Collagen is overproduced or under-produced relative to the surrounding skin level. The color of scar tissue can vary from pink to the color of the person's skin or red to dark brown. Among all types of scars, hypertrophic scars and keloid scars as well as surgical scars are the most unsightly and difficult to treat (Juckett & Hartman-Adams, 2009; Wade & David, 2019).

There are many invasive and non-invasive scars treatment and prevention options available for consumers. Clinical treatment options for both hypertrophic and keloid scars include surgery, scar injected steroids, radiotherapy, cryotherapy and laser therapy. Non-invasive therapy includes silicone gels and creams, topical vitamin E creams (and other moisturizers), massage therapy, pressure therapy, topical steroids, counseling and polyurethane patches (Leventhal, Furr & Reiter, 2006). Moisturizing emollient and humectant creams and moisture-retentive dressings such as silicone sheets and fluid silicone gel have been shown to be beneficial for itching scars, and can also reduce the size and pain or discomfort associated with scars as well as improving their appearance. Studies have shown that, after wound healing, water still evaporates more rapidly through scar tissue and may take over a year to recover to pre-wound levels (Suetake, *et al.* 1996). Silicone products may help to prevent excessive scar formation by restoring the water barrier through occlusion and hydration of the stratum corneum and need to be used as soon as the wound/suture is healed (Mustoe, 2008).

There are numerous anti-scarring agents available over the counter, including silicone dressings, onion extract, and vitamin E based remedies, and also prescription drugs as corticosteroids, 5-fluorouracil, and bleomycin. There is a multitude of commonly used over the counter scar treatment products that have little evidence-based efficacy (O'Brien & Pandit, 2006; Shih, Waltzman & Evans, 2007). Among these, silicone dressings, onion extract, and vitamin E based remedies rank as the top selling products, despite lacking an evidence base (O'Brien & Pandit, 2006; Morganroth, Wilmot & Miller, 2009; Shih, Waltzman & Evans, 2007).

In this work the analysis of Polish pharmaceutical market offer of anti-scarring and scar reducing products was conducted. The topical products: cosmetics, medical devices and drugs available in the Polish pharmaceutical market were analyzed. The aim of work was to estimate the kinds of physicochemical forms and active ingredients used in products as well as to analyse of marketing communication used by producers.

## The methodology of analysis

The analysis of the pharmaceutical market offer of topical anti-scarring and scar reducing products was carried out in pharmacies in January-March 2021. The analysis proceeded in five stages:

Stage 1: Review the pharmaceutical market offer of leading brands of anti-scarring and scar reducing products available in the Polish market.

Stage 2: Division of anti-scarring and scar reducing products into 3 categories: cosmetics, medical devices and drugs.

Stage 3: Division of anti-scarring and scar reducing products into available physicochemical forms.

Stage 4: Carrying out a detailed analysis of the active ingredients of anti-scarring and scar reducing products based on their Latin and English names present in the INCI (the International Nomenclature of Cosmetic Ingredients) composition indicated on the packages.

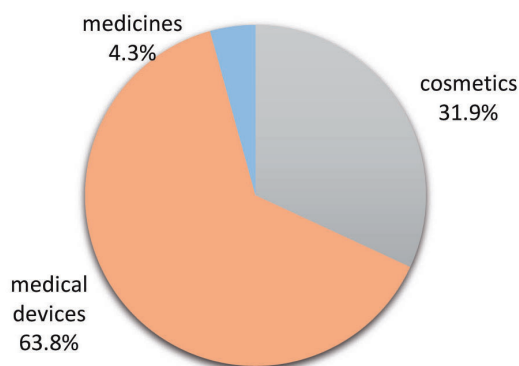
Stage 5: Analysis of main marketing communication concerning the cosmetic properties of topical anti-scarring and scar reducing products.

## Results and discussion

### The kinds of topical anti-scarring and scars reducing products available in Polish pharmaceutical market

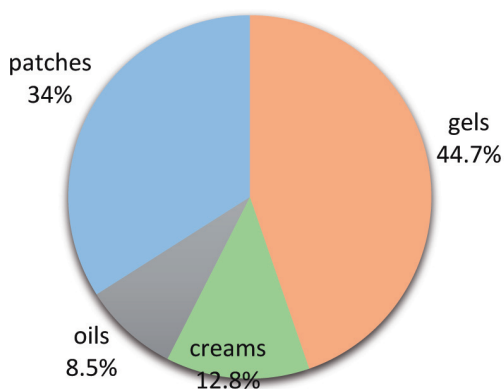
The analysed topical anti-scarring and scars reducing products were classified according to legal status of products (Fig. 1) into: medical devices, medicines, cosmetics, and physicochemical forms (Fig. 2) into: gels, creams, oils, patches.

The biggest group of products are medical devices (63.8%), occurring mainly in form of gels and patches, and cosmetics (31.9%), occurring in form of gels and creams. Gels are the biggest physicochemical forms of anti-scarring and scars reducing products (44.7%).



**Figure 1. Legal status of topical anti-scarring and scars reducing products**

Source: own study.



**Figure 2. Physicochemical forms of topical anti-scarring and scars reducing products**

Source: own study.

### Anti-scarring and scar reducing active ingredients

A detailed analysis of the chemical composition of anti-scarring and scars reducing products was carried out. Particular attention was paid to active ingredients that have a fundamental influence on the caring properties of the products. 64 active ingredients were identified in products. The content of active ingredients in the analysed products ranges from one to several. The most popular active ingredients turned out to be polysiloxanes especially in medical devices (mainly in patches), and plant derivatives in cosmetics and medicines.

Silicone-based products are widely and readily available, relatively inexpensive and have long been used for hypertrophic scar prophylaxis treatment. Silicone gels contain polysiloxanes – long chain silicone polymer which cross link with

silicone dioxide. It spreads as an ultrathin sheet and works 24 hours per day (Sawada & Sone, 1990). Silicone gel increases hydration of stratum corneum and thereby facilitates regulation of fibroblast production and reduction in collagen production. It results into softer and flatter scar. Silicone gel protects the scarred tissue from bacterial invasion and prevents bacteria-induced excessive collagen production in the scar tissue. It reduces itching and discomfort associated with scars (Choi, *et al.* 2015; Poston, 2000; Suetake, *et al.* 2000).

Plant extracts, such as onion extract, chamomile, calendula, Asian pennywort, sea buckthorn, aloe, tamarind, red clover and paper mulberry turned out to be very popular ingredients in anti-scarring cosmetics and medicines. Ye, *et al.* (2015) showed in their studies that a number of plants with medical properties have been studied for their effectiveness in the prevention of scarring due to content of some compounds such as quercetin, resveratrol, oleanolic acid, epigallocatechin gallate, curcumin, and many others. Quercetin has been shown *in vitro* to reduce proliferation in fibroblasts derived from keloid scars and alter intracellular signalling pathways and collagen synthesis (Long, Zeng & Zhang, 2006; Phan, *et al.* 2004; Phan, *et al.* 2005). Resveratrol has been shown to reduce fibroblast cell proliferation through cell cycle arrest in fibroblasts derived from hypertrophic scars and normal skin fibroblasts and induce apoptosis (Zeng, *et al.* 2013). Oleanolic acid is a naturally occurring triterpenoid compound with a number of biological properties including anti-inflammatory and anti-tumour effects (Chakravarti, *et al.* 2012; Yang, *et al.* 2012). It was found to significantly inhibit hypertrophic scarring (Wei, *et al.* 2011). Epigallocatechin gallate (EGCG) has been demonstrated to improve re-epithelisation in a chronic wound model and the structural stability of collagen was shown to be enhanced with EGCG (Goo, *et al.* 2003; Kim, *et al.* 2008). Curcumin has been shown in a rat wound healing model to increase contraction and reduce wound healing time (Panchatcharam, *et al.* 2006). *In vitro* and *in vivo* studies have shown that a number of 'natural' therapeutic agent and strategies may play a role in the future treatment of scarring, particularly hypertrophic scarring (Mehta, Branford & Rolfe, 2016).

The most popular plant extract turned out to be an onion extract. There are *in vitro* studies suggesting that onion extracts may accelerate wound healing by exerting a number of effects on mast cells and fibroblasts in the inflammatory cascade, and by decreasing inflammation (Pawlikowska-Pawlega & Gawron, 1995; Ross, *et al.* 2001). A topical gel containing onion extract and allantoin has been available for more than 60 years treating, preventing, and reducing dermatologic scars and keloids (Willital & Heine, 1994). Onion extract has anti-inflammatory, anti-microbial, anti-proliferative, and regenerative activities (Sidgwick, McGeorge & Bayat, 2015), while allantoin shows keratolytic, hydrating, epithelizing, and anti-irritant activities (Araujo, *et al.* 2010). Several clinical trials have confirmed that gel with onion extract and allantoin is well tolerated and helps prevent pathological



scarring and improves preexisting scars (Aysan, *et al.* 2010; Beuth, *et al.* 2006; Ho, *et al.* 2006; Hosnuter, *et al.* 2007; Khadziiski, Diakov & Petrova, 2001; Willital & Simon, 2013).

Vitamin E is also commonly used in commercially available anti-scarring products. Some reports had claimed that its topical application might accelerate wound healing and enhance the cosmetic outcome of postsurgical scars via its antioxidant properties. More recently, small clinical studies reported positive data when vitamin E was used alone or in combination presurgery, postsurgery (Perez, *et al.* 2010; Zampieri, *et al.* 2010) and in systemic sclerosis related digital ulcers (Fiori, *et al.* 2009).

Heparin is also commonly used in commercially available anti-scarring products. It has inhibitor effect on fibroblast proliferation, it increases tissue hydration as well as reducing induration and irritation. Heparin accelerates wound healing (Arikana, *et al.* 2005). It has been determined that complex: heparin, allantoin and onion extract cause inhibition on human fibroblast cultures (scar, keloid) depending upon its antiproliferative effect, reduces synthesis of collagen and proteoglycans and prevents polymerization of collagen (Beuth, *et al.* 2006; Saliba, 2001).

Plant oils such as almond oil, avocado oil, borage oil, camelia oil, jojoba oil, macadamia oil, meadowfoam oil, olive oil, safflower oil, shea butter, soya oil, sunflower oil, wheat oil are also commonly used especially in anti-scarring body oils. Due to fatty acids content, plant oils are an excellent basis for cosmetics. They firm and moisturise the skin and make it soften. The test of products containing plant oils showed that they provide a long-lasting, soft and supple skin feeling, caring effect and improve the skin appearance and that scars appear less pronounced (Bielfeldt, *et al.* 2018).

Essential oils such as chamaemelum nobile oil, lavender oil, rosy oil, rosemary oil are also commonly used especially in anti-scarring body oils. Essential oils are substances comprised of volatile and fat-soluble secondary metabolites, which can be extracted from different parts of the plant. Secondary metabolites present in essential oils can have biological activity on the healing mechanism by decreasing the retraction time and helping in wound healing. Essential oils give promising results in skin wound healing, by influencing the mechanisms involved in the inflammatory, proliferative and remodeling phases (Gushiken, *et al.* 2016).

Nacre and snail secretion filtrate are also used in anti-scarring and scars reducing products. The data support the use of low concentrations of nacre in aesthetic formulations, with the potential for high concentrations to cause changes in skin and scar cells which may have impact on efficacy. It has beneficial effects on the skin with enhanced wound healing (Agarwal, *et al.* 2014). Snail secretion filtrate gives a positive reaction to test for protein contents, comprising amino acids and proteins which play role in cell regeneration and growth. The animal protein

content of snail slime has a high biological value in wound healing and in the inhibition of inflammatory process (Perez, Dina & Iwang, 2012).

The analysis of the chemical composition showed that active ingredients are precisely selected and correspond to the actual needs of the target group of users for whom these products are designed. The products contain above-mentioned ingredients and show anti-scarring and scar reducing effects. Depending on the brand, the content of active ingredients ranges from one to several.

## **Marketing communication of anti-scarring and scars reducing topical products**

The analysis of marketing communication of cosmetic properties of anti-scarring and scar reducing topical products showed that the key cosmetic claims used by producers differ depending on the legal status of topical anti-scarring and scars reducing products. It means that cosmetics have different communication (claims) than medical devices and medicines. It is related to the law, as delimitation between cosmetic products and other product categories is important. The presentation of the product (including all communication mediums) and the manufacturer's intended purpose should ensure that the cosmetic product falls within the definition from Regulation (EC) No 1223/2009 on cosmetic products. A cosmetic is used for e.g. cleansing the skin, changing its appearance and keeping it in good condition. In turn, medical devices and medicines can restore, correct or modify physiological functions of the skin.

The most popular declarations of cosmetics are:

brightens, softens and smooths the scars,

- makes the skin more elastic and bright,
- improves the appearance of scars,
- restores flexibility,
- smooths the scars,
- supports the reduction of the scars,
- accelerates the regeneration of the damaged skin structure,
- maintains proper skin moisture,
- soothes the feeling of itching,
- regenerates the skin and improves its appearance.

The most popular declarations of medical devices and medicines are:

- effective in the treatment of burn, traumatic and surgical scars,
- stimulates the formation of connective tissue,
- counteracts non-physiological scar growth,
- makes the scar more flexible,

- improves the appearance and elasticity of scars,
- prevents scarring,
- prevents the formation of hypertrophic scars,
- prevents transepidermal water loss,
- softens, flattens and smoothes scars,
- reduces discolorations, itching and pain,
- improves hydration,
- improves blood supply to the tissue,
- soothes irritations,
- regenerates the tissue,
- has anti-inflammatory properties,
- has bactericidal properties.

With reference to claims and active ingredients, cosmetics manufacturers are required to meet the expected regulatory conditions. The analysis confirmed that the claims concerning properties of cosmetics do not go beyond the definition of a cosmetic.

## Conclusion

Scarring following surgery or trauma is difficult to predict, and both physicians and their patients are highly concerned with minimizing scar appearance and value even small improvements in scarring as clinically meaningful. Next to specific surgical techniques and appropriate general aftercare of fresh wounds, a multitude of scar gels, creams, patches, and ointments are available on the Polish pharmaceutical market and are being promoted for scarless wound caring (cosmetics) and wound healing (medicines, medical devices). Next to well-known silicone-based products, many plant extracts, vegetable and essential oils as well as vitamin E, heparin, nacre and snail secretion filtrate have been highlighted as potential anti-scarring ingredients. There are many reports on the positive effect of these ingredients on the appearance of scars, but there are also those in which their beneficial effects have not been proven.

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## EVALUATION OF SELECTED FUNCTIONAL PROPERTIES OF COSMETIC PREPARATIONS WITH THE ADDITION OF MORINGA, SPIRULINA AND CHLORELLA EXTRACTS

Martyna Pietrzak, Katarzyna Michocka<sup>1</sup>

### Abstract

Increasingly popular natural cosmetics, which consumers reach for, are preparations with the addition of algae, among others. with the addition of spirulina and chlorella. The high content of vitamins and micro- and macroelements gives green algae nutritional properties, which is used not only in cosmetics, but also in dietary supplements, as well as moringa, which is also included in the superfood, a cosmetic discovery of recent years. The task was to compare selected application properties of self-made creams and shampoos with the addition of extracts from: moringa, spirulina and chlorella. The assessment was made by analyzing the organoleptic properties of the preparations, sensory tests of the emulsions, measuring the hydration of the epidermis with the use of SkinAnalyzer and the application properties of shampoos. The research material used were creams and shampoos prepared in the laboratory with the use of powdered products – plant extracts of moringa, chlorella and spirulina. We have prepared three emulsions and three shampoos with selected additives. A selected group of consumers participated in the study.

### Keywords:

moringa, spirulina, chlorella, cosmetic emulsions, shampoos, functional properties

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of of Technology and Instrumental Analysis, Poland; [katarzyna.michocka@ue.poznan.pl](mailto:katarzyna.michocka@ue.poznan.pl)

## Introduction

Food products contain many cosmetically valuable substances, and what is more, they can also be used internally, providing consumers with the necessary nutrients, vitamins and microelements. Raw materials that are used in food products are increasingly used on the cosmetics market, creating an environmentally friendly trend in cosmetics. A modern consumer of cosmetics has a highly developed ecological awareness, and therefore consciously seeks an active and effective natural cosmetic. The use of plant extracts in cosmetic formulations gives the possibility of obtaining much better effects than the application of single biologically active compounds. For the production of natural cosmetics, mainly selected plant materials are used, rich in active ingredients such as: vitamins, micro- and macroelements, proteins, amino acids, carbohydrates, phospholipids, natural antioxidants and preservatives.

Manufacturers are looking for new ingredients in cosmetic preparations among exotic additives. A perfect example are the algae or the moringa tree, from which mainly seed oil is obtained, not forgetting the leaves, which contain a huge amount of nutrients. As in the case of alg-spirulina and chlorella, moringa contains a large amount of chlorophyll, which has a beneficial effect on the human body. Despite the high content of active ingredients, these plants are more often used as dietary supplements than as cosmetic products. Producers on the cosmetics market offer a small amount of cosmetics with the addition of the above-mentioned plants, and their prices vary from a few to even several hundred zlotys.

## “Green” cosmetic raw materials

Consumers buy cosmetics of natural origin, mainly due to fashion, trend and lifestyle. Therefore, there are more and more products on the market containing, for example, herbs, algae and many other plant materials that perfectly care for the appearance of the skin, smooth out wrinkles, brighten and moisturize the skin and strengthen nails and hair. They also help fight skin diseases. Due to these properties, plant raw materials are essential in the recipes of natural cosmetic products.

The growing interest in healthy and natural cosmetics is a perfect complement to the concept of “naturalness” of human life. Natural raw materials are usually environmentally friendly, have a gentle effect on the skin, and what’s more, they stimulate the natural functions of skin renewal, nourish, beautify, nourish and protect (Ariede, *et al.* 2017, Kiełtyka-Dadasiewicz, 2016; Priyadarshani & Rath, 2012).



Natural cosmetics, which are increasingly used by consumers, are cosmetics with the addition of algae, among others with the addition of spirulina and chlorella. Products with the addition of algae on the cosmetics market are becoming more and more common. They contain substances that have a beneficial effect on the skin and the entire human body. They have large amounts of plant dyes with antioxidant properties, which is why they are often used in anti-aging preparations and cosmetics for the care of mature skin. They are ingredients that support slimming and cellulite removal. The high content of vitamins and micro- and macroelements gives green algae nutritional properties, which is used not only in cosmetics, but also in dietary supplements, similarly to the superfood moringa, a cosmetic discovery of recent years.

