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Contents

Substantiation of hemp seeds storage and processing technologies for functional, dietary and specialty products. Review

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Abstract

Introduction. Analytical researches of the composition and quality of hemp seeds, their storage and processing methods and technologies for the production of functional, dietary and specialty products are presented.

Materials and methods. The subjects of research are aspects of the composition of industrial hemp seeds and its storing; peculiarities of production of hemp food products (oil, kernel, flour et al.); aspects of using hemp seeds and its derivative products. Research methods are the analysis of scientific works.

Results and discussion. Hemp seeds contain more than 30% of oil and about 25% of protein, a variety of minerals (Ca, Mg, P, K, S, Fe, Zn, etc.), dietary fiber and biologically active substances. The component composition and biological value of hemp seeds depend upon the region and growing conditions. Sustainable storage conditions are seeds moisture content of 8–11%, temperature of 14–18 °C and relative humidity of 50–55%. Products derived from processing of hemp seeds are oil, kernel, flour and protein concentrate. Oil is mainly extracted from seeds by mechanical pressing. Hemp oil contains fatty acids such as linoleic, linolenic and γ-linolenic acids, with the latter promoting the formation of γ-globulin, which has an important function in the human immune system. Hemp oil tocopherols act as antioxidants in alimentary, dietary and specialty products. Seed shelling machine did the hemp seeds de-hulling. The resulting product is rich in essential amino acids. Flour, fiber and protein concentrate are produced from hemp cake. Publications deal superficially with the relationship between the factors of material preparation, production process variables of hemp products, storage conditions and time in terms of the content of functional and biologically active components. Utilization of hemp seeds and their derivatives enhances the biological and nutritional value, functional and sensory properties of foods. Further research on the use of drugs to regulate the antimicrobial and antioxidant properties of functional, dietary and specialty products is of paramount importance.

Conclusion. The article substantiates the relevancy of using the presented theoretical, scientific and practical insights in integrated solutions for the processing of environmentally sound industrial and medical hemp seeds.

Introduction

There is an increased interest of producers and consumers in products from industrial hemp seeds.

This requires increased scientific and practical knowledge and accelerated efforts in the systemic research into the integrated processing of seeds for the production of functional and specialty foods, dietary supplements and drugs.

Materials and methods

Materials

The subjects of research are:

- Aspects of the composition of industrial hemp seeds;
- Aspects of storing hemp seeds;
- Peculiarities of production of hemp food products (oil, kernel, flour, protein concentrates)
- Aspects of using hemp seeds and its derivative products.

Methods

Research methods are the analysis of scientific works.

Results and discussion

1. Aspects of the composition of industrial hemp seeds

Hemp seed (Figure 1) is a one-seeded fruit – a roundish egg-shaped nutlet consisting of an outer hard coat and a kernel located in the middle, surrounded by a thin film of dark green color. The seed has two cotyledons, a radicle and a budlet, which have grown together into a single whole – the embryo. The bulk of the nutrients in hemp seeds are concentrated in the embryo. Hemp seeds contain more than 30% of oil and about 25% of protein, as well as a fair amount of minerals, scarce coarse dietary fiber (cellulose, hemicellulose, pectin, and lignin) and biologically active substances (phospholipids, fatty acids, and vitamins). Hemp seeds mainly include edestin protein, as well as nitrogen-containing substances such as nucleic, choline and a small amount of trigonelline. In addition, 37 chemical elements are found in hemp seeds, of which calcium, magnesium, phosphorus, potassium, sulfur are dominant, as well as a small amount of iron and zinc (Sukhorada et al., 2009; Shewry et al., 2000; Yufriakova. et al., 2020). Shashkarov with co-authors (2016) additionally emphasizes the presence of rare earth elements in hemp seeds such as thorium, selenium, molybdenum, zirconium and beryllium.

Figure 1. Hemp seeds

Comparison of the composition and quality indicators of hemp seeds from different parts of the world is shown in Table 1.

Table 1

Composition and quality characteristics of hemp seeds

Component	Content, %, in hemp seeds of different region of production				
	Pakistan (Anwar et al., 2006)	**Russia (Serkov et al., 2011)**	**Canada (Vonapartis et al., 2015)**	**Ukraine (Oseyko et al., 2019)**	**USA (Lan et al., 2019)**
oil	26.9–31.5	30.24	26.9–30.6	33.3±0.5	24.3–28.1
linoleic acid*	56.5–60.5	78.60	59.7	54.8–56.9	-
α-linolenic acid*	16.9–20.0	19.52	17.0	16.0–18.5	-
protein	23.0–26.5	21.3	23.8–28.0	22.5±0.15	32.7–35.9
fiber	17.0–20.5	17.71	-	32.3±0.2	-

* from the total amount of fatty acids.