## Moringa

*Moringa oleifera* (*Moringa oleifera*) is a medium-sized tree belonging to the Moringaceae family, which includes a total of 13 species. It is called the “tree of life” or “the miracle tree” due to its numerous pro-health properties. Moringa is also referred to as horseradish tree because its roots have a taste similar to horseradish. This plant comes from the areas of Pakistan, Bangladesh, Afghanistan and India, which are its main producer (Grdeń, Kulczyński, Gramza-Michałowska, 2017; Munirat, *et al.* 2016).

Moringa fruits are low in calories and contain: dietary fiber, magnesium, potassium and vitamin C. The seeds are rich in protein, fat, calcium, iron, phosphorus and vitamin E. Moringa leaves, in turn, provide primarily calcium, potassium, phosphorus, vitamin A and C. Studies in an animal model show high bioavailability of folate in leaves. The high nutritional value of the leaves is due to the presence of many bioactive compounds. Moringa leaves are also a source of flavonoids and carotenoids (Piejko, 2016). Moringa chlorofil – also contains a green dye, which supports the processes of purification of the body – to form a strong connection with toxic, limiting their reach tissue and facilitates excretion (Mietkowska, 2018; Munirat, *et al.* 2016). Most frequently occurring amino acids are: leucine, lysine, glycine, valine, alanine, arginine, glutamic acid and aspartic acid. Moringa leaves also contain omega-3 and omega-6 fatty acids. The dominant acids are  $\alpha$ -linolenic acid and linoleic acid. Among the saturated fatty acids, palmitic acid is mentioned first of all. The seeds contain slightly different fatty acids, i.e. oleic, palmitoleic, stearic and arachidonic (Grdeń, *et al.* 2017).

In medicine, *in vivo* and *in vitro* tests confirm the hypolipemic, antiplatelet, hypotensive and hypoglycemic effects. Moreover, the described plant exhibits anti-inflammatory properties, contributing to the inhibition of the production of pro-inflammatory cytokines. On the other hand, microbiological studies indicate

that the extracts obtained from this plant inhibit the development of pathogenic bacteria and fungi (Piejko, 2016). It contains 46 antioxidants, which contributes to increasing protection against infections, cancer and pollution of the body. Powdered moringa leaves affect the metabolism of proteins and increase the sensitivity of tissues to insulin, while the high content of methionine has a beneficial effect on metabolism and fat accumulation in the liver (Mietkowska, 2018, Munirat, *et al.* 2016).

Moringa oil has antioxidant, smoothing and softening properties. It is also used as a base preparation for face and body massage. Moringa oil illuminates the skin and prevents it from sagging (Piejko, 2016). Moringa perfectly cleanses the skin, removes excess sebum and make-up residues from its surface, and prevents the formation of blackheads. It is a component of deeply moisturizing cosmetics, smoothing wrinkles, lightening discoloration, regenerating the epidermis and preventing premature skin aging. It removes the effects of fatigue and is an excellent product for irritated, dry and acne-prone skin ([www.olej-moringa.pl](http://www.olej-moringa.pl)).

Plant extracts show antiseptic and anti-inflammatory properties – they facilitate the healing of the skin after mechanical abrasions, burns, insect bites), cosmetic and dermatological treatments (chemical peels, deep exfoliation, retinoid treatment). They soothe and accelerate the healing of the epidermis also in the case of sun damage (Piejko, 2016). Seed oil works very well in the care of dry, damaged and weak hair, because it contains nutrients, vitamins and minerals. It facilitates detangling, styling and also helps in the fight against dandruff. What is more, moringa strengthens the hair structure and reduces the hair's susceptibility to chemical and mechanical damage ([bliss.natemat.pl](http://bliss.natemat.pl)). It can also be used as a serum for damaged hair ends, preventing them from splitting and breaking (Munirat, *et al.* 2016; Piejko, 2016). Moringa is a plant that is very often used in cosmetic products. Even in chain drugstores, you can find various types of cosmetics with the addition of this extract. The most widely used oil is moringa, which is mainly added to hair care products. There are many moringa shampoos and masks on the cosmetic market. These products usually do not exceed PLN 90.

## Spirulina

Spirulina is a simple, unicellular micro-algae, whose name comes from the word spiral, because that just has the shape of this alga observed under a microscope. It is a trade name for cyanobacteria belonging to the order of Oscillatoriales. It occurs naturally in lakes Chad in Africa and Texcoco in Mexico ([www.bio-med.pl/Spirulina.pdf](http://www.bio-med.pl/Spirulina.pdf)). The biggest commercial cultivation of spirulina are located in the United States, Thailand, India, Taiwan, China, Pakistan, Burma, Greece and Chile (Jękot, Rzewińska, Hałaszuk, 2016).

Spirulina is very rich, in proteins about 60–70% of dry weight, which contain essential amino acids, such as leucine, isoleucine, valine, lysine, methionine, threonine, tryptophan and phenylalanine. This algae is therefore a source of wholesome protein that contains all the amino acids necessary to maintain nitrogen balance in adults (Bogdański, *et al.* 2013; Ragusa, *et al.* 2021). About 5–8% of the dry matter consists of fats, of which 40% are glycolipids. There are many types of carbohydrates in the composition of spirulina, some of which are responsible for stimulating the immune system and supporting DNA repair. The human digestible polysaccharides are glucosamine, glycogen and rhamnose (a natural plant polysaccharide). These are carbohydrates that do not make you hypoglycemic. Additionally, spirulina is an excellent source of many vitamins and minerals. Among them, B vitamins and  $\beta$ -carotene should be mentioned (Bogdański, *et al.* 2013; Nege, Masithah & Khotib, 2020). Algae contains vitamins such as: C, E, PP (niacin) and B vitamins (B1, B2, B6 and B12). It is also a rich source of minerals: P, Fe, Ca, K, Na, Mg. Spirulina contains dyes valuable for the body: chlorophyll and phycocyanine – it is a bioactive, blue dye belonging to phycobylins, which is mainly responsible for its ability to neutralize free radicals, and is also needed for the proper functioning of the liver and digesting amino acids (Jękot, *et al.* 2016; Nege, Masithah & Khotib, 2020; Ragusa, *et al.* 2021).

Spirulina affects the carbohydrate and lipid metabolism of the body – it can lower the concentration of total cholesterol, LDL cholesterol and triglycerides. It affects the reduction of blood glucose and blood pressure. What's more, it changes the antioxidant status and improves the body's efficiency. This alga increases the production of red tissues, which is why it is recommended for people suffering from anemia (Bogdański, *et al.* 2013; Ragusa, *et al.* 2021). The high content of vitamin B12 has a positive effect on the proper functioning of the nervous tissue, and vitamin E reduces the risk of cancer (Bieżyński, Krajewski & Zdrojewicz 2018; Nege, Masithah & Khotib, 2020).

Spirulina is used in the production of cosmetics, nutritional supplements, it provides food for organisms living in water, enriches water reservoirs with oxygen and regulates access to sunlight. As a dietary supplement, it is characterized by high bioavailability at the level of 85–95% (Kępska, Olejnik, 2014). It is also used in sanitary products, paper towels, plasters, blotting papers and in pharmaceutical products, e.g. for abrasions of the epidermis, scratches, cuts of eczema. Alga is a source of proteins in moisturizing and soothing cosmetics (Jękot, *et al.* 2016). It is one of the most popular additives to cleansing masks because it has an enzymatic and nourishing effect. The purpose of its application is to nourish the skin and prevent severe irritation (Barabasz-Krasny, *et al.* 2016). What is more, it has a strong cleansing effect. The same properties will have a tonic with algae or a peeling, which not only removes dead epidermal cells, but also exhibits the delicate properties of enzymatic peeling, which allows you to remove other

biological impurities that cannot be removed mechanically. It can be used for bathing, because it prevents fungal diseases and contains a large amount of minerals, which allows you to remove the feeling of heavy legs and restores the balance of tired skin ([www.spirulina.pl](http://www.spirulina.pl)). Spirulina reduces damage caused by UV radiation, inhibits the activity of matrix metalloproteinases, and also contributes to the increase in collagen and elastin expression in fibroblasts. It has properties that regulate the work of sebaceous glands, improves the lipid barrier of the skin, so it is perfect for people with dry skin. It improves skin tone, mineralizes, regenerates and nourishes it. It has anti-aging, cleansing, anti-inflammatory properties and accelerates wound healing (Kucia, 2017).

The plant pigments it contains – phycoerythrin, phycocyanin and chlorophyll – protect against the harmful effects of UV radiation on the skin, thus significantly delaying the aging process (Bieżyński, *et al.* 2018).

Spirulina increases the activity of the immune system and has antiviral properties. It has a beneficial effect on sugar levels, in the treatment of cardiovascular diseases by improving lipid levels and reducing blood pressure (Ekiert, Dochniak, 2015). It reduces the negative impact of the environment, protects the gastric mucosa and supports the detoxification of the body (Pielesz, 2010).

## Chlorella

Chlorella is a single-celled algae that belongs to the plant kingdom (eukaryotes). The name of the alga comes from the green pigment chlorophyll, which enables the photosynthesis process to be carried out. *Chlorella vulgaris* is the most abundant species. The largest producers of biomass from Chlorella algae are Germany, Japan and Taiwan (Silva, *et al.* 2019; Siti, Novi & Oktarina 2021; Starmach, 1989). Chlorella is green in color and spherical in shape. It contains one large, cup-shaped chloroplast. Algae reproduction occurs by dividing the cell into autospores. It does not form a colony, and its size ranges from 2 to 12 microns and contains over 70 substances of high biological importance for the human body. What is more, it is so small that we can only see it under a microscope. As previously mentioned, its green color is due to a large amount of chlorophyll, which is the most abundant of all algae (Silva, *et al.* 2019; Siti, Novi & Oktarina 2021; Bieżyński, *et al.* 2018).

Chlorella contains a growth factor – called Chlorella Growth Factor. It is produced in the process of photosynthesis and is obtained from the nucleus of the algae. It consists of valuable substances that support the natural growth of cells. Moreover, it protects the genetic material of human cells, accelerating repair processes, and thus increasing the cells' ability to self-clean and self-repair (PurellaFood\_Top10\_list.pdf).

Chlorella is a source of bioactive ingredients. It is rich in amino acids, nucleic acids, protein, fats, carbohydrates, fiber, sugars, vitamins – vitamin A, B vitamins,

vitamins C, E and K, as well as minerals – zinc, phosphorus, iodine, magnesium, manganese, potassium, selenium, sodium, calcium and iron ([www.chlorella.pl](http://www.chlorella.pl)). This algae is a source of fats in the form of glycolipids, phospholipids and free fatty acids. In addition, it contains oleic acid, palmitic acid and linolenic acid, which are a component of the skin's lipid coat. Fiber is found in the cell walls of the algae. It contains pigments such as  $\beta$ -carotene, astaxanthin, canthaxanthin and lutein (Pinkowska, Posz, Silva, *et al.* 2019; Siti, Novi & Oktarina 2021; Stebel, 2016). This single-celled organism is also famous for its very high content of chlorophyll. It delays the processes of cellular oxidation, has anti-aging properties, eliminates free radicals, accelerates wound healing, protects against harmful radiation and reduces unpleasant odors (Czepak, *et al.* 2009). It can multiply very quickly – up to four times a day. This affects the growth of probiotic bacteria in the intestines after consuming algae. Accelerate the regeneration of the intestinal epithelium, the nervous system and the brain, and also increases the production of DNA and RNA, by protecting our genetic code and has anti-aging properties (Wierzbicka, 2016).

Chlorella is a plant that has health-promoting properties and is widely used in medicine. It lowers high blood pressure, cholesterol and glucose levels in people suffering from diabetes. It works, inter alia, immunostimulating against viruses, bacteria and cancer. This algae contains glycoproteins that have anti-inflammatory and antibiotic properties, activate immunological processes, lower blood pressure and cholesterol levels, and reduce the negative effects of excess ultraviolet radiation (Czepak, *et al.* 2009; Silva, *et al.* 2019; Siti, Novi & Oktarina 2021). The rich composition of chlorella makes it widely used in cosmetics that have regenerating, rejuvenating and firming effects. As already mentioned, chlorella contains vitamins E and C, valuable for the body, which protect the skin against free radicals and stimulate metabolic processes occurring in it, e.g. collagen synthesis, strengthen the walls of blood vessels, and increase the skin's resistance to external factors. Thanks to the content of vitamins such as B1, B2, B6 and B12, it is used for the care of seborrheic complexions (PurellaFood\_Top10\_list.pdf). This alga is a precursor of vitamin A, which is involved, among others, in the regulation of the processes of cell renewal and exfoliation of the epidermis and collagen synthesis. It also contributes to smoothing and improving skin elasticity and reducing fine lines (Pinkowska, 2016). The composition of algae also includes zinc, calcium, iron, magnesium and silicon, which improve the condition of the skin, hair and nails (PurellaFood\_Top10\_list.pdf). Alga is used in moisturizing preparations, gel eye foundations, cosmetics for make-up removal and anti-wrinkle creams. It also acts as an antioxidant and improves the condition of the skin (Pinkowska, 2016).

Chlorella is also a source of biomass in many industries, which is used as a renewable resource. This algae can assimilate carbon dioxide from air polluted

with exhaust fumes and act as “bio-fertilizers” (by binding atmospheric nitrogen) or feed additives. In addition, it is applicable to the bioremediation of the aquatic environment, thanks to the easy absorption of phosphorus and nitrogen compounds from municipal and industrial wastewater. Algae thus contribute to the protection of open reservoirs against the eutrophication process. Their natural biosorptive properties can also be used to remove toxic metal ions (Makowska, 2017). Of all the extracts described above, chlorella has the least use in cosmetics. It mainly comes in pill or powder form as a dietary supplement. Unlike spirulina, it is more applicable in face creams.

## Materials and methods

The aim of the study was to compare selected application properties of self-made creams and shampoos with the addition of extracts from moringa, spirulina and chlorella.

The scope of the research included:

- evaluation of organoleptic properties of preparations (appearance, color, smell, consistency),
- sensory analysis of emulsions,
- measurement of epidermal hydration using the SkinAnalyzer.
- assessment of application properties of shampoos

The research material used were creams and shampoos prepared in the laboratory with the use of powdered products – plant extracts of moringa, chlorella and spirulina. 3 creams (Table 1 composition) and 3 shampoos (Table 2 composition) with selected additives (moringa product number I, spirulina – II and chlorella-III) were prepared.

## Research material

In order to prepare the cream, the fat phase containing the appropriate amount of eucerin was prepared. The composition of the water phase consisted of appropriate amounts of glycerin, water and panthenol. Moringa, Chlorella, and Spirulina powder was added to the water, respectively. The two phases were then placed separately in a water bath and heated to 85°C while stirring the baguette. After dissolution, the fat phase was added to the aqueous phase and mashed until homogeneous.

For the preparation of shampoos with the appropriate powdered extracts of moringa, chlorella and spirulina, the surfactants Sulfurocanol L255/Ib and

Rokamid KAD were added to the water. The whole was heated in a water bath to the temperature of 85°C, while stirring gently with a baguette. Then glycerin and powdered extract of moringa, chlorella and spirulina, respectively, were added. The entire content was mixed until a homogeneous consistency was obtained. The solution was thickened gradually by adding sodium chloride to it – after each portion, the solution was thoroughly mixed with a baguette until the appropriate viscosity was obtained.

**Table 1. Composition of prepared cosmetic emulsions**

Ingredients (Name, INCI, Company)	Composition [%]
Eucerin (petrolatum – 95%, cetyl alcohol – 3%, cholesterol – 2%); INCI: Petrolatum, Cholesterol, Cetearyl Alcohol; www.zrobsobiekrem.pl	46.00
Glycerine; INCI: Glycerin; www.ecospa.pl	3.00
Water; INCI: Aqua	49.75
Panthenol (75% solution) INCI: Panthenol; www.ecospa.pl	1.00
Extract respectively from: Moringa (INCI: Hydrolyzed Moringa Oleifera Seed Extract-powder) company Astron; Chlorella (INCI: Chlorella Vulgaris Powder); company MyVita, Spirulina (INCI: Spirulina Platensis Powder); company Aliness	0.25

**Table 2. Composition of prepared shampoos**

Ingredients (Name, INCI, Company)	Composition [%]
Sulforocanol L225 / I Texapon NSO (27% SLES) (INCI: Sodium Laureth Sulfate); <a href="https://www.products.pcc.eu/pl/">https://www.products.pcc.eu/pl/</a>	40.0
Rokamid KAD (INCI: Coconut Fatty Acid Diethanolamide); <a href="https://www.products.pcc.eu/pl/">https://www.products.pcc.eu/pl/</a>	3.00
Glycerine (INCI: Glycerin) www.ecospa.pl	2.00
Sodium chloride	2.50
Extract respectively from: Moringa (INCI: Hydrolyzed Moringa Oleifera Seed Extract-powder) company Astron, Chlorella (INCI: Chlorella Vulgaris Powder) company MyVita, Spirulina (INCI: Spirulina Platensis Powder) company Aliness	0.25
Water	55.00

## Results and discussion

### Organoleptic evaluation of creams

Table 3 presents the results of the organoleptic characteristics of the tested creams. Nine women aged 25–54 and two men aged 28 and 60 participated in the study. All persons carrying out the assessment of appearance, color and odor had dry skin.

**Table 3. Organoleptic characteristics of the tested creams**

Characteristic	I. Cream with the addition of moringa	II. Cream with the addition of spirulina	III. Cream with the addition of chlorella
<b>Appearance</b>	Homogeneous mass without lumps and bubbles, visible powdered moringa leaves, heavy consistency	Homogeneous mass without lumps and bubbles, visible spirulina extract	Homogeneous mass without lumps and bubbles, no impurities
<b>Color</b>	Light green	Light green	Green
<b>Fragrance</b>	Absence	Absence	Absence

Source: own study.

Comparing all three creams, it can be noticed that they differ in both color and appearance. In the case of the moringa cream and the chlorella cream, the mass is uniform, but there is an extract that has not completely dissolved during mixing. These creams are light green in color, with the difference that the moringa cream has a warm shade of green, and with the addition of chlorella – cool. The common feature of all the emulsions is that they are odorless. The color of the cream with spirulina is much darker than the rest of the preparations. It is also noticeable that the cream is smoother than cream with the addition of moringa and cream II with the addition of spirulina.

The testers highly rated the level of skin lubrication after applying the creams, assigning from 4.6 to 4.8 points out of five possible. Good lubricating effect is also confirmed by the results of the SkinAnalyzer device – model SK-02, reaching the fifth degree of lubrication in the tested creams. The moisturizing effect of the epidermis was found in all three emulsions, however also in this category the product with spirulina achieved the best results, reaching the 4th and 5th degree of hydration. In most of the surveyed people, after using the emulsion with spirulina, the skin changed its type from dry to normal.



## Organoleptic evaluation of shampoos

Table 4 shows the results of the organoleptic assessment performed by five people, including three women and two men. Characteristic features of shampoos were examined: appearance, color, smell and consistency. Additionally, a pH measurement was performed, the value of which was 5.5 in all tests.

Based on the organoleptic evaluation, it was found that the shampoos with the addition of algae: spirulina and chlorella contain a sediment that did not dissolve during the preparation of the shampoos. The prepared products differed in both color and smell. Moringa shampoo has a light color compared to shampoos No. I and No. III, which are dark in color. In the case of fragrance, only the shampoo with spirulina had an unpleasant odor, while the rest of the shampoos were odorless. None of the products contained visible impurities. The testers agreed that when applied, shampoos with moringa, chlorella and spirulina, in contact with water, produce long-lasting foam, and the effect after washing the hair is satisfactory.

**Table 4. Organoleptic characteristics of the tested shampoos**

Characteristic	I. Shampoo with the addition of moringa	II. Shampoo with the addition of spirulina	III. Shampoo with the addition of chlorella
<b>Appearance</b>	Homogeneous mixture, clear, without sediment, visible powdered moringa leaves, no impurities,	Homogeneous mixture, visible sediment – spirulina extract, no impurities,	Homogeneous mixture, visible precipitate – Chlorella extract, no impurities
<b>Color</b>	Light green	Dark green	Green
<b>Fragrance</b>	Absence	Absence	Unpleasant
<b>Consistency</b>	Medium	Medium	Medium

Source: own study.

## Sensory analysis of creams

In the sensory evaluation of the creams, the ten most important features of the emulsions were taken into account, which were given points on a scale from 1 to 5, where 1 is the worst and 5 is the best. Nine women aged 25–54 and two men aged 28 and 60 participated in the evaluation. Based on the collected data, a sensory profile was created for each of the products. The results presented in Table 5 are the arithmetic mean of the grades given by the people participating in the study.

The parameters of the creams were assessed very similarly. Summing up all the evaluation criteria, the cream with the addition of chlorella obtained the highest

number of points with a result of 39.8 points. Creams with moringa and chlorella fared slightly worse, reaching 37.8 and 36 points.

**Table 5. Sensory evaluation of the analyzed creams (average results)**

Characteristic	I. Cream with the addition of moringa	II. Cream with the addition of spirulina	III. Cream with the addition of chlorella
Pillow effect	3.2 ±0.01	2.8 ±0.03	4.0 ±0.01
Uniformity	3.8 ±0.06	4.2 ±0.06	5.0 ±0.06
Consistency	4.2 ±0.06	3.4 ±0.06	4.0 ±0.10
Adhesion	4.6 ±0.06	4.0 ±0.00	4.8 ±0.06
Distribution	4.2 ±0.06	4.2 ±0.00	4.8 ±0.00
Viscosity	4.4 ±0.06	3.8 ±0.05	3.8 ±0.06
Greasiness and greasing	4.8 ±0.06	5.0 ±0.10	4.8 ±0.06
Absorption	3.8 ±0.06	3.6 ±0.05	3.6 ±0.10
Smoothing	4.8 ±0.015	5.0 ±0.10	5.0 ±0.12
<b>Total</b>	<b>37.8</b>	<b>36.0</b>	<b>39.8</b>

Source: own study.

### Measurement of the degree of hydration and lubrication of the epidermis with the SkinAnalyzer device

Five people participated in the study, including three women aged 25, 37 and 54 and two men aged 28 and 60. In the first stage, before the application of the cream, the SkinAnalyzer model SK-02 FURE was applied to the skin of the forearm to check the degree of hydration and lubrication of the skin, as well as its type. In the second stage, the cream was spread with a fingertip, then the device was re-applied and the results were read after 15 minutes. The results of the skin hydration level are read based on the number of displayed columns. Results below the third column are dry skin (water percentage less than 33). However, the result between the 4th and 6th column means normal skin (33–39% water). Scores greater than 7 (> 39%) columns indicate moisturized skin. The results of the degree of skin lubrication are also read on the basis of the number of displayed columns. The result below the second column is dry skin. However, the result between the 3rd and 8th columns is normal skin. Results above column 9 are oily skin.

After applying three creams, the results confirm their moisturizing effect. Cream with spirulina had the highest values (4 and 5) degree of hydration, while cream

with chlorella and moringa had slightly lower values (3–5). When comparing with the results before applying the emulsion, each of those who tested the formulation could see an increase of 2–3 units after testing the creams. In the case of the second parameter, which is the level of epidermis lubrication, in all people after applying three creams: moringa, chlorella and spirulina, the results indicate the fifth level of lubrication, i.e. achieving a normal skin type. Measurements of the level of epidermis hydration before the application of the creams showed that all the people who participated in the study had a dry skin type and the level of hydration was less than 33%. In the case of the measurement of epidermal lubrication, the value of the examined persons was 1, which also means that the skin is dry. The analysis with the SkinAnalyzer device showed that the skin requires care in the studied women and men.

### Evaluation of the properties of shampoos

The aim of the research was to analyze the functional properties of shampoos with the addition of three extracts of moringa, chlorella and spirulina, and to evaluate their effectiveness. The consumer opinion survey was conducted in the form of a consumer test (survey attached). 12 women aged 25–55 and three men aged 25, 28 and 60 participated in the study. Each person tested all three shampoos identically during the hair washing steps. People participating in the study answered the questions included in the questionnaire by marking the squares with the words “yes” or “no” and evaluating the shampoo using an adjective scale, consisting in determining a given criterion for assessing the shampoo with the words, in this case: “very good”, “average” “And” weak “. Each shampoo received a separate survey with the same questions. The questions asked were designed to find out what consumers think about the characteristics (such as foamability, spreadability, softness, dry hair, and easy detangling) and the effects of individual shampoos when using them.

The responses obtained in the survey indicate that the tested shampoos are definitely more suitable for the care of men’s hair and for the care of undyed hair. For women with color-treated hair, shampoos with moringa, chlorella and spirulina make the hair dry and coarse after application. This may be the result of the use of SLES surfactant in the shampoo formulation, which exhibits significant degreasing properties. There were also difficulties detangling the hair. In most people, the hair after washing was medium soft and without shine. In terms of foaming properties, the moringa shampoo fared the best, while the other algae shampoos, according to the majority of respondents, foamed moderately. No problems with the distribution of shampoos on the scalp and hair were noted during the testing of the products, with the exception of three people who had long colored hair.

## Conclusions

### Creams

In the opinion of consumers participating in the study (10 product parameters were assessed, such as adhesion, consistency, uniformity, pillow effect, spreading, absorption, stickiness, greasiness, oiling), the emulsion with spirulina turned out to be the best preparation, while the other two creams – with chlorella and moringa, scored worse. The analysed preparations differed in appearance and colour. In the case of the creams with algae it was observed that the extract was not completely dissolved when the preparation was mixed. The cream with spirulina obtained the best hedonic properties, as no residues of powdered extract were observed in its mass. The prepared emulsions have moisturizing and greasing properties. The products are ideal for people with dry skin. No major differences in use were observed between people of different age groups, so the creams can be used by both young and older consumers.

### Shampoos

The consistency of all shampoos was rated as medium. The tested products were a homogenous mixture, without impurities. Only in the case of the product with moringa no precipitate appeared, while small amounts of powdered moringa leaves were noted. Shampoos with moringa and chlorella were odourless, whereas the shampoo with spirulina had an unpleasant aroma. The pH of the shampoos was determined at 5.5, which is the optimum pH for both the scalp and the hair. All products showed foaming ability during use. The shampoo with moringa had the best foaming properties and was rated “very good”, whereas the shampoos with chlorella and spirulina were rated “average”. The foaming ability of the hair cleanser is an important attribute, as an abundant and fine lather facilitates thorough distribution of the cleanser on the hair surface.

Based on consumer reviews, the prepared shampoos are excellent for men’s hair and for the care of uncoloured hair. The products are not suitable for people with colour-treated hair, as they dry it out. There was also a problem with combing hair after use. In terms of hair softness after washing, respondents rated the products as giving “medium” and “poor” softness. The shampoo with moringa performed best in this category. When it came to the ease of spreading the shampoos during washing, the majority of testers said that all formulations were easy and pleasant to apply. Only those with long, coloured hair reported difficulty in spreading the product.

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## EFFECT OF BOTOX AND “BOTOX-LIKE” PREPARATIONS FROM THE CONSUMER’S POINT OF VIEW

Katarzyna Wybieralska<sup>1</sup>, Izabela Chłodzińska<sup>1</sup>

### Abstract

In purpose to have of firm, smooth skin, consumers are eagerly reaching for anti-wrinkle creams. This is due to the market offer offering “eternal” youth, suitable lifestyle but also from greater consumer awareness about their body, skin, tissues and the way antioxidants work. Injection methods of fighting wrinkles, such as the use of tissue fillers or botulinum toxin, are also gaining more and more popularity. Cosmetic companies develop technologies that allow the non-invasive use of active compounds, which cause an effect similar to the effect of botox.

The aim of the study was to evaluate the effects of preparations that guarantee results similar to aesthetic medicine treatments with the use of botox. Cosmetics with botox-like substances (argireline, herbal botox, DMAE, bee venom, snake venom, conotoxin, stoichiol) regularly used are an alternative for consumers who do not like invasive care methods. The effects of botox-like substances were discussed by comparing the feelings of people taking part in the study and by studying the information gathered about specific preparations. The analysis of consumers’ preferences regarding the use of botox treatments and the application of preparations rich in compounds which, according to the producers, guarantee strong anti-wrinkle effects was also performed.

### Keywords:

botox, botox-like preparations, aesthetic medicine treatments, consumer feedback

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Technology and Instrumental Analysis, Poland; *k.wybieralska@ue.poznan.pl*

## Introduction

### Botulinum toxin – application

**Aesthetic medicine.** Botulinum toxin is mainly used to eliminate wrinkles caused by too much tension or the habitual, constant contraction of facial muscles. These muscles become paralyzed under the influence of the toxin. Only type A toxin BTX-A (Botulinum toxin A is one of the highly effective neurotoxins produced by the bacterium *Clostridium botulinum*) is used for the treatment. The substance is injected in a solution with physiological saline in the amount of 20–50 units, which depends on the individual characteristics of the patient, as well as the degree of contraction of the muscle. The injection is made into the muscle responsible for the formation of the wrinkle, as well as in its close vicinity. Immediately after injection, it is inadvisable to massage the injection area, assume a horizontal position of the body or fly by plane. These activities could lead to the displacement of the substance and the asymmetry of the face. The first effects become visible 24–48 hours after the injection of the skin, but the final effects become visible after 2–3 weeks. Smoothing lasts 3–6 months. This time depends on the place of administration, preparation or individual patient characteristics. After this time, the treatment can be repeated until a permanent effect is obtained. The toxin only works in the muscle into which it has been injected, leaving the rest of the muscles fully active. The main indications for the treatment are: horizontal forehead wrinkles, the so-called lion's wrinkle, crow's feet, or smoker's wrinkles. Of course, treatment may not always be performed. Contraindications include: pregnancy, cancer, swallowing disorders, bacterial skin diseases, muscle atrophy, allergies, taking medications that reduce acetylcholine secretion (Noszczyk, 2016; Placek, 2016; Przylipiak, 2017; Satriyasa, 2019). Smoothing wrinkles with the described method also causes positive side effects. It has been observed that the treatment improves the skin's protective functions by reducing TEWL (transepidermal water loss) while improving skin hydration. There is also a reduction in the amount of sebum and an increase in the amount of collagen fibers.

**Primary hyperhidrosis.** The treatment with botulinum toxin consists of a few to a dozen intradermal injections in the areas of hyperhidrosis. They can be subcutaneous, but then you should use a fine needle. A dose of 100 units is given and the procedure is painless. The effects begin to appear after 72 hours, but the full effect becomes apparent after 7–14 days and lasts for 6–9 months. The toxin acts on sympathetic fibers that do not regenerate as quickly as the motor fibers (Noszczyk, 2016; Placek, 2016; Przylipiak, 2017).

**Migraine headaches.** Treatment of migraine with botulinum toxin has only been used for a few years and is carried out in patients with chronic migraine who do not respond to other treatments. Such treatment is based on the finding that one



of the causes is the constant excessive tension in the muscles of the neck and head, which is eliminated by the injected toxin. The dose is selected individually so as not to reduce the possibility of movement. The effects become visible after a few hours, but the final effect is visible after 3–7 days and lasts for 3–6 months. In half of the cases, such treatment completely eliminates the symptoms of the disease, and in half of the cases it quite visibly reduces them (Noszczyk, 2016; Przylipiak, 2017).

**Eye defect – strabismus.** The procedure consists in injecting a small amount of the toxin into the selected antagonistic oculomotor muscle contributing to the wrong positioning of the eye and is performed under local anesthesia. The substance reduces the contraction of the muscle, so the knob positions the right way. The effects are visible in most patients (Noszczyk, 2016; Przylipiak, 2017).

**Gummy smile.** This smile is manifested by the visibility of the gums in the upper dental arch. It is not a disease, but for many people it is aesthetically disturbing. The toxin is injected into the circular muscle of the mouth and reduces contraction in that muscle. The result is the lack of visibility of the gums when smiling (Noszczyk, 2016; Przylipiak, 2017).

**Accelerating the healing of difficult-to-heal wounds and preventing scars.** Large, long scars that cause pain and contracture qualify for treatment with the toxin. The skin is punctured on both sides of the lesion as close to the center as possible. The dose used is 4 units. The treatment should be performed in 3 or 4 series at intervals of 6–8 weeks. The result is a softening and disappearance of the scar (Noszczyk, 2016; Placek, 2016; Przylipiak, 2017).

**Hyperactive rumens.** This phenomenon manifests itself in the grinding of the teeth at night and the formation of a „square” face through the growth of the rumen. The procedure is performed to protect the enamel, as well as for aesthetic reasons. Botulinum toxin is administered after 2 or 3 pricks in the center of the taut rumen on both sides. 3–4 doses are used. The effect should be visible after one treatment, but it can be repeated after 2 weeks in order to weaken the muscle faster (Noszczyk, 2016; Placek, 2016; Przylipiak, 2017).

**Excessive seborrhea.** Botulinum toxin inhibits the productivity of the sebaceous and sweat glands, which reduces the multiplication of bacteria. Indirectly, it also increases the migration of keratinocytes, thanks to which the hair follicles are not clogged. When treating oily skin, intradermal punctures are made into the skin surface (Noszczyk, 2016; Placek, 2016; Przylipiak, 2017).

**Possible side effects.** Type A botulinum toxin is a relatively safe substance used in aesthetic medicine. However, there are side effects due to the way the toxin is injected and the development of an inflammatory reaction. They are topical and occur up to several days after the procedure. Usually these effects are mild and

temporary. Before the procedure, the doctor collects a detailed medical history of each patient, which reduces the number of side effects. The most common are: viral and bacterial infection in the puncture area, hematomas, bruises, erythema, pain, drooping eyelids, and even respiratory disorders or aspiration pneumonia. There are also isolated cases of death after administration of botulinum toxin, but they were associated with cardiovascular diseases, and the relationship with the substance used directly has not been demonstrated (Noszczyk, 2016; Placek, 2016; Przyłipiak, 2017; Surowiak, 2011; Zbrojkiewicz, Lebedowska & Błońska-Fajfrowska, 2018).

## „Botox-like” substances

Botox-like substances belong to the group of neurocosmetics. They can be defined as: „a topically applied, non-toxic, non-reabsorbable product that influences the activity of the dermal nervous system or modulates the activity of cutaneous neurotransmitters” (def. Prof. Misery). Substances acting in a similar way to botox reduce the tension, play the complete relaxation of the facial muscles. This allows you to reduce the appearance of wrinkles. Additionally, these compounds can have a lifting, moisturizing and stimulating effect on the formation of collagen and elastin. Manufacturers declare that after using them, the skin should be smooth, taut, moisturized, brightened, without signs of fatigue (Dragomirescu, *et al.* 2014; Rizzi, *et al.* 2021).

**Argirelin.** The most popular substance among botox-like products. As the skin ages, the amount of peptides present in it decreases. Argireline (acetyl hexapeptide 3) is a peptide that works very similar to botulinum toxin. 3-Acetyl hexapeptide blocks muscle contraction by acting on acetylcholine. It works precisely by preventing this neuromuscular neurotransmitter from leaking into the synaptic space of the motor plate. This product is not introduced into the skin by injection, it is applied on the surface in the form of creams, serums, masks or even ointments. The advantage of the substance is the low probability of side effects, because it is a non-toxic substance and has been clinically tested thoroughly. It is estimated that it reduces the depth of wrinkles by 30% in 30 days when using a substance with a concentration of 10%. This peptide, under the name Argirelina, is sold by the Lipotec laboratory from Barcelona (Sokołowska-Wojdyło, 2018; Zielińska, 2015).

**„Herbal Botox”.** Acmeallaoleracea plant extract, containing mainly substances from the group of alkylamides – spilanthol. Its composition also includes: 3-lipoic acid, P-sitostenone, scopoletins, vanillic acid, transferulic acid and trans-isoferulic acid. The leaves, roots and buds of the plant are mainly used to make the extract, because they contain the most alkylamides, which reduce muscle tension, while smoothing wrinkles. This effect is possible thanks to the perfect migration of

spilanthol through the individual layers of the skin reaching the muscle itself. It is a substance that can also be used by injection, it will give a better and faster effect, but it is not necessary. An additional effect is skin firming. The substance works in the same way as botulinum toxin, but the effect is lighter and shorter. You can get a great smoothing of wrinkles, so the name „herbal botox” is not a marketing gimmick. Studies have shown that it is not a toxic substance, does not cause any side effects, and has no contraindications to its use (Mrożek-Szetela, 2017).