As can be seen from Table 1,

- Ukraine has hemp seeds with the highest content of oil (33.3%) (Oseyko et al., 2019), with the United States having the smallest (24.3–28.1%) (Lan et al., 2019);
- United States have hemp seeds with the highest content of protein (32.7–35.9%) (Lan et al., 2019), with Russia having the smallest (21.3%) (Serkov et al., 2011);
- Ukraine has hemp seeds with the highest content of fiber (32.3%) (Oseyko et al., 2019), with Russia having the smallest (17.71%) (Serkov et al., 2011).
- The ash content in hemp seeds from different regions ranges from 5.0 to 7.6% (Anwar et al., 2006; Serkov et al., 2011; Vonapartis et al., 2015; Lan et al., 2019).

Comparative analysis of the composition of mineral substances in hemp seeds from different parts of the world is shown in Tables 2 and 3.

Table 2

Content of macroelements in hemp seeds

Macroelement	Content in hemp seeds of different region of production		
	Russia (Serkov et al., 2011)	Ukraine (Oseyko et al., 2019)	USA (Lan et al., 2019)
Phosphorus, g/kg	1.11	8.9	4.1
Calcium, g/kg	0.28	0.9	-
Potassium, mg/kg	1.07	-	-
Magnesium, g/kg	-	2.4	3.4

Table 3

Content of microelements in hemp seeds

Microelement	Content in hemp seeds of different region of production		
	Romania (Mihoc et al., 2013)	Ukraine (Oseyko et al., 2019)	USA (Lan et al., 2019)
Iron, mg/kg	130-164	74.7	46.7
Zinc, mg/kg	42-57	56.1	28.2
Cobalt, mg/kg	-	0.5	-
Manganese, mg/kg	89-108	59.4	169.1
Copper, mg/kg	10-12	-	29.0
Nickel, mg/kg	1.6-6.1	-	41.0
Chromium, μg/kg	598-877	-	-
Molybdenum, μg/kg	265-652	-	-
Lead, μg/kg	217-626	-	-

As can be seen from Tables 2 and 3,

- Hemp seeds from Ukraine (Oseyko et al., 2019) and Russia (Serkov et al., 2011) were found to have the highest (8.9 g/kg) and the lowest (1.1 g/kg) phosphorus content, respectively;
- Hemp seeds from Romania (Mihoc et al., 2013) and USA (Lan et al., 2019) were found to have the highest (130–164 and 42–57 mg/kg) and the lowest (46.7 and 28.2 mg/kg) content of iron and zinc, respectively;
- Hemp seeds from the USA (Lan et al., 2019) and Ukraine (Oseyko et al., 2019) were found to have the highest (169.1 mg/kg) and the lowest (59.4 g/kg) manganese content, respectively.

According to summarized data, the component composition and biological value of hemp seeds depend upon the region and growing conditions, and, obviously, on the seed material quality. Breders should pay due attention to the improvement of existing varieties of industrial hemp and development of new ones based on their primary and functional purpose.

2. Aspects of storing hemp seeds

As hemp seeds are stored, their composition and quality characteristics change, which affects their further processing. Among the main parameters that affect the shelf life of hemp seeds are moisture content, temperature and storage time.

Klevtsov with co-authors (2015) determined the physical and mechanical properties of hemp seeds: 1000 kernel weight, bulk weight, moisture content, density of seeds, angle of repose, static and dynamic friction coefficients, flowability and intergrain space. This allows addressing practical issues. Physical and mechanical properties of hemp seeds were assessed based on the characteristics of grain masses. These properties have a bearing on the organization of seed harvesting and further processing of hemp seeds. Samples of hemp seeds of Ukrainian varieties Zolotonos'ki 15 and YuSO-31 had a bulk density ranging from 513 to 586 kg/m^3; 1000 kernel weight of 15.2–17.7 g; static internal friction coefficient of 0.47–0.55; dynamic external friction coefficient of 0.30–0.52; static external friction coefficient of 0.29–0.37. The data show that hemp seeds are extremely free flowing materials. The characteristics that determine the flowability of hemp seeds can be used to simulate their behavior as they move by gravity through sieves, containers, and the like.

Sacilik with co-authors (2003) proved that the higher moisture content of industrial hemp seeds correlates with the higher 1000 kernel weight, the angle of repose and the friction coefficient.

Sova with co-authors (2019) investigated the quality of industrial hemp seeds at the stages of post-harvest handling, namely: seeds from stems (sample 1), seeds from the bunker of a combine harvester (sample 2), seeds after primary cleaning on an OVS-25 grain cleaner (sample 3), seeds after drying on a stationary grain dryer (sample 4), and commercial seeds after sorting on the PETKUS K531 GIGANT grain cleaner (sample 5). Samples 4 and 5 had the moisture content not exceeded 11%. The seed purity of the mechanically harvested sample 2 was less than that of the manually harvested sample 1. The mass fraction of oil in samples 2 and 3 decreased in comparison with that of sample 1. The increased acid value of oil in seed samples 2, 3, and 4 as opposed to sample 1 is attributed to the grain damage during their mechanical treatment. Following sorting, the acid value of oil in seeds decreased. Sample 1 was found to be the most resistant to oxidative deterioration during storage. Mechanized harvesting of hemp seeds requires stages of cleaning, sorting and wet seed conditioning. The identified imperfection of the drying process affected the increase in the peroxide value of oil. A decrease in the 1000 kernel weight was common for all post-harvest handling stages as opposed to sample 1.