**DMAE.** The full name of this ingredient is dimethylaminoethanol. It is a precursor to acetylcholine. It has a relaxing effect on the muscles, which allows them to contract less and at the same time eliminate wrinkles. It is a product that works mainly on „crow’s feet” and also on „smoker’s lines”. It also acts as an antioxidant, slowing down the aging processes of the skin. After its application, a facelift of flabby skin is visible. The problem with using this ingredient is the pH at which its action is most effective. Needs an alkaline pH close to 10, which is a very alkaline environment. Such a base has a bad effect on the skin, irritating it and causing allergies. The pH of the skin is slightly acidic, therefore most care substances have a neutral, slightly alkaline or acidic pH, which makes the effects of DMAE among such products uncertain. The studies that have been conducted show, unfortunately, the effect of DMAE on reducing the number of cells responsible for the formation of collagen. Not all detailed studies have been carried out yet, which does not define dimethylaminoethanol as an unfit product (Sokołowska-Wojdyło, 2018; Zielińska, 2015).

**Bee venom.** Apitoxin – a complex of toxic peptides and proteins. It consists of: apamine, adolapine, dopamine, norepinephrine, histamine and mellitin, which is 50% in the whole venom. Its action is based on the skin’s reaction to its presence. The place of application is perceived as inflammation, which causes vasodilation to oxygenate and nourish the skin. In addition to smoothing caused by skin tension, it also brightens. However, the exact mechanism is unknown. It has not yet been thoroughly tested, so not all side effects are known. However, it is a strong allergen that can cause allergic reactions in about 2% of people in the world (Han, *et al.* 2015; Zielińska, 2015).

**Viper Venom.** It is a synthetic substance that is a multi-component complex. It mimics the action of the natural viper peptide – Wagler’s alarm (Templeviper). The substance is also known as „real agekillingeffect”, which delays action of cells responsible for skin aging. Smoothing is achieved by inhibiting nicotinic acetylcholine receptors. Systematic use of 4% SYN AKE has a smoother effect than 10% argireline. Clinical trials also confirm the absence of side effects. However, you should be careful with the frequency of using this substance, because with more frequent application than specified by the manufacturer, swelling or itching of the skin may appear (Sokołowska-Wojdyło, 2018; Zielińska, 2015).

**Conotoxin.** The substance contains peptides obtained from sea snails of the cone family (Conidae). The action of conotoxin is based on the blockade of the muscle contraction mechanism at the cellular level. These peptides do not allow sodium cations to travel through the cell wall. In order for the substance to be used in cosmetology, the paralyzing effect was removed. In addition to the clear smoothing, you can also observe the relaxation of the skin (Mrożek-Szetela, 2017; Sokołowska-Wojdyło, 2018; Zielińska, 2015).

**Stoechiol.** It is a natural substance obtained from Spanish lavender (Butterflylavender), also known as butterfly lavender. It has a smoothing, lifting, antioxidant and protective effect. It relaxes muscle spasms by secreting beta-endorphins. It also influences better skin density, sealing the epidermis and increasing the amount of lipids. All this allows you to visibly smooth out wrinkles. Thanks to its natural origin, it is very well absorbed by the skin, it can also be used in people with sensitive skin (Sokołowska-Wojdyło 2018; Żukowska & Bodnar, 2019).

## Application properties of cosmetics with the addition of “botox-like” substances

The aim of the study was to analyze the application properties of cosmetics with the addition of selected active ingredients intended for mature or demanding skin. The task was to assess the effects of preparations that guarantee results similar to aesthetic medicine treatments with the use of botox.

## Material and methods

The 7 substances described above were selected for the experiments. Argirelin and DMAE were obtained from MCCM, conotoxin – BingoSPA, viper venom was produced by Murier, „herbal botox” by BioPlant Natura, bee venom from Dietesthetic, and stoechiol – Resibo. 3 women in different age groups (especially selected test group) participated in the study (Table 1).

Cosmetics were applied to cleansed and toned skin. 2 ml of the preparation were used. Each cosmetic was tested separately (one cosmetic was tested for 7 days, followed by a day off, and then the next cosmetic was tested for the next 7 days). The products were patted on the entire surface of the face and left for 12 hours. At the designated time, the effects of the substances were assessed on the basis of the feelings of the examined women and the noticeable changes.

**Tabel 1. Characteristics of the study participants**

Women 1	Women 2	Women 3
<p><b>Age – 24 years</b>  <b>Combination skin with a visibly oily T-zone, Transverse forehead wrinkles, Firm skin.</b></p>	<p>Age – 47 years,                      Combination skin with a tendency to dry out,                      Numerous surface wrinkles all over the face, mainly on the forehead and around the eyes,                      A few deep wrinkles, especially on the surface of the forehead,                      The skin gently sagged around the cheeks.</p>	<p>Age – 80 years,                      Dry skin,                      A dozen or so superficial wrinkles,                      Numerous deep wrinkles all over the face,                      The skin was sagging mainly on the cheeks and around the eyes,                      Gently sunken eye sockets.</p>

## Results

Based on the study, it can be concluded that argireline, herbal botox, snake venom and bee venom show similar effects to botox, and the first effects are visible after 24 hours. The remaining substances: DMAE, conotoxin and stoechiol do not show any spectacular effects after 7 days of use. This does not mean, however, that these substances do not have an anti-wrinkle effect, most likely they need longer, systematic use. Similarly, their short-term application doesn't provide the skin with the expected moisturizing effect.

Results of the test analysis of organoleptic features presents the similar results to the application tests. Substances that work like a Botox have been best received by consumers. The exception is DMAE, which obtained much better marks in the study of the cosmetic properties in relation to the results of application for wrinkles, and „herbal botox”, which received low scores in this study. This may be due to the form in which it was used. „Herbal Botox” has been tested in the form of an oil that is absorbed relatively long compared to other substances. It also leaves an oily glow that is unpleasant for everyone. Research has shown that the viper venom and argireline turned out to be the best received by consumers and having the closest effect to Botox.

### Botox and its substitutes – consumer preferences

The aim of the study was to analyze the preferences of consumers regarding of the use of botox treatments and the application of preparations rich in compounds that, according to the producers, guarantee strong anti-wrinkle effects.

## Methods

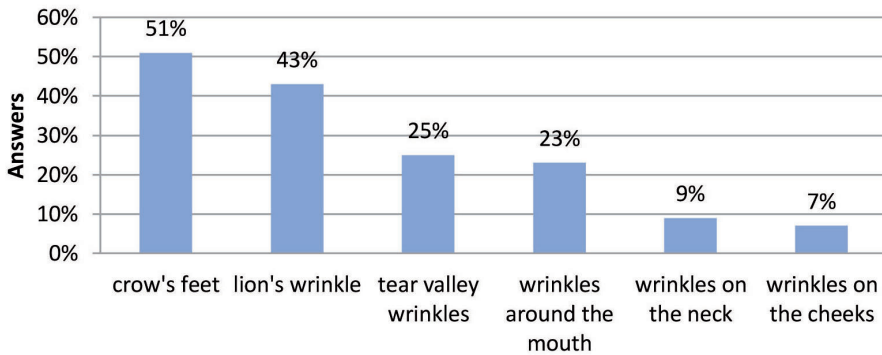
The study was conducted in January 2020 and in the first quarter of 2021 using the Forms page available on Google Drive and was disseminated on websites and groups associating people interested in cosmetology, aesthetic medicine or skin care. The form was also distributed in a friendly aesthetic medicine office. The author's survey was to check how consumers care for aging skin, what they know about using Botox, as well as the popularity of the studied substances. The respondents also answered questions about the desired and undesirable effects after surgery or using substances at home. The study involved 216 people, both men and women, over the age of 25, because it is at this age that the production of collagen and elastin begins to decline, and this is when the subject of skin aging becomes important for the respondents.

### Respondents characteristics

In the study were participated 216 people. Women dominated as they constituted as much as 95% of the respondents. Only 8 respondents it's men. The respondents were divided into 4 age groups: 25–30 years old, 31–40 years old, 41–50 years old, over 50 years old. The youngest group turned out to be the most numerous, i.e. 25–30 years old (60%). People over 50 were more willing to answer on the spot in the office, but it was the smallest group – 15 people. Classifying the respondents in terms of earnings, it can be concluded that the largest group were people earning PLN 2,000-5,000, there were as many as 55%, then up to PLN 2,000 – 30% and over PLN 5,000 – 20%. The largest part of the respondents (35%) live in cities with more than 500,000 inhabitants, not much less, because 25% live in the countryside.

### Skin aging and care

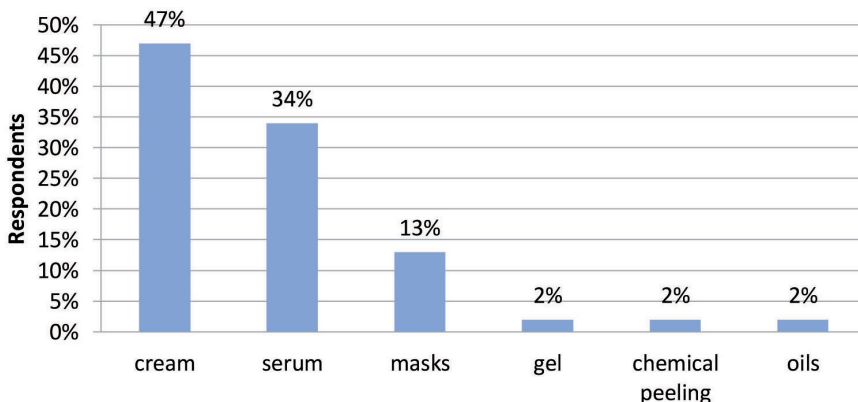
In the questionnaire, consumers were asked, inter alia, about the estimated age at which they noticed the first wrinkles on their face. It turns out that most people notice wrinkles at the age of 25. Interestingly, even the younger ones notice the first signs of aging on their face. Most likely, these wrinkles are not caused strictly by the aging of the skin, but rather by the psychological campaign of the media, advertisements for the need to be eternally young. They can also be the so-called gravity wrinkles. The respondents were asked to indicate the type of the most bothersome wrinkles. They could select up to 3 answers. The results are shown in Figure 1.



**Figure 1. The most problematic wrinkles according to the respondents**

The most disturbing wrinkles turned out to be crow’s feet (51%) and the lion’s wrinkle (43%). Then, although to a much lesser extent, i.e. less by 20%, the respondents indicated tear valley lines (25%). The next ones are the wrinkles around the mouth (23%) and the wrinkles on the neck (9%). The least disturbing were the wrinkles on the cheeks (7%).

It turned out that 55% of respondents use anti-wrinkle cosmetics in their daily care. By listing a specific type of cosmetics, the respondents listed two or more examples. In response, creams (47%) and serums (34%) dominate, followed by masks (13%), gels, chemical peels and oils turned out to be the least popular in care – all 2% each, Figure 2. More than half, 53.1 % stated that it is difficult to determine the effect of cosmetics, because the skin remains in the same state after their application, changes were not observed. The next group are people who do



**Figure 2. Types of anti-wrinkle cosmetics used by the respondents**

not see the good effects of the products used (26.25%). The smallest number of people (20.65%) perceive the positive effects of anti-wrinkle substances. When asked about the age to start using anti-aging cosmetics, the respondents indicated that they were 25 (26%). It is connected with the age of noticing the first wrinkles, which is referred to in the literature as 25, because this is when the skin aging process begins. All respondents were asked to provide answers about the use of anti-wrinkle treatments in a beauty salon or an aesthetic medicine clinic. 103 people stated that they had not used such services yet.

### Assessment of botox and botox-like substances

Aim of the survey was also to analyze consumer preferences regarding the preparation rich in compounds, the main task of which is to delay the effects of skin aging. 45.6% of people declared the use of botulinum toxin to reduce wrinkles. The rest (54.4%) did not use this substance.

The next question was intended for people who performed at least one injection of a tissue filler – botulinum toxin. Responders were asked to say if they noticed and if so what side effects of the treatment. The results are shown in Figure 3.

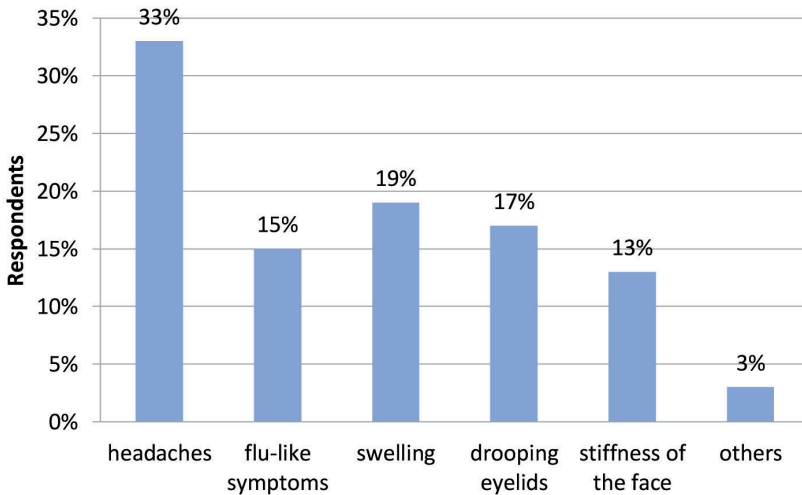
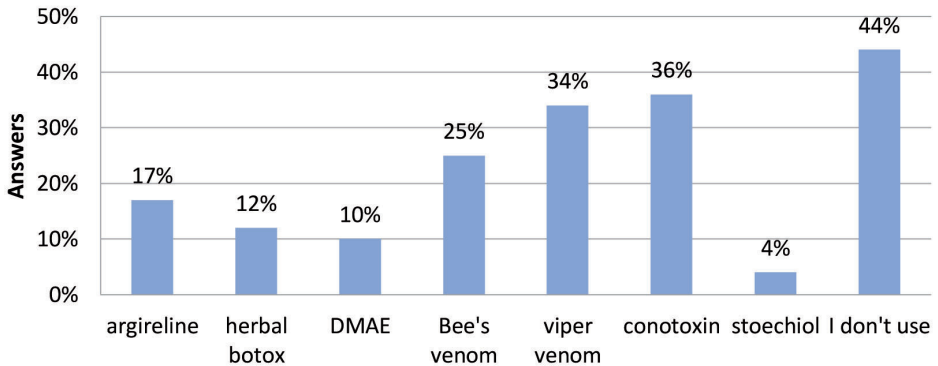


Figure 3. Side effects of botulinum toxin signaled by the respondents

When analyzing the respondents' answers, it can be concluded that very few people reported negative effects of the procedure. There are only 15% of such people. Each of them listed at least 2 side effects. The most popular were headaches (33%), followed by flu-like symptoms, swelling, eyebrow drooping – all



at 17%, and the inability to move the injected area – 16%. The vast majority of respondents who have ever had an injection of botulinum toxin to reduce wrinkles (80.6%) were satisfied with the procedure and repeated it for a lasting effect. The group of satisfied people who did not repeat the procedure and those who were dissatisfied constituted 9.7% of the respondents. The respondents were also asked about their knowledge of the botox-like ingredients (Figure 4). Consumers could select all known substances.



**Figure 4. Knowledge of botox-like substances**

A large group of respondents didn't know any of the mentioned substances (44%). The most popular of these turned out to be conotoxin (36%), snake venom (34%), bee venom (25%), argireline (17%), DMAE (10%), herbal botox (12%) and stoechiol finally (4%).

Another question related to the popularity of treatments with the use of the above-mentioned substances. The vast majority of respondents have never had a treatment or used a home care product with the "botox like" preparations. This group constituted as much as 85% of the respondents. Only 15% tested substances that smoothen wrinkles when used on the surface.

Exactly half of the respondents did not notice any effect after using this type of preparation. The next group are people who noticed the positive effects of cosmetics, but other than their expectations – moisturizing etc., not anti-wrinkle (30%). Only 24% of the respondents assessed the effect as satisfactory.

It was also checked whether clients noticed any side effects when using the tested substances. The responses of the respondents show that botox-like substances did not cause any undesirable symptoms among them.

## Conclusions

- The respondents notice the first signs of skin aging at the age of 25, although they do not declare using anti-aging cosmetics before the age of 30.
- The users of botox treatments are satisfied with the results.
- The risk of side effects of botox is very small, according to the respondents.
- Botox-like substances are not very popular among Polish consumers.
- Only 20% of respondents declared the application of preparations with the “botox-like” ingredients and were satisfied with the effect of the treatments.
- According to the respondents, the use of such preparations does not cause side effects, which means that they can be proposed as safe for human skin.
- The factor limiting the willingness to buy/use these cosmetics is mainly the price, the cost of the treatment about PLN 350 and the need for frequent application.
- The cost of the botox treatment varies between PLN 400 and PLN 1500. The effect is visible after one treatment, but if the patient wants to preserve the smoothing, the treatment must be repeated.
- Beauty salon clients using botox injections didn't express the will to apply the „botox like” substance, even like as a means of supporting the previously obtained effect.

Research has shown that most of the substances called „botox-like” are effective and intense, almost like botox. However, there are substances such as conotoxin and stoechiol that have a weak anti-wrinkle effect. Despite the very good effects of other preparations, these cosmetics are not popular among consumers, and the vast majority of them prefer botox. Surveys show that most of the people who know „botox-like” preparations are not satisfied with their action. These consumers, in spite of skin injections and the possibility of experiencing pain during the procedure, choose a fast and long-acting botox.

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## THE GROWING IMPORTANCE OF SUSTAINABLE PACKAGING DESIGN

Jana Gondárová<sup>1</sup>, Malgorzata A. Jarossová<sup>1</sup>

### Abstract

Plastics are a major element of the modern economy with countless applications in both the industrial and consumer area. They are a cheap, lightweight, durable and widely used material in many industries. Within the EU, plastics are mostly used as a packaging material (e.g. water bottles). Packaging represents approximately 40% of plastics production and 61% of all waste generated from plastics. Plastic packaging is also the type of packaging with the lowest recycling rate in the EU (42%) compared to other materials. The design of packaging is crucial for recyclability of plastic packaging. The article aims to show the strategy of selected companies to the circular economy as well as to identify new sustainable packaging introduced by these companies in the field of food, cosmetics and cleaning products. The methods of *analysis* and *synthesis* were applied to obtain theoretical backgrounds on this issue. Using these methods, we analysed information gained from domestic and foreign scientific sources, European legislation and websites of companies. We can conclude that using sustainable packaging is an opportunity to gain a competitive advantage between companies, as consumers begin to notice non-ecological behaviour among producers and prefer products' packaging that cares for our planet.

### Keywords:

plastics, packaging, food, cosmetics, cleaning products, sustainability, circular economy

<sup>1</sup> University of Economics in Bratislava, Department of Marketing, Slovakia; [jana.gondarova@euba.sk](mailto:jana.gondarova@euba.sk); [malgorzata.jarossova@euba.sk](mailto:malgorzata.jarossova@euba.sk)

## Introduction

Packaging holds a lot of functional benefits related to product protection and transport efficiency. Next to these practical benefits, consumers use packaging to evaluate products and brands, particularly in the case of fast-moving consumer goods (Hertenstein, Platt, & Veryzer, 2005; Orth & Malkewitz, 2008). A serious downside of packaging is that it is usually discarded directly after product use and unavoidably adds to our environmental footprint. Each European citizen generates approximately 160 kg of packaging waste. It means that packaging is an important issue when considering ecological inefficiency. One way to lower this environmental weight is to use ecologically-designed packaging (Magnier & Schoormans, 2015).

Nowadays a lot of materials used for short-term and disposable packaging application are still non-biodegradable but many companies have noticed the growing sensitivity of consumers to social problems, reflected by their interest in environmental concerns, e.g. the usage of sustainable packaging materials that are eco-friendly and safe for consumers (European Union, 2019; Herbes, 2020; Jerzyk, 2016; Muthuraj, *et al.* 2018).

Recent innovations enable manufacturers to produce sustainable packaging with a conventional look that does not signal sustainability. For example, Unilever employs MuCell® technology which uses 15% less plastic thanks to gas injection that creates gas bubbles in the middle layer of the bottle wall, reducing the density of the bottle and the amount of packaging required. Coca-Cola uses PlantBottle® technology, in which up to 30% of recyclable PET is made from plant materials. It is not always easy for consumers to understand that structures based on recycled materials are more ecologically friendly. Indeed, although these packages are designed to lower environmental impacts, they can easily be misunderstood because they look conventional. Package sustainability claims are especially important in the case of such packages (Magnier & Schoormans, 2015).

Plastic is the standard material for a huge number of applications. For example, plastic is often used for packaging because it's able to fulfil an impressive range of functions – from making sure products stay undamaged while being transported and used, through to providing the right amount of product whenever it is needed. Since plastic packaging weighs less than other alternatives, it can even reduce the carbon footprint created during transport. This means that plastic is not bad in and of itself – it is a valuable material. The problem comes from the way in which plastic is used, and that is where the concept of a circular economy comes into play (Henkel, 2021a).

Part of the European Union's action plan related to the circular economy via modifying the ways of using plastic materials, is the European strategy for plastics,

adopted in January 2018, which builds on existing measures to reduce plastic waste. The EU's plastics strategy aims to transform the way plastic products are designed, produced, used and recycled in the EU and it's a key element of Europe's transition towards a carbon neutral and circular economy. Its *objectives* involve e.g. reducing marine litter, greenhouse gas emissions and our dependence on imported fossil fuels, supporting more sustainable and safer consumption and production patterns for plastics and transforming the way plastic products are designed, produced, used and recycled within the EU. The specific *actions* to be taken to achieve the above-mentioned goals, with maintaining the matter of packaging materials in sight, include the following measures: new rules on packaging to improve the recyclability of plastics and increase the demand for recycled plastic content, measures on bio-based, biodegradable and compostable plastics, scaling up support for innovation, with a financial support to develop smarter and more recyclable plastics materials, to make recycling processes more efficient, and to trace and remove hazardous substances and contaminants from recycled plastics (European Commission, 2018). With the problematics of the ecological friendliness of plastic materials used for goods' packaging alongside with the European strategy for tackling this issue in mind, the article provides an overall information on actions and focus of two eminent European corporations – producing food, cosmetics or cleaning products – regarding their “sustainable plans” concerning plastic packaging: Swiss food giant Nestlé and German cosmetic/drogistic enterprise Henkel.

The article aims to show the strategy of selected companies to the circular economy as well as to identify new sustainable packaging introduced by these companies in the field of food, cosmetics and cleaning products.

## Nestlé's sustainable packaging strategy

Nestlé's strategy towards a sustainable packaging – including the use of plastic materials – aims towards the three following goals: functional, safe (i.e. food-grade) and environmentally friendly packaging (Nestle, 2019a). “We need packaging to keep food safe, protect it during transportation, extend shelf life and reduce waste. However, these requirements should not come at the expense of the planet, which is why we are innovating solutions with stronger sustainability credentials” (Nestlé, 2021b).

As we can see, a very specific aspect that Nestlé as well as other food processing merchants need(s) to take into account in comparison to, for example, the Henkel company, is the safety of their products for a human consumption, a factor that overruns all the other interests when designing the products' packaging. When putting the objectives of food safety and ecological value of products' packaging

together, companies such as Nestlé are bound to face (often considerable) obstacles, including the impediments of using plastic packaging materials. “Most plastics are difficult to recycle for food packaging, leading to a limited supply of food-grade recycled plastics.” (Nestle, 2020a). However challenging the process, Nestlé has publicly set it as its commitment to make 100% of its packaging recyclable or reusable by 2025, with a particular focus on avoiding plastic-waste (Nestle, 2019b) and reaching so-called “plastic neutrality”.

Needless to mention that this action-plan may have been driven by the extensive criticism of the Nestlé company after releasing that Nestlé is being the world’s third biggest “plastic polluter” together with Coca-Cola and PepsiCo (The Guardian, 2020). Considering the topic of a sustainable packaging in general, the relatively wide range of Nestlé’s practices involves the following actions:

- reducing packaging material (reducing the amount of PET in plastic bottles),
- using recycled packaging material (replacing virgin plastics with recycled materials, increasing the amount of recycled PET in plastic bottles),
- using recyclable packaging material (phasing out all non-recyclable or hard to recycle plastics),
- using bio-based and bio-degradable packaging material,
- applying reusable and refillable packaging systems,
- labelling product packaging with recycling information to help consumers dispose of it in the correct way,
- establishing the Nestlé institute for packaging sciences with the purpose of developing and evaluating different sustainable packaging materials,
- cooperating with external partners to develop new packaging materials and solutions,
- funding start-up companies developing innovative packaging solutions,
- joining the New Plastics Economy, an initiative which brings together the key stakeholders to rethink and redesign the future of plastic.

As Andrew Morlet, the CEO of Ellen MacArthur Foundation (initiator of the New Plastic Economy) sums up: “By eliminating the plastics we don’t need, innovating in areas like reuse models and new materials, and circulating the plastics we *do* need – also in more challenging food grade applications – we can create an economy where plastic never becomes waste” (Tecnaplastics, 2020).

In the following section of the article, we would like to focus our attention on three of the above-mentioned measures undertaken by Nestlé in their effort to support circular economy through sustainable packaging arrangements: recyclable packaging, reusable packaging, refillable systems.

## Recyclable (paper) packaging

In July 2019, Nestlé has announced the launch of its YES! snack bars in a new recyclable paper wrapper (Figure 1). In a breakthrough innovation, for the first time a confectionery bar has been packaged in paper using a high-speed flow wrap technology. This launch has unlocked the potential for recyclable paper packaging to be widely used in the confectionery industry. Up until now, high-speed production of shelf-stable snacks was only achieved using plastic films and laminates. Now paper can be used at large scale while guaranteeing product quality and freshness over the entire shelf life. The paper is made from sustainable sources, certified by the Forest Stewardship Council (FSC) and The Program for the Endorsement of Forest Certification (Nestle, 2019c).



**Figure 1. Nestlé recyclable packaging for YES bars**

Sources: <https://www.worldbakers.com/product/nestle-launches-yes-plant-protein-bars/>.

In January 2021, Nestlé announced that following the YES! bars, also its popular Smarties brand is now using recyclable paper packaging for its confectionery products worldwide. This represents a transition of 90% of the Smarties range, as 10% was previously already packed in recyclable paper packaging. Smarties (Figure 2) is the first global confectionery brand to switch to recyclable paper packaging, removing approximately 250 million plastic packs sold globally every year. The new paper packaging is sourced sustainably and is made of a coated paper, paper labels or a carton board. Information about how to properly dispose



**Figure 2. Nestlé recyclable packaging for Smarties**

Sources: <https://www.nestle.com/media/news/smarties-first-global-confectionery-brand-recyclable-paper-packaging>.



of this packaging is also included on its labels to increase consumer awareness. Nestlé has also adapted their existing manufacturing lines to allow for the careful handling that is required for paper (Nestle, 2021b).

Another fact deserves our attention. Previous name “Smarties” was “Lentilky”. After 110 years, the Czechs will stop producing Lentilky. Lentilky have been produced in the Czech Republic since 1907. They appeared on the market much earlier than competing M & M’s, which only debuted in 1941. From 2021, Lentilky will be manufactured exclusively in Hamburg, Germany. The reason is to change the recipe and packaging. They are supposed to contain less sugar and more chocolate. Supposedly, only the factory in Hamburg can meet the requirements of the new recipe. Another reason is change the packaging. It will now be packed in paper tubes, and the machines that enable this are only available in Hamburg (Przemysl spożywczy, 2020).

## Reusable packaging

Following the recycled packaging strategy, Nestlé also started to offer *reusable containers* for its Nesquik cocoa powder, Ricoré chicory and coffee drink, as well as Chocapic Bio cereals in partner-ship with the French retailer Carrefour and Loop, a global circular company (Figure 3). Consumers can access the cocoa, coffee and chicory drink, and cereal products in reusable stainless-steel containers. Once consumed, the empty containers are collected from the consumer’s home or dropped off by the consumer in the stores. The containers are then cleaned, refilled and put back in Loop platform’s circular system (Nestle, 2020b).



**Figure 3. Nestlé reusable packaging for Nesquik, Ricore and Chocapic**

Sources: <https://www.nestle.com/media/news/nesquik-ricore-chocapic-bio-reusable-containers-loop-carrefour>.

A similar concept has also been applied at the beginning of this year (2021) on two flavours of the Häagen-Dazs ice cream in the province of Ontario, Canada (Figure 4). The vanilla and coffee-flavoured ice cream can be bought in a double-walled steel packaging, which – according to the Nestlé’s representatives – “keeps

it at an optimal temperature from the time it's filled until the first delicious scoop. The canister design ensures that when opened, the ice cream melts more quickly at the top than at the bottom of the container, ensuring that every scoop is in perfect condition. The ice cream are now in zero-waste packaging” (Nestle, 2021c). At present, also all Häagen-Dazs ice cream bar *cartons* are recyclable.



**Figure 4. Nestlé reusable packaging for Häagen-Dazs icecream**

Sources: <https://www.corporate.nestle.ca/en/media/pressreleases/allpressreleases/ontario-residents-can-now-enjoy-iconic-häagen-dazs-flavours-new-reusable>.

## Refillable systems

At the beginning of 2020, Nestlé also started piloting refillable dispensers for soluble coffee (on the Swiss market), as part of its efforts to reduce single-use packaging (Figure 5). Consumers can bring reusable containers to purchase different types of Nescafé coffee, while also being able to digitally access product information that is typically found on packaging (such as ingredients, nutritional values and shelf life). Hélène Lanctuit, R&D Packaging Lead at Nestlé, declares: “Packaging plays a key role in maintaining food safety during a product’s shelf life. This means that whenever new packaging systems are explored we need to ensure that our products can be delivered to consumers in a safe and hygienic manner. These dispensers are novel because they incorporate smart technology which allows us to ensure product safety, and also guarantee the freshness and traceability of our products” (Nestle, 2020c).



**Figure 5. Nestlé refillable systems for soluble coffee**

Sources: <https://www.nestle.com/randd/news/allnews/nestle-pilots-reusable-refillable-dispensers-reduce-single-use-packaging>.

## Henkel's sustainable packaging strategy

The Henkel company's plastics strategy covers three core pillars:

- using materials from sustainable sources,
- using smart design,
- and closing the loop.

According to Henkel company only when packaging is designed with the least material possible; is recyclable or reusable; is made of recycled content, and is designed with context in mind it is fully sustainable.

Under the first pillar sits Henkel's work to use less material in the first instance, and to source plastics from renewable (non-fossil-fuel-based) or recovered sources. This objective also touches the second – smart design – pillar. Henkel's business uses a “reduce, replace, rethink” hierarchy when innovating at the design stage (Edie, 2020). Henkel's sustainable goal is that by 2025, 100% of their packaging will be recyclable, reusable or compostable – excluding adhesive products where residue may affect recyclability (Henkel, 2018a). “Reusability is clearly in our focus – some of our products already have refills, which come in flexible packs that, by weight, use up to 90% less plastic than the primary packaging,” declares Thorsten Leopold, the international director of packaging technology at Henkel. To that end, Henkel recently also invested in Trumans – an American startup utilising refillable bottles and concentrated products in the cleaning sector. The business is also working with retailers to develop an in-store refill station model for some of its most popular liquid products. Away from reuse, smart design innovations piloted by Henkel include plastic that maintains a black hue without the carbon pigment that makes it hard-to-recycle and software which enables users to scan any plastic packaging and receive an overview of its recyclability.

The latter of these innovations was designed to combat the fact that there is no clear, universal definition of ‘recyclable’ – due to variation in factors such as infrastructure availability between nations, states or even towns – and made open source because not every business will have the capacity or knowledge to assess recyclability alone. The desire to help drive plastics progress beyond Henkel's own operations is evident not only through the software project, but the company's participation in collaborative initiatives such as the New Plastics Economy Global Commitment, the Alliance to End Plastic Waste and Germany's Rezyklat-Forum.

According to Thorsten Leopold, the biggest benefit of taking part in such initiatives is a better engagement with policymakers. “While we are a large company, being part of industry and cross-industry collaborations means having an even larger voice,” he said. “What will help the whole industry is having a harmonised set of definitions, guidelines and waste collections and recycling systems. Of course, this won't happen across the globe overnight... but differing systems make it

harder for an international company like us to design packaging which is context-appropriate and our role cannot be ignored” (Edie, 2020).

In the following part of the article, we outline several of Henkel’s achievements in the field of sustainable, ecologically friendly cosmetics and cleaning products’ packaging.

## The EasyD4R® software tool for evaluating the recyclability of packaging

Recyclable packaging is a prerequisite for a functioning circular economy. In order to quickly and reliably determine the recyclability of new packaging, Henkel has developed the software tool EasyD4R® in 2020. Its goal is to quickly and accurately assess the recyclability of packaging as early as the first stages of product development. Other companies and organisations can use this software free of charge. Henkel has won Best Practice sustainability award from Packaging Europe with the software in 2020 (Henkel, 2021b).

## Henkel invests in chemical recycling

In 2019, Henkel and the packaging manufacturer Alpla jointly produced bottle bodies based on chemically recycled plastic for the first time (this pilot project uses Perwoll bottles, Figure 6). By using chemical recycling, material made from fossil resources can be replaced by recycled material made from plastic waste. The packaging made from these chemically recycled materials has the same quality as packaging based on virgin plastic (Henkel, 2019a).



**Figure 6. The packaging made from these chemically recycled materials for Perwoll bottles**

Sources: <https://www.henkel.com/press-and-media/press-releases-and-kits/2019-10-17-first-henkel-bottles-made-of-chemically-recycled-plastic-991126>.

## Refill stations are gaining importance

While recycling remains an important factor for sustainability as a whole, refill stations and systems for reusing packaging are also becoming increasingly important. In addition to new product forms and the use of recycled plastics in the packaging, Henkel is also using refill stations. The concept is simple: customers buy a container once and fill it with detergent or dishwashing liquid. As part of a pilot project, Henkel has set up refill stations in selected test markets in the Czech Republic (in November 2019). Customers can refill liquid detergents, fabric softeners, dishwashing liquids, shampoos and shower gels in these stores. The “gas station” system was designed so that the customer selects an empty bottle of the required product when they enter the store and then they scan its code at the station. A label is also printed to inform the customer of the product’s expiry date. When the product is used up, the customer takes the empty bottle back to refill it.

In November 2020, the Beauty Care Professional brand Authentic Beauty Concept launched the first vegan refill bar on the European market (Figure 7). Customer uses the refill bar to fill a bottle made from 92 percent recycled plastic with the required product. Once the client has used it all up at home, the empty bottle can be brought back to the salon to be refilled. This new process prolongs the life of single-use bottles, cutting plastic consumption and encouraging a circular economy (Henkel, 2020a).



**Figure 7. Authentic Beauty Concept refill bar**

Sources: <https://www.henkel.com/press-and-media/press-releases-and-kits/2020-11-06-authentic-beauty-concept-offers-the-1st-vegan-refill-bar-1128022>.

## Henkel product packaging with “Social Plastic”

Henkel was the first global fast-moving consumer goods company to team up with social enterprise Plastic Bank one year ago. The goal: collecting plastic waste before it enters the ocean. At the collection centres in Haiti, one of the poorest

countries in the world, the local population can return collected plastic waste and exchange it for money, goods, or services. The so called “Social Plastic” is then integrated back into the plastic value chain. Now for the first time, Henkel included this Social Plastic in its packaging. The collected plastic is sorted, processed and then integrated into recycling value chains as Social Plastic – material that has been verified by the Plastic Bank to indicate that the collectors received an above-market price for the plastic waste (Henkel, 2019b).

## The perfect duo: CARDBOX packaging packs detergents of Henkel company

Henkel decided on an alternative packaging solution for their Duo-Caps and Power-Mix Caps detergents from Greiner Packaging and Cardbox Packaging – a combination of cardboard and plastic, which helps to reduce the plastic consumption. Its innovative patented tear-off system makes it easy to separate the cardboard wrap from the plastic container and recycle it. A ratio of plastic in the packaging has been distinctly minimised, while retaining the container’s stability by the cardboard wrap (Cardbox packaging, 2021; Figure 8).



Figure 8. CARDBOX packaging packs detergents of Henkel company

Sources: <https://www.cardbox-packaging.com/the-perfect-duo-cardbox-packaging-packs-detergents-of-henkel-company>.

The Cardbox Packaging plant in Wolfsberg, Austria, has been certified in the field of corporate social responsibility (CSR), the international auditing company EcoVadis, and has won a silver medal. EcoVadis evaluates the company’s approach to sustainable development on the basis of 21 criteria relating to environmental protection, working conditions and human rights, ethics and sustainable development. EcoVadis’ methodology is based on international CSR standards, such as the Global GRI Reporting Initiative, the UN Global Compact and ISO 26000. The resulting analysis summarizes our company’s strengths and weaknesses in terms of our policies, activities and results, and also provides recommendations for improving activities and procedures that will further strengthen our social responsibility (Cardbox packaging, 2021).

## Conclusions

Plastics, including plastic packagings, are an important material in our economy and our daily lives. However, they can have serious negative effects both on the environment and on human health, which is where the concept of a circular economy comes into play. In January 2018, the European strategy for plastics – part of the European Union’s action plan related to the circular economy via modifying the ways of using plastic materials – has been adopted with one of its main objectives being to apply new rules on packaging, including improvement of the recyclability of plastics and an increase of demand for recycled plastic content in products’ packaging.

The strategy of achieving and preserving a sustainable progress (not only) via altering the ways in which plastic packagings are designed and used, has already been adopted by a number of European enterprises, including two of the business giants: Swiss Nestlé and German Henkel. Their approach in the “eco – plastic – packaging” area can be summarised into the following actions: reducing packaging material in general; using recycled, recyclable, reusable and/or compostable packaging materials (including plastics). More specifically, both Henkel and Nestlé are, for example, investing into the use of refill stations: Nestlé for their Nescafé coffee and Henkel for their liquid detergents, fabric softeners, dishwashing liquids, shampoos and shower gels, both companies reporting a highly positive feedback from customers in all the test markets.

The ultimate goal for plastic packaging of both companies can be put using the words of Andre Molet of the New Plastics Economy initiative with which both Nestlé and Henkel co-operate in their efforts: “By eliminating the plastics we don’t need, innovating in areas like reuse models and new materials, and circulating the plastics we do need – also in more challenging food grade applications – we can create an economy where plastic never becomes waste”.

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## DETERMINATION OF OXYGEN TRANSMISSION RATE OF PACKAGING MATERIALS USING DYNAMIC ACCUMULATION METHOD

Wojciech Kozak<sup>1</sup>

### Abstract

Barrier properties are one of the most important features of packaging materials from the point of view of the protective function of the packaging. This feature may relate to the degree of gas, water vapor or other factors, e.g. light permeability. It determines the exposure of the packed product to these external factors, and thus directly affects the protection and maintenance of product quality. For this reason, the aim is to achieve the best possible barrier properties of the packaging materials used.

Among the factors that may penetrate the packaging material, oxygen is of particular importance, as it contributes to many unfavorable changes (e.g. oxidation) in the packed products, especially food products. The parameter most often used to determine the degree of oxygen permeation through the packaging material is the so-called oxygen transmission rate (OTR). Various methods and devices are used to determine it.

The paper presents one of the newest methods of measuring OTR, namely the so-called dynamic accumulation method (DA), which uses selective fluorescence to measure the amount of oxygen permeated. The measurement methodology, equipment, sample measurement results and comparison with other OTR measurement methods used so far were presented.

**Keywords:** oxygen transmission rate, OTR, dynamic accumulation method, DA, packaging, barrier properties, packaging materials quality

<sup>1</sup> Poznań University of Economics and Business, Institute of Quality Science, Department of Industrial Products and Packaging Quality, Poland; [wojciech.kozak@ue.poznan.pl](mailto:wojciech.kozak@ue.poznan.pl)

## Introduction

Oxygen permeability or permeation, is one of the most important parameters characterizing the suitability (and thus quality) of packaging materials for packaging oxygen-sensitive products, in particular foodstuffs. Knowledge of it allows correct selection of packaging material for the production of packaging for a specific product and the envisaged packaging technique and preparation for consumption, so as to protect the quality of the packed products in the best possible way, as well as forecast its potential changes (Baele, *et al.* 2019; Singh, Wani & Langowski, 2017). It also allows you to assess the barrier properties of modern packaging materials and packaging, including those based on the so-called biopolymers (Shakeel, 2018).

Currently, the largest share in the market, next to paper packaging, is plastic packaging, replacing other traditional packaging materials, such as glass or metal. This is mainly due to the lower price and weight of plastic packaging, as well as the ease of forming them and thus the possibility of giving them any shape, which is particularly important from the point of view of the attractiveness of the packaging, and thus the product packed in it. Unfortunately, plastic has also flaws. Unlike glass or metal packages, packages made of plastics show permeability to gases present in the atmosphere of the package or its surroundings. Gases (including oxygen) diffuse through the packaging material along the concentration gradient. The rate of their diffusion is in most cases related to the internal structure of packaging materials, which in turn is a derivative of their chemical structure (Lagaron, 2011; Singh, Wani & Langowski, 2017). This speed is of great practical importance because it indirectly affects the stability of packaged products stored on store shelves, in particular the shelf life of the food. Suitable barrier properties are important in packaging processes, especially in modified atmosphere packaging (MAP) technique (Lee, 2021). It requires the packaging to maintain a stable atmosphere composition. Only if this basic condition is met, the packaging can effectively protect the product packed in it. It is possible when we know the barrier properties of the packaging materials used and thus we can properly select them for the intended packaging technique. Hence, oxygen permeability is a factor that must be taken into account when designing packaging. The use of different materials for the production of packaging is directly related to significantly different properties of the packed products. The barrier properties of polymer-based packaging materials depend primarily on their structure (crystalline, amorphous). In addition, other factors are also important, such as the method of obtaining polymers, their thermal, mechanical and physical treatment (e.g. orientation, plasticization), type of chemical groups present in the polymer (polar, non-polar), degree of cross-linking, glass transition temperature, packages forming method (Buntinx, *et al.* 2014; Jost & Stramm, 2016; Siracusa, 2012). Besides the aforementioned characteristics of the material, also other

factors may influence its barrier properties. The most important factors are the external factors surrounding the packaging, in particular temperature and relative humidity (Robertson, 2009).

Oxygen permeability can be defined as the degree to which oxygen permeates through a given packaging material (Brandrup, Immergut & Grulke, 2003). In other words, it is the rate at which oxygen diffuses through a particular medium, in this case a polymer (Robertson, 2012). If the packaging material is free from defects (perforations, cracks, inclusions), diffusion is the only process responsible for permeation. This means that the oxygen dissolves in the polymer first from the side where the oxygen concentration is higher and travels towards the side where the oxygen concentration is lower and then releases. The diffusion of a specific gas is related to the size, shape and polarity of its molecule. Another important aspect is the degree of crystallization of the polymer through which the gas diffuses. Gases are generally insoluble in crystals (Kofinas, Cohen & Halasa, 1994). Therefore, the more crystalline the polymer is, the better the barrier properties it has. Unfortunately, most of the polymers obtained from crude oil or gas processing have a limited proportion of the crystalline phase and thus insufficient barrier properties. Therefore, these materials must be modified, for example by metallization, coating or bonding with other materials to form laminates, multilayer (co-extruded) films or composite materials (e.g. nanocomposites) (Hrnjak Murgic, *et al.* 2015).

Measurement of oxygen permeability allows, among other things, to assess the effectiveness of the applied method of modifying packaging materials in terms of improving their barrier properties it is also commonly used tool for packaging material quality control and verification (e.g. for compliance with the specification) (Singh, Wani & Langowski, 2017).

## Oxygen transmission rate (OTR)

A commonly used unit in measuring gas permeability is the gas transmission rate (GTR). In case of oxygen permeability, the abbreviation OTR (oxygen transmission rate) or less often  $O_2TR$  is used. This value informs about the amount of oxygen permeating through a given surface of the tested material in a given time. The amount of oxygen permeated is most often given in cubic centimeters, while the area it permeates is 100 square inches (a unit mainly used in the United States) or 1 square meter. The amount of oxygen permeated is based on a time of 24 hours. Hence, the OTR unit is  $cm_3 O_2 / 100 in^2 \cdot 24 h$  or more often  $cm_3 O_2 / m_2 \cdot 24 h$ . It is common practice to include oxygen partial pressure difference during the measurement in the results (usually 0.21 or 1 atmosphere) depending on the oxygen content in the carrier gas to be used (Welt, 2015).

Sometimes the so-called oxygen permeability coefficient (OPC) is used, which expresses the theoretical permeability of a given material per unit of thickness. In practice, this factor is obtained by multiplying the OTR value of a given material by its actual thickness.

## Commonly used OTR measuring methods

One of the oldest (but still used) methods of determining gas permeability through packaging materials is the so-called manometric method (ASTM D1434-82 2015; ISO 15105-1 2007; ISO 2556:1974). In this case, a decrease or increase in gas pressure is measured depending on which side of the sample (placed in special permeation cell) the pressure gauge is mounted on. The pressure change is converted to the volume of gas passing through a given surface area of a given packaging material. There is also volumetric variant of this method, with the difference that instead of measuring the change in pressure, the change in volume is measured.

Those methods are universal because it is possible to determine the permeability of various gases (including oxygen) with the use of the same measuring equipment, but are not as accurate as other methods of gas permeability measurement (Siracusa, *et al.* 2015).

Currently the most commonly used OTR determination method is the so-called coulometric method also known as carrier gas method (Müller, *et al.* 2017). In this case the amount of permeated oxygen is measured by a coulometric sensor (ASTM D3985-05 2010; ASTM F1307-14 2014, ASTM F1927-14, 2014, ISO 15105-2, 2003). Measurements are carried out in a special measuring chamber in which the analyzed sample is mounted. On one side of the tested material there is a flow of so-called test gas containing oxygen (most often it is a mixture of nitrogen with a certain amount of oxygen), on the other side a flow of primary inert, so-called carrier gas (most often nitrogen of high purity) is used, which “collects” the oxygen molecules permeating through the test sample. In testing materials with expected high barrier properties, pure oxygen is often used as the test gas. It is important to maintain the same gas flows on both sides of the test sample throughout the measurement. Such an arrangement should assure the same pressure on both sides of the tested sample and make the method isostatic. Consequently, oxygen concentration gradient should be always constant, so the method can be called steady state. During the OTR measurement the carrier gas containing oxygen permeated through the sample is then directed to a coulometric detector whose signal is proportional to the amount of oxygen. The coulometric method also allows the measurement of oxygen permeability through packaging and closures. Coulometric method is very popular, but requires the use of significant amounts

of special and thus expensive technical gases. In addition, it is necessary to constantly control the gas flow on both sides of the test sample, which is difficult as the amount of gases in the cylinders decreases. Another issue is that it does not reflect the actual process of oxygen permeation through the packaging material, because the gases used during the measurements flow at a certain speed and usually in opposite directions, and thus the gas permeation is not natural, but to some extent forced. It is also not suited for barrier properties determination of highly permeable materials (e.g. porous).

Apart from above mentioned OTR measurements methods, it is also possible to indirectly define oxygen permeability as the ambient oxygen ingress rate (AOIR) into the package (Larsen, Kohler & Magnus, 2000). An electrochemical oxygen meter or a chromatograph is used for this. However, it is an invasive method, and therefore it is subject to error.

## Dynamic accumulation OTR measuring method

In real conditions only natural diffusion takes place during the penetration of oxygen through a given material. This condition is met by another method of measuring oxygen permeability, namely so-called dynamic accumulation (DA) or unsteady state method using fluorescent oxygen measurement to determine changes in the oxygen concentration within the test (accumulation) chamber with the sample (packaging material) or within packaging as the oxygen permeation process progresses (Abdellatief, *et al.* 2014; Abdellatief & Welt, 2013; ASTM F2714-08, 2008; ASTM F3136-15 2015; Siro, Plackett & Sommer-Larsen, 2010; Welt, 2015). Hence, such a way of measurement seems to be a more appropriate method for measuring oxygen permeability through packaging materials, because in this case oxygen permeation occurs in a natural, unforced manner, as in packaging on store shelves. The measuring chamber is similar to that used in the coulometric method. It consists of two parts which are separated during the measurement by a sample of the tested material placed between them. During the measurement, one of the parts containing the optical oxygen sensor is filled with an inert gas, for example nitrogen, and the other part uses ambient air as the oxygen-containing carrier gas. In measuring the permeability of high barrier materials, it is possible to fill said chamber with a gas mixture with a higher oxygen content or even pure oxygen. Such a procedure is used to speed up the measurements. At predetermined intervals, the oxygen concentration is measured in the part of the chamber containing the oxygen indicator until the increase in oxygen concentration is constant over time. The oxygen permeability through the tested material is calculated on the basis of the change in oxygen concentration in a given time, for a given sample area and the volume of the

measuring chamber. This method is very simple, and therefore user-friendly and economical – a small amount of technical gases is used for measurements (the measuring chamber has a volume between 5 to 7 cm<sup>3</sup> depending on particular unit). Next advantage is that having more test cells it is possible to measure OTR of multiple packaging materials in parallel with just one oxygen meter. As the sample results of studies carried out by different researches show, OTR of various packaging materials determined using DA method is comparable with OTR results achieved using coulometric (steady state) method (Table 1).

**Table 1. OTR measured using dynamic accumulation (DA) vs steady-state method**

Sample type	Dynamic accumulation (DA) method		Coulometric (steady state) method	
	OTR [cm <sup>3</sup> /m <sup>2</sup> /day]	±95 CI*/SD**	OTR [cm <sup>3</sup> /m <sup>2</sup> /day]	±95 CI*/SD**
High barrier film	6.8	0.5*	7.4	0.3*
Medium barrier film	840	30*	780	70*
Low barrier film	9700	200*	9600	700*
Hostaphan® RN 100 (PET) 0% RH	16.4	0.7**	16.3**	0.5**
Hostaphan® RN 100 (PET) 50% RH	13.5	0.7**	13.9	0.3**

Source: based on (Abdellatif & Welt, 2013; Müller, *et. al.* 2017).

Additionally, with the use of an appropriate adapter, it is possible to measure the oxygen permeability of whole packaging (e.g. bottle or container). This is particularly important because such packages have variable thickness and it is not possible to take a sample of the package with a uniform and therefore representative thickness. An interesting area of application of this method of measuring oxygen permeability is the assessment of the barrier properties maturation containers, oak barrels and closures of bottles used in the wine industry (Dieval, Vidal & Aagaard 2011; Nevares, del Alamo-Sanza, 2021; Nevares, *et al.* 2014). In addition to packaging, this method is also used to determine the oxygen permeability of contact lenses, which is a specific quality indicator that determines their compatibility with the eyeball and comfort of use (Perez-Ortiz, *et al.* 2007). OTR measurements using the DA method are possible thanks to commercially available devices offered on the market by such companies as Oxysense (USA), AMTEK MOCON (USA), Presens (Germany).

## OTR measurement methodology using dynamic accumulation method

### OTR measurement of films/laminates

First, appropriate samples of the material to be tested are prepared by cutting them out in the form of 6.5 cm squares with scissors, guillotine or special cutter. Then the test sample is placed in the aforementioned test chamber, which is made up of two aluminum parts, connected by a hinge. The sealing of the sample/chamber connection is achieved by the use of vacuum grease, which is spread by hand or with an applicator on the surface of the top part of the measuring chamber around the edge of the circular cavity. The area of this cavity corresponds to the actual area of the sample through which oxygen is permeating. Additionally, an oxygen indicator is placed in the cavity of the upper part of the measuring chamber. The second, lower part of the measuring chamber has a similar recess around which an O-ring is attached, which, during measurements, adheres to the test sample. Therefore, there is no need to additionally seal this part of the measuring chamber with grease. The total volume of the upper measurement part (cavity, inlet and outlet channels) is factory determined and permanently stamped on each measurement chamber so that it can be entered into the software and on this basis allow the amount of oxygen permeated to be calculated. After the upper part of the measuring chamber is greased and the test sample is placed, the chamber is closed and additionally tightened by two hand screws. The next step is to fill the upper part of the measuring chamber with oxygen-free inert gas. It is equipped with two valves (inlet and outlet) which must be open during this operation. One of the two valves is connected to a gas source (for example a nitrogen cylinder) through a flow meter (set at 0.05–0.1 l/min), and then “rinses” the top of the measuring chamber until the oxygen-free atmosphere is achieved. After this the inert gas supply is shut off by turning off the inlet valve first, and then outlet valve as quickly as possible. The order in which the valves are closed is extremely important. Closing the outlet valve first may compress the inert gas filling the upper part of the measuring chamber, which will disturb the natural course of the oxygen permeation process during the measurement, and in extreme cases may damage the test sample. After closing the above-mentioned valves, the inert gas source is disconnected and the initial oxygen concentration within the test chamber is measured. Before that, important measurement parameters should be determined, namely thickness of the test sample (in micrometers), the volume of the measuring chamber used (in cubic centimeters) and the oxygen content in the mixture constituting the source of oxygen (in percent). If air is used as the source of oxygen, both valves at the bottom part of the measuring chamber should be opened for the entire duration of the measurement. If a gas mixture with a higher oxygen content or pure oxygen



is used, then the lower part of the chamber is filled with them, just like the upper part, with an inert gas. Subsequent measurements of the oxygen concentration in the upper chamber can be performed manually or as automatic measurements, but the time intervals between successive measurements should be previously determined. During the duration of the measurement, oxygen begins to penetrate the tested material and at some point the rate at which it penetrates becomes constant. The end of the test is considered to be the moment when, at least three consecutive measurements have been taken (the coefficient of determination  $R^2$  is 0.95 or more). In the case of materials with medium and low barrier properties ( $OTR > 1000 \text{ cm}^3/\text{m}^2 \text{ 24 h}$ ), the frequency of measurements should be increased, because the moment of the permeation equilibrium occurs much faster in this case. On the other hand, for high-barrier materials ( $OTR < 10 \text{ cm}^3/\text{m}^2 \text{ 24 h}$ ), the frequency of measurements should be reduced, because changes in the oxygen concentration in the upper part of the measuring chamber occur very slowly.

Due to the significant influence of temperature and humidity on gas permeability, oxygen permeability measurements should be carried out in rooms with controlled conditions (preferably air-conditioned), compliant with the subject standards for testing barrier properties of packaging materials. Most often, tests of this type are carried out at a temperature of  $23^\circ\text{C}$  and at specified relative humidity (RH), for example 0.50 or 70% (ASTM D3985-05 2010; ASTM F1927-14 2014).

## OTR measurements of whole packages

As already mentioned, the DA method is also suitable for OTR measurements of whole packages. They are most often performed for bottles made of plastic, it is also possible to measure the oxygen permeability by other forms of packaging, for example containers, cups, trays, etc. In the case of bottles, a special adapter placed on the neck of the bottle can be used, enabling the achievement of the oxygen-free atmosphere inside the bottle, which is necessary for the proper OTR measurement. This adapter consists of two screwed together metal plates, the upper of which is equipped with two valves enabling the change of the atmosphere inside the bottle and an oxygen indicator. The lower part slides over the neck of the bottle (under the thread or flange) and is screwed together with the upper part by means of four screws. This part has a V-shaped cutout, which enables the same adapter to be fitted to bottles with different neck diameters. Measurement of oxygen permeability through a package is based on the determination of oxygen concentration changes inside it over time. The above-mentioned permeability measurement adapter is placed on the bottle-shaped packaging, with a special sealing glue applied to the contact surface of the bottle with the top plate of the adapter. If, due to the shape of the packaging, it is not possible to use the said

adapter, another solution should be used to obtain an oxygen-free atmosphere in the packaging, and additionally, an oxygen indicator should be placed in it. In this case, a custom made adapter to a given type of packaging can be made on your own or by ordering it. Yet another, simpler solution is the use of two lines (inlet and outlet) terminated with needles, which are inserted into the wall of the tested package through a self-sealing septum, guaranteeing tightness at the point of insertion of the needle also after its removal. The thickness of the needles should be selected experimentally to easily break through the wall of the package, the permeability of which we want to determine. If the measurements are made of opaque packaging, it is necessary to use special adapter for invasive measurements. Another method can be successfully used for transparent and opaque packaging in the form of cups or trays. Namely, the tested packaging should be glued with the edges (to which, when closing the packaging in the packaging process, a welded laminate or aluminum foil, the so-called platinum is attached), to a specially prepared glass plate with attached oxygen sensor and two holes enabling the installation of the inlet and outlet valves. Plate size and valve hole spacing must fit the dimensions of the package, it is preferable to leave a certain margin so that the plate protrudes slightly beyond the outline of the package. It is important to select the appropriate adhesive that connects the tested package to the glass plate. You should follow the type of plastic from which the packaging is made, and before starting the appropriate measurements, make a trial connection of the packaging with the glass plate. If the obtained connection is too weak or leaky, another type of adhesive should be used. After preparing the packaging for testing, i.e. placing the oxygen indicator in it (in the case of bottles) and installing (depending on the needs) the aforementioned additional equipment enabling filling the tested packaging with oxygen-free gas (adapter, needles, glass plate, etc.), the appropriate OTR measuring test of a given package can be started. Initially, the package is filled with oxygen-free gas in order to obtain an oxygen-free atmosphere. Then the gas supply is cut off by closing the valves or removing the needles from the package and the initial measurement (starting oxygen concentration) is made. Subsequent measurements of the oxygen concentration, allowing to determine the rate of its penetration, are performed analogously as previously described OTR measurements of packaging materials in the form of films or laminates. As in the case of testing oxygen permeability through whole packages, it is not possible to determine their uniform thickness (thickness is not uniform in different places of the package) or to precisely determine their surface, the test result relates to the declared or measured capacity of the tested package, which is included in the OTR calculations.

## Conclusions

The presented DA method is an interesting alternative to other OTR determination methods. The principle of the method makes it reflect the process of oxygen permeation through the packaging material taking place in real conditions better than other commonly used methods. The test is quite simple, uses minimal amounts of technical gases, which makes the measurement cheaper. The results obtained during the research are similar to those obtained with the use of other OTR determination methods. The dynamic accumulation method is suitable for testing packaging materials with a wide range of barrier properties, including porous materials intended for breathing products such as fruits and vegetables. The latter is not possible with other methods. A certain disadvantage of the method is relatively long measurement time (the longer the higher barrier properties of tested material are), but in return the measurement is closer to the natural diffusion process taking place in the real packaging. However, a promising way to shorten the OTR measuring time of high barrier materials using DA method has already been found (Welt, 2015).

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## THE ROLE OF PRODUCT DESIGN IN THE GLASS INDUSTRY

Renáta Ševčíková<sup>1</sup>

### Abstract

The success of innovative design-driven practices that drive sustainable long-term growth in business revenues and company performance has been demonstrated in many studies. The glass industry in Slovakia has a long tradition, whether in the form of the production of utility glass or technical glass. The aim of the paper is to investigate the best practices using product design for innovation in glass industry to get better understanding of how design supports firm competitiveness. We have chosen Rona, the leading utility glass producer with long tradition in Slovakia. The firm thanks to technological know-how and cooperation with designers, competes with world glass producers. The paper was elaborated using a case study method, which resulted in several recommendations for the use of product design to achieve a sustainable competitive advantage. The analysed firm has been paying great attention to the design of its products since the first half of the 20th century. Internal designers are in daily contact with the company, they are perfectly acquainted with its technology and thus have been able to harmonize commercial requirements with individuality and design. An open-minded approach and following new trends, can lead to the excellent position in the international glass industry.

**Keywords:** product design, glass industry, competitive advantage

<sup>1</sup> University of Economics in Bratislava, Department of Marketing, Faculty of Commerce, Bratislava, Slovakia; [renata.sevcikova@euba.sk](mailto:renata.sevcikova@euba.sk)

## Introduction

The Slovak glass industry belongs to the manufacturing industries, which have a long tradition and extraordinary importance and position in the national economy of the Slovak Republic. It is based on a relatively available raw material base. The glass industry produces glass and mineral fibers and glass fiber products, packaging glass and packaging glass for pharmaceutical products – ampoules, vials, utility glass and unique glass art objects.

Glass is the only material that can be recycled indefinitely. Thanks to glass recycling a considerable number of raw materials is saved, and natural resources are preserved. Even though the glass industry is highly energy intensive and produces a relatively large volume of emissions, recycling glass is, in addition to saving raw materials, also a way to reduce emissions and thus an important way to carbon neutrality.

Due to shortening product life cycles and the rapid introduction of new products, companies today face an ever-increasing need for rapid, efficient product design and development (Wang, Mo & Tseng, 2018). Product design has been defined in various ways in the marketing literature, but is generally considered a three-dimensional element that includes dimensions of aesthetics, functionality, and symbolism (Bettels & Wiedmann, 2019). Design is a structured process that transforms creative ideas into specific products, services, and systems, and as such, combines creativity with innovation. As part of the innovation process, design has the potential to significantly contribute to improving the brand image, increasing sales and profitability of the company. Creativity and design can thus be linked to innovation, as creativity is the first to contribute to the dissemination of the creative ideas that are available, and design gives a better chance of successfully commercializing creative ideas in the form of new products (Mlákay, 2015).

Companies that invest in design tend to be more innovative and profitable and grow faster than companies that do not invest. Recent studies (Chiva, Alegre, 2009; Hertenstein, Platt & Veryzer 2005; Kootstra, 2009) show that design-driven companies perform better in innovation. These studies also show that companies that place design at a strategic level, or as an internal process, come up with new products faster than companies that do not have a design policy in place. The design transforms thoughts and ideas into practical and attractive propositions for users and consumers and represents a powerful tool of differentiation for companies in the competition. An increasing number of companies are integrating industrial or product design into the process of developing their products to gain a competitive advantage in the market. Studies suggest that design budgets in European and American companies are growing by 8 to 20% per year (Gemser & Leenders, 2001). Industrial design is one of several key areas that are crucial for new product development along with research and development (R&D), marketing,

manufacturing, and sales. Industrial design contributes to the development of new products by better connecting the customer with the product, through ease of use, capabilities, and appearance of the product. Awareness of the role that industrial designers play in new product development has increased over the last two decades (Hertenstein, *et al.* 2005).

Borja de Mozota (2006) introduced the concept of four design tasks. (1) Design as a differentiator – a source of competitive advantage in the market through brand value, customer loyalty, price setting and customer orientation. (2) Design as an integrator – a means to improve the development processes of new products and services. (3) Design as a transformer – a means of creating new business opportunities, improving the company's ability to cope with change and as a tool for better interpretation of society and the market. (4) Design as a good business – a source of increased sales and higher margins, greater brand value, greater market share, better return on investment.

The effective use of design and design resources available to the company in accordance with its goals is represented by design management. It is directly related to the placement of design in the organizational structure, to the identification of specific design knowledge that is relevant to solving key issues of management and education of managers in the field of effective use of design (Knošková, 2014). The European Commission considers design management to be a competence that falls under innovation management, recognizing that companies need the ability to innovate to respond to new market opportunities and threats (Kootstra, 2009).

In 2001, the Danish Design Center developed The Design Ladder that illustrates and evaluates the use of design in companies. It is based on the hypothesis that there is a positive relationship between higher revenues, the use of design in the early stages of the development process and the placement of design in the overall business strategy of the company. It consists of four steps (Danish Design Center, 2001):

- STEP 1: No design. Design is an invisible part of product development, and this task is not handled by trained designers. The solution is based on the participants' ideas about good function and aesthetics. Users' views play little or no role in this process.
- STEP 2: Design as a styling. Design is perceived exclusively as the final stage of surface treatment (styling), whether in relation to product development or graphic design. The task can be performed by professional designers, but it is usually in charge of people with other professional backgrounds.
- STEP 3: Design as a process. Design is not the result, but an approach that is integrated in the initial phase of the development process. The solution is problem-driven and user-driven and requires the involvement of a wide



range of skills and functions, such as process engineers, materials engineers, marketers, and administrators.

- **STEP 4: Design as a strategy.** The designer cooperates with the owners and management of the company on a complete or partial reassessment of the business concept. The key is to focus on the relationship of design to the company's business visions and required areas of business.

Many companies today do not use design in a conscious, systematic, or strategic way. This is even though design as an innovative activity seems to be particularly suitable for small and medium-sized enterprises, given the relatively low capital requirements and rapid return on investment (Kootstra, 2009). Design is a powerful tool for nationalities and companies willing to add value to their products or services and to be competitive in both local and global market. However, especially small and medium-sized enterprises (SMEs) are still not aware of the potential benefits of design (Raulik-Murphy, 2010).

## Methodology

The aim of the paper is to investigate the best practices using product design for innovation in the glass industry to get better understanding of how design supports firm competitiveness. We have chosen Rona, the leading utility glass producer with long tradition in Slovakia. The firm thanks to technological know-how and cooperation with designers, competes with world glass producers. The paper was elaborated using a case study method.

## Results

### Historical development of the Rona company

The glassworks in Lednické Rovné was founded at the end of the 19th century as the last largest glassworks of the Viennese company "Jozef Schreiber and nephews" (Joseph Schreiber & Neffen). The glassworks originally specialized in the production of sheet glass. A year later, production was reoriented to the production of utility pressed glass, and at the end of 1894, the production of hand-blown glass under the Rona Crystal brand began. An important part of quality products is very good craftsmanship, thanks to which the brand has gained a significant place in the world market. A substantial part of the production was a hotel cup of simple shapes with an emphasis on the elegance of the line. The standard part of the assortment consisted of table sets designed for everyday use. The first technique of finishing the cup in Lednické Rovné was grinding. At the beginning of the last century, the glassworks was the largest and most modern

glass company and produced quality glass under the “Rona Crystal and Kaiser Crystal” brand. After 1918, the product range was expanded by the well-known cylinders for kerosene lamps. In 1942, the glassworks became independent under the name “Slovenské sklenné huty”. After liberation (1945) it was under the National Administration, in 1946 nationalized and renamed the Slovak Glassworks n.p. Subsequent privatization in 1995 became a limited liability company called B.D.S. Ltd. with its registered office in Lednické Rovné. In 1996, it became a joint stock company at the request of the founder. In 2000, it was renamed RONA TRADING, a.s. and a year later it was already operating under the name RONA, a.s. (Osuská, 2012; RONA 2021).

Thanks to its results, the joint-stock company was one of the most important exporters of glass products in 2001, not only in terms of product range and volume, but also its own contribution to the gradual improvement of product quality. During the introduction and development of the most modern technologies, there was a modern development of artistic design of products and production techniques. Thanks to the supervision of experienced experts, the modern and technically equipped company produces glass, which is exported to the whole world under the Rona brand. The glassworks in Lednické Rovné currently supplies more than forty million pieces of glass annually to different parts of the world. Some products are subject to high demands on design and quality, which Rona a.s. meet, as evidenced by the fact that in 2009 it received a quality certificate from Loyd’s Register Quality Assurance. Today, the joint-stock company continues its business activities from previous periods. Its goal is to continue the completion of the sales network after taking over foreign trade and at the same time to build its own import – distribution companies in some places, with the aim of increasing the quality of service up to retail channels. Rona also has its own companies, Rona Deutschland GmbH (Germany), RONEX “M” (Russia), RONA IMPORTS Ltd. (UK), which offer glassware for households, restaurants, and hotels. Skill work glassworks and its own quality products, the glassworks quickly gained international importance, while most of the production was exported abroad (Osuská, 2012; RONA 2021).

### **The role of design in glass innovation**

The company’s strategy is mainly market orientation. The primary goal is quality, attractive assortment, customer service and especially customer satisfaction. Motivation, qualification of staff and mastery of demanding technologies are the basis of the company’s competitive advantage. It is not only a modern application of glass forming machines, but also a regular improvement of all phases of the production process in order to achieve the best production properties. The new “pulled foot” technology, which has been introduced using new unique production lines, makes it possible to produce very highly exclusive cups. This technology

consists of a cup (upper part) and a foot (lower part of the cup), which form a uniform unit, which increases its quality and durability. The used production technologies increase the physical resistance of the offered assortment.

Great attention is also paid to the composition of glass so that it meets today's above-standard requirements for performance. Rona produces crystal-quality glass, with hardness as the main attribute for washing in dishwashers and excellent optical properties being high-quality properties of the products. It is necessary to mention another fact that is very topical today. Ecology and environmental criteria are applied in all areas of the company's activity.

In the 20th century, it was not customary for companies to keep in-house designers. Ron's glassworks had two top ones: Karol Hološek and Jaroslav Taraba. They spent decades in daily contact with the factory. They were given the space to get to know the technology perfectly, enriched the glass craft and significantly shifted the design of the glass. They were co-creators of the technology to produce beverage sets with a drawn knife, which influences production to this day and is very successful. This made it possible to realize a shape that corresponds to the aesthetic intentions of the design. At present, both internal designers and external designers are dedicated to design in the glassworks. One of the external designers is Patrik Illo, who as a renowned designer specializing in drinking glasses and interior items for commercial use has won a number of international awards.

## Conclusions

The aim of the paper was to investigate the best practices using product design for innovation in glass industry to get better understanding of how design supports firm competitiveness. We have chosen Rona, the leading utility glass producer with long tradition in Slovakia. The firm thanks to technological know-how and cooperation with designers, competes with world glass producers. The paper was elaborated using a case study method, which resulted in several recommendations for the use of product design to achieve a sustainable competitive advantage.

The analysed firm has been paying great attention to the design of its products since the first half of the 20th century. The strong position of the company's internal designer positively impacts the quality and perception of products. Internal designers are in daily contact with the company, they are perfectly acquainted with its technology and thus have been able to harmonize commercial requirements with individuality and design. The important role of design is underlined by organizing international glass symposia and cooperation with external designers and students. An open-minded approach and following new trends, can lead to the excellent position in the international glass industry. For several years now, the

Rona company has succeeded in combining useful properties with quality design. The client is crucial, thanks to the designers, Rona helps the customers to realize their own ideas about the products as well as suitable decorations. Creativity, innovation, and functionality are the basic pillars on which the company stands, and as the company's director says: „The future is already being decided today”. Rona has been implementing design in its development process for decades. It uses the latest technology, invests in innovation, and constantly strives to move forward and not stagnate.

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## APPLICATION OF DETOUR NUMBER IN PREDICTION AQUATIC TOXICITY OF ALKYL BENZENE SULFONATES

Ryszard Zieliński<sup>1</sup>

### Abstract

We study a relationship of the partition coefficient between n-octanol and water ( $\log KO/W$ ) and the structure of anionic surfactants belonging to sodium salts of alkylbenzene sulfonates. We correlate  $\log KO/W$  values for 23 surfactants with the number of carbon atoms (B) in the side alkyl chain of surfactant, characteristic volume (VX) and the topological index known as Wiener's number (WN) as well as detour number (DD) of the surfactant molecule.

We have found a good correlation between experimental and computed values of  $\log KO/W$  for a series of the surfactants having various structures of the alkyl part of the molecules and the resulting correlation equation is:

$$\log KO/W = -0.16210 \cdot B + 0.026947 \cdot VX + 0.000783 \cdot DD - 6.1793$$

It was found that the developed correlation equation allows for prediction  $\log KO/W$  sodium salts of alkylbenzene sulfonates with the maximum error of 0.18 units. Furthermore, we correlate computed DD values for pure isomers of 20 sodium salts of alkylbenzene sulfonates and experimental aquatic toxicity data for those surfactants published in literature. We have found that the aquatic toxicity of tested surfactants against goldfish species can be predicted with the maximum error of 0.13 pEC50 units using the following relationship

$$pEC50 = 0.001979 \cdot DD + 1.133$$

**Keywords:** surfactant, safety, toxicity, topological indices, detour matrix, partition coefficient

<sup>1</sup> Poznań University of Economic and Business, Institute of Quality Science, Department of Technology and Instrumental Analysis, Poland; [r.zielinski@ue.poznan.pl](mailto:r.zielinski@ue.poznan.pl)

## Introduction

Surfactants can be defined as group of amphiphilic chemical structures consisting of two parts: hydrophilic polar head and hydrophobic tail. The hydrophilic polar head group of the surfactant molecule provides a solubility of the molecule in water or other polar liquids. Based on the ability of the polar head groups to dissociate into ions all surfactants can be divided into two main groups: nonionic surfactants and ionic surfactants (Zieliński, 2017). Among a group of ionic surfactants we can find: anionics, cationics, zwitterionics, catanionics and mesoionics compounds. On the other hand, the hydrophobic moiety of the surfactant molecule is responsible for solubility of the whole molecule in nonpolar systems. It usually contains a long hydrocarbon chain of unbranched or branched structure.

In aqueous solutions at low concentrations of surfactants their molecules can exist in the monomeric form, while above a certain surfactant concentration called the critical micelle concentration (CMC) surfactant molecules can exist in form of monomers and associates called micelles. For a given surfactant the numerical value of the CMC in aqueous solution depends on several factors such as temperature and the presence of salts in the solution (Zieliński, 2017). The main factor affecting the numerical value of the CMC is mostly related to the hydrophobicity of the surfactant molecule.

The environmental risk of surfactants, especially ionic ones, requires toxicity measurements (Lechuga, *et al.* 2016). It is known that different test organisms used in toxicity experiments have different sensitivity to the toxics, therefore it is necessary to establish the most appropriate organism to classify the surfactant as very toxic, toxic, harmful or safe, in order to establish the maximum permissible concentrations in aquatic ecosystems. Prediction of the fate and potential harmful effect or toxicity of surfactants in the environment is needed by some legislation systems like Registration, Evaluation Authorisation and Restriction of Chemicals (REACH). It is known that a successful prediction requires information on hydrophobicity of surfactant molecules which is used to describe the relationship between the chemical structure and the physicochemical properties and biological activity of surfactants. In most of practical applications the n-octanol and water solvent system has been widely accepted as a model system and it is used in medicinal chemistry and toxicology (Leo, Hansch & Elkin, 1971) for evaluation of hydrophobicity and the toxicity of the substance. It was reported in literature that the numerical value of the partition coefficient ( $\log K_{O/W}$ ) is correlated with toxicity of chemicals (Roberts & Costello, 2003; Roberts, 2004; Zieliński, 2017). Therefore, the value of  $\log K_{O/W}$  for any molecule in this system can be used as a source of information on its potential toxicity. In most of applications of surfactants as active components of pharmaceutical, cosmetic or household chemistry products, the numerical value the partition coefficient ( $\log K_{O/W}$ ) as well as some toxicity

information is needed. In medicinal chemistry the value of  $\log K_{o/w}$  is routinely used to estimate oral (Lipinski, *et al.* 1997) and skin (Edwards & Price, 1997) bioavailability of drug candidates. On the other hand ecotoxicologists use it to model acute and chronic toxicity to aqueous species (Cronin & Mark, 2006; Kaiser & Esterby, 1991) and potential for bioaccumulation (Bintein, Devillers & Karcher, 1993). According to literature reports on skin toxicity (Lémery, *et al.* 2015), there is a definite difference between toxicity of ionic and non-ionic surfactants. It was reported that ionic surfactants are the most toxic if they are soluble in water, while crystalline ionic surfactants of low solubility show low toxicity. In the case of skin toxicity the sign of the charge, anionic or cationic, does not matter.

In this work we study a correlation between aquatic toxicity data of various isomers of sodium salts of alkyl benzene sulfonates and their chemical structure. To describe the structure of isomeric surfactants we use topological indices known as the detour number (DD) and characteristic volumes ( $V_x$ ) of the molecules. We use both  $V_x$  and DD values of the surfactants to correlate them to available in literature experimental values of the octanol-water partition coefficient ( $\log K_{o/w}$ ) and toxicity ( $pEC_{50}$ ) to goldfish.

## Experimental

### Surfactants

In this work we investigate effect of chemical structure on the numerical value of the partition coefficient and aquatic toxicity of several anionic surfactants of the general structure presented in Figure 1.

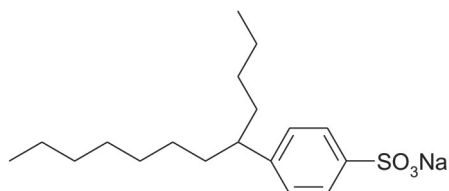


Figure 1. Chemical structure of sodium salts of alkylbenzene sulfonates

## Methods

### Characteristic volume

One can expect that toxicity of the organic molecules should depend on the size and shape of the molecules and its lipophilicity. The size of the molecules can be



easily described by means of molecular weight, molecular volume or characteristic volume of the molecules. The characteristic volume ( $V_x$ ) is the quantity proposed by McGowan (McGowan & Mellors, 1986; McGowan, 1990; Sowad & McGowan, 1992) and it can be estimated basing on a number of physical properties of the molecule extrapolated to the temperature of absolute zero. Therefore, the numerical value of  $V_x$  can regarded as a measure of the real volume occupied by the atoms present in a molecule of a considered chemical structure.

The  $V_x$  value is obtained by simply summation of numerical contributions attributed to atoms, bonds and rings present in a given chemical structure. In this work we use the numerical values of atoms, bonds and rings contributions developed by McGowan (McGowan & Mellors, 1986; McGowan, 1990; Sowada & McGowan, 1992) for the calculation of hydrophile-lipophile balance values of surfactants. We use them to estimate the molar volume of the molecules of several anionic surfactants belonging to a geometrical isomers of sodium salts of various alkylbenzene sulfonates.

### Wiener's number

To describe the shape of the molecule one can use numerical values of the topological indices. Wiener's number (WN) is one of such topological indices and it is defined as the sum of the distances between any two carbon atoms in the molecule and it is expressed in terms of the total number of carbon-carbon bonds. The numerical value of the Wiener's number reflects the degree of the incompactness of the molecule. For the purpose of surfactant species the Wiener's number can be redefined as the sum of the distances between any two non-hydrogen atoms present in the surfactant molecule. From practical point of view it is expressed in terms of the total number of bonds between any two non-hydrogen atoms. In this work we have to assume that the distance between any two neighboring non-hydrogen atoms can be approximated by the length of the carbon-carbon atoms, regardless if in the real molecule we have the single, double or triple bond between the considered atoms. It can be calculated from the following formula

$$WN = \sum_{i < j} D_{ij}$$

where  $D_{ij}$  is the number of bonds between the "i" atom and the "j" atom.

## Detour number

Detour number (DD) is another topological index of the molecules which can be defined as the sum of the longest distances between any two carbon atoms in the molecule and it is expressed in terms of the total number of carbon-carbon bonds. The numerical value of the detour number is another measure of the incompactness of the molecule. For the purpose of surfactant species the detour number can be redefined as the sum of the maximum distances between any two non-hydrogen atoms present in the surfactant molecule. The numerical value of detour number can be calculated from the following formula

$$DD = \sum_{i < j} DD_{ij}$$

where  $DD_{ij}$  is the number of bonds between the "i" atom and the "j" atom.

It should be noted, however, that for any molecule without any ring in the chemical structures the value of the detour number is equal to the Wiener's number (DD=WN).

## Toxicity data

We have studied 23 anionic surfactants belonging to sodium salts of alkylbenzene sulfonates with various isomers of alkyl moiety having a total length of octyl, decyl, dodecyl, tridecyl and tetradecyl without or with one branching in the hydrophobic moiety at the alpha carbon atom and correlated them to the partition coefficient values.

For another group of 20 anionic surfactants belonging to sodium salts of alkylbenzene sulfonates with various isomers of alkyl moiety having the total length of nonyl, decyl, dodecyl, and tridecyl without or with one branching in the hydrophobic moiety at the alpha carbon atom and correlated them to the aquatic toxicity data.

## Results

Table 1 contains experimental and computed values of the partition coefficient ( $\log K_{o/w}$ ) between n-octanol and water for some surfactants belonging sodium salts of alkylbenzene sulfonates. In the following part of this work we have used the following notation of the surfactant hydrophobic group structure:  $N = A + B$ , where N is the total number of carbon atoms in the hydrophobic moiety of the surfactant, A is the number of carbon atoms in the main alkyl chain and B is the number of carbon atoms in the side alkyl chain of the surfactant. Therefore, the structure shown in Figure 1 is noted as 8-4. Table 1 contains computed values of

the characteristic volume ( $V_x$ ), Wiener's number (WN), detour number (DD) and polarity number (PN) for 23 anionic surfactants belonging to a homologous series of sodium salts of alkylbenzene sulfonates. The structure of the surfactants listed in Table 1 is given using the following A-B notation, where A indicates the number of carbon atoms in the longest alkyl chain of the surfactant molecule while B is the number of carbon atoms in the shorter alkyl group attached to the alpha-carbon atom of the surfactant.

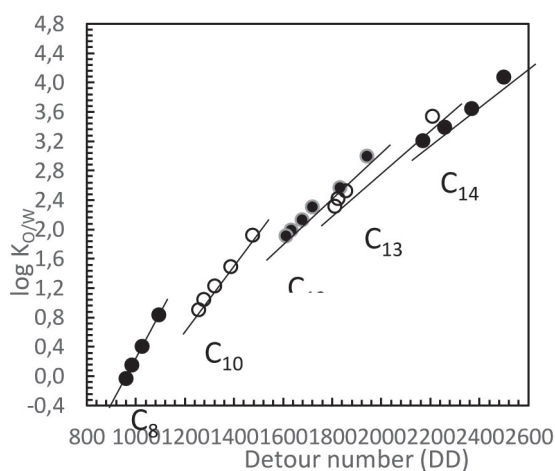
**Table 1. Experimental and computed values of the partition coefficient ( $\log K_{O/W}$ ) between n-octanol and water for some surfactants belonging sodium salts of alkylbenzene sulfonates**

Surfactant	$V_x$ [cm <sup>3</sup> /mol]	WN	DD	$\log K_{O/W}$ (exp.)	$\log K_{O/W}$ (calc.)
8-0	222.72	902	1094	0.84	0.679
7-1	222.72	836	1028	0.41	0.465
6-2	222.72	792	984	0.15	0.269
5-3	222.72	770	962	-0.03	0.089
10-0	250.9	1260	1476	1.92	1.738
9-1	250.9	1172	1388	1.49	1.507
8-2	250.9	1106	1322	1.23	1.293
7-3	250.9	1062	1278	1.05	1.096
6-4	250.9	1040	1256	0.91	0.917
12-0	279.08	1702	1942	3.00	2.862
11-1	279.08	1592	1832	2.57	2.614
10-2	279.08	1504	1744	2.31	2.364
9-3	279.08	1438	1678	2.13	2.169
8-4	279.08	1394	1634	1.99	1.972
7-5	279.08	1372	1612	1.91	1.793
13-0	293.15	1957	2209	3.54	3.450
9-4	293.15	1605	1857	2.53	2.526
8-5	293.15	1572	1824	2.42	2.338
7-6	293.15	1561	1813	2.32	2.168
14-0	307.26	2248	2500	4.08	4.058
13-1	307.26	2106	2358	3.65	3.793
12-2	307.26	2006	2258	3.39	3.545
11-3	307.26	1918	2170	3.21	3.314

Source: author's calculation.

As can be seen in data listed in Table 1 for surfactants having the same value of the A + B sum the numerical values of the molar characteristic volumes of the surfactant are the same (see for example for A + B = 12), but the value of both WN and PN as well as the experimental values of  $\log K_{o/w}$  are different. Therefore, we expect that all for molecular parameters ( $V_x$ , WN, DD and PN) may contribute to the observed value of  $\log K_{o/w}$ .

Figure 2 shows the relationship between experimental values of logarithm of the partition coefficient ( $\log K_{o/w}$ ) and the detour number (DD) for several isomeric forms of sodium salts alkylbenzene sulfonates. The numerical values of  $\log K_{o/w}$  were computed using author's method (Zieliński, 2017) based on various experimental data available in literature.



**Figure 2. Comparison between experimental values of  $\log K_{o/w}$  and detour number for several sodium salts of alkylbenzene sulfonates**

Source: author's calculation.

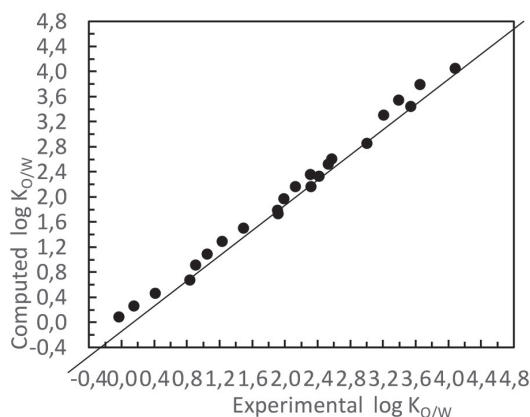
As can be seen in Figure 2, in each case a linear relationship is observed for a given total number of carbon atoms present in the hydrophobic moiety of molecules. It should be noted, however, that the slope for substances having various molecular weights are slightly different and its value decreases with the increase the size of the molecules. Therefore, this finding cannot be used for a prediction of the  $\log K_{o/w}$  values and toxicity for other surfactants belonging to the same homologue series.

Figure 3 presents a graphical comparison between experimental and computed values of logarithm of partition coefficient  $\log K_{o/w}$  for several isomeric forms of sodium salts alkylbenzene sulfonates. The computed numerical values of  $\log K_{o/w}$  were obtained using the total number of carbon atoms in the shorter alkyl chain

(N), characteristic volume ( $V_x$ ), detour number (DD) of the surfactant listed in Table 1. All these quantities can be easily calculated basing on the chemical structure of the surfactant molecule. As can be seen in Figure 3 we have found a good correlation between experimental and computed values of both  $\log K_{o/w}$  for a series of anionic surfactants belonging to sodium salts of alkylbenzene sulfonates having various structures of both alkyl part of the surfactant molecules. The resulting correlation equation obtained by means of the least squares method using Solver add-in (De Levie, 1999; De Levie, 2012) incorporated in MS Excel 2016 spreadsheet is as follows:

$$\log K_{o/w} = -0.16210 \cdot B + 0.026947 \cdot V_x + 0.000783 \cdot DD - 6.1793$$

This correlation equation describes the diagonal of the plot shown in Figure 2. As can be seen almost all experimental values of  $\log K_{o/w}$  are located on the diagonal or close to the diagonal of the plot. Also in this case such a good correlation ( $r^2 = 0.9921$ ,  $N = 23$ ,  $f = 19$ ) between  $\log K_{o/w}$  and tested variables were obtained using the number of carbon atoms in the side chain (B), the characteristic volume ( $V_x$ ), and detour number (DD) of the surfactant indicates the importance of the considered contributions to the experimental values of  $\log K_{o/w}$ . It was found that the application of this equation based on the detour number to sodium salts of alkylbenzene sulfonates allows for prediction  $\log K_{o/w}$  with the maximum error of 0.18 units.



**Figure 3. Comparison between experimental and computed values of logarithm of partition coefficient ( $\log K_{o/w}$ ) for several sodium salts of alkylbenzene sulfonates.**

Calculation based on detour number.

Source: author's calculation.

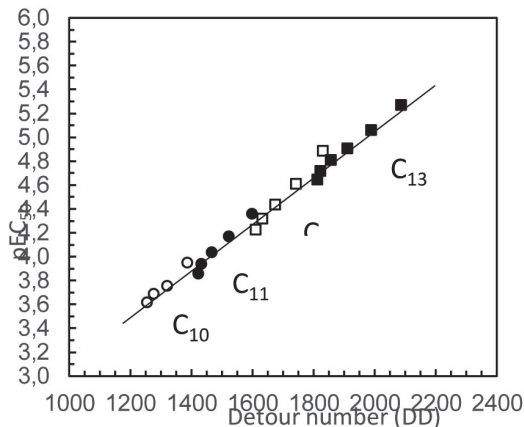
In the following part of this work we correlate information on the structure surfactants expressed by means of the detour number (DD) to corresponding

aquatic toxicity data. Table 2 contains computed values of the detour number (DD) as well as experimental (Roberts & Costello, 2003) and calculated aquatic toxicity data (pEC<sub>50</sub> based on molar concentration) against goldfish for pure isomers of 20 anionic surfactants belonging to a homologous series of sodium salts of alkylbenzene sulfonates.

Continuing our research interest in QASR related to toxicity of surfactants we correlate the detour numbers and corresponding experimental aquatic toxicity data for surfactants published in literature. Figure 4 presents a graphical comparison between experimental values toxicity (pEC<sub>50</sub>) of 20 chemically pure various isomers of sodium salts alkylbenzene sulfonates and computed values of the detour number (DD).

$$pEC_{50} = (0.001979 \pm 0.00051) \cdot DD + (1.133 \pm 0.085)$$

This correlation equation represent a straight line shown in Figure 4. As can be seen almost all experimental toxicity values (pEC<sub>50</sub>) are located on or very near the straight line in shown the plot. It is somewhat unexpected that the straight line is common for surfactants having different molecular weights and various shapes. Such a good linear correlation ( $r^2 = 0.9880$ ,  $N = 20$ ,  $f = 18$ ) obtained between toxicity data (pEC<sub>50</sub>) and detour number (DD) indicates the importance of the DD value in description and prediction toxicity data expressed in pEC<sub>50</sub> units.



**Figure 4. Correlation between detour number (DD) computed for various structural isomers of sodium salts of alkylbenzene sulfonates and experimental aquatic toxicity data (pEC<sub>50</sub> based on molar concentration) determined for goldfish**

Source: author's calculation based on toxicity data reported by Roberts & Costello (2003).

Detailed analysis of the data presented in Table 2 allows us to conclude that results of our study indicate that the detour number of surfactants allows for

prediction their aquatic toxicity against tested goldfish species with the maximum error of 0.03 pEC<sub>50</sub> units.

**Table 2. Value of detour number (DD) and experimental (exp.) and calculated (calc.) aquatic toxicity data (pEC<sub>50</sub>) against goldfish for pure isomers various sodium salts of alkylbenzene sulfonates**

Surfactant	DD	pEC <sub>50</sub> (exp.)	pEC <sub>50</sub> (calc.)
9-1	1388	3.95	3.879
8-2	1322	3.76	3.749
7-3	1278	3.69	3.662
6-4	1256	3.62	3.618
10-1	1599	4.36	4.297
9-2	1523	4.17	4.146
8-3	1467	4.04	4.035
7-4	1434	3.94	3.970
6-5	1423	3.86	3.948
11-1	1832	4.89	4.758
10-2	1744	4.61	4.583
9-3	1676	4.44	4.449
8-4	1634	4.32	4.366
7-5	1612	4.23	4.322
12-1	2088	5.27	5.264
11-2	1989	5.06	5.068
10-3	1912	4.91	4.916
9-4	1857	4.81	4.807
8-5	1824	4.72	4.742
7-6	1813	4.65	4.698

Source: author's calculation.

One can suppose that our finding graphically presented in Figure 4 can be applied not only for prediction toxicity of this group of surfactants but this observation seem to be more general. It is likely that similar correlations between detour number and aquatic toxicity should hold for various geometrical isomers of another groups of surfactants based on only very few experimental toxicity data.

## Conclusions

In this work we point out to a potential application of McGowan's characteristic volume, as well as two topological indices known as Wiener's number (WN) and the detour number (DD) of some anionic surfactants in prediction of the value of the partition coefficient ( $\log K_{O/W}$ ). We have successfully applied them to various surfactant molecules belonging to a group of sodium salts of alkylbenzene sulfonates. We have found a good correlation between experimental and computed values of  $\log K_{O/W}$  for geometrical isomers of a series of anionic surfactants belonging to sodium salts of alkylbenzene sulfonates having various structures of alkyl part of the surfactant molecules. A slightly better predictions of  $\log K_{O/W}$  values were found when the detour number (DD) was used. It was found that the application of this developed correlation equation based on the detour number to sodium salts of alkylbenzene sulfonates allows for prediction  $\log K_{O/W}$  with the maximum error of 0.18 units.

In another study it was found that the detour number (DD) is linearly correlated to the aquatic toxicity ( $pEC_{50}$  based on molar concentration) of various isomers of anionic surfactants belonging to sodium salts of alkylbenzene sulfonates. It was concluded that the detour number of surfactants allows for prediction its toxicity in against tested goldfish species with the maximum error of 0.13  $pEC_{50}$  units.

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