The method of harvesting hemp seeds was found to affect the change in its physical and chemical parameters. Thus, the water level indicator for manually harvested seeds (sample 1) was 2.5% less than for mechanically harvested seeds (sample 2). Mass fraction of oil in sample 1 was 0.7% more than that of sample 2. Mechanized harvesting of hemp seeds was found to increase the acid and peroxide values of oil extracted from samples 1 and 2 by 0.22 mg KOH/g and 0.65 ½ O mmol/kg, respectively. Thus, harvesting and drying of hemp seeds deserve special attention (Sova et al., 2019).

Lukianenko with co-authors (2009) revealed the extent to which the main factors of drying hemp seeds (layer thickness, air flow rate, flow temperature and layer mixing) affected the process time and quality indicators of the product. The germination capacity and quality indicators of hemp seeds were most influenced by the air temperature above 60 °C in the drying zone.

According to (Small et al., 2012), hemp seeds of Canadian varieties were exposed to a combination of four temperature regimes (20, 5, -20 and -80 °C) and three seed moisture

indicators (11, 6 and 4%) for 66 months. Storage of hemp seeds with a moisture content of 11% at 20 °C was found to reduce the germination capacity to zero in less than 18 months. A decrease in temperature to 5 °C and moisture content to 6% had a positive effect on the survival of seeds. There has been no evidence of benefit from oxygen-free storage.

It was found in (Mishchenko, 2013) that the longer storage time of hemp seeds of Ukrainian varietiesresulted in the lower germination energy and capacity of seeds. Germination capacity showed a rather sharp decline after three years and was actually lost after four years under normal storage conditions.

Pariharwith co-authors (2014) investigated the effect of moisture, temperature and storage time on the germination capacity and survivability of hemp seeds released in India. The study was carried out with a combination of moisture content indicators (5, 7, 8, 10 and 12%), temperature indicators (environment, 15 and -20 °C) and various storage periods (0, 3, 6, 9, 12, 18, 24 and 36 months). The critical moisture content was 5% and was found to increase to 7% at a storage temperature of 15 °C, and to 12% at -20 °C. With a moisture content of 5 and 7%, the survivability of hemp seeds was maintained for up to 36 months of storage, and with 8% up to 12 months of storage. A complete loss of survivability was reported after 24 months of storage of hemp seeds with a moisture content of 12%, whereas a germination capacity decrease of more than 40% was observed after storage of hemp seeds for 36 months at 15 °C.

Suriyonga with co-authors (2015) revealed the effect of storage conditions on the quality of hemp seeds grown in Thailand. The hemp seeds were packed in aluminum foil and a polypropylene bag. Seeds packed in aluminum foil were stored at room temperature and at temperatures of 15, 4 and -4 °C, and seeds in the polypropylene bag were stored at room temperature. The hemp seeds underwent a monthly quality control for 12 months. As a result, hemp variety, storage conditions and shelf life, and interactions between these parameters were found to influence seed quality. During storage, the moisture content of hemp seeds packed in the polypropylene bag varied with moisture control. Germination capacity and energy of seeds packed in both types of materials did not change for 6 months of storage at room temperature, with a germination energy decrease of 30% observed during 8-12 months of storage. It should be noted that the germination energy of hemp seed samples stored at temperatures of 15, 4 and -4 °C for a year remained almost unchanged. Therefore, a temperature of 15 °C (cold room) was suggested as the optimal storage condition for hemp seeds.

When stored, grain produces heat and moisture due to the vital activity (respiration) of grain mass (seeds, microorganisms, kernels, and impurities) and oxidation of organic substances. In addition, grains and seeds can absorb water vapor and gases from the environment. The degree of moisture absorption by the grain mass predetermines its hygroscopicity, which depends on the colloidal, physical, and structural properties of seeds (Oseiko, 2006).

Special attention should be given to the sorption properties of seeds when stored under various conditions, since it is the high oil content of oilseeds that makes their equilibrium moisture content significantly lower than that of grain crops. According to the research findings (Klevtsov, 2015), the most active moisture absorption was found to occur at a temperature of 25 °C and a relative humidity of 80%, with the lowest equilibrium moisture content recorded in seed samples at a temperature of +5 °C and a relative humidity of 50%. The equilibrium moisture content of hemp seeds is higher than that of flax seeds. As a result, hemp seeds can be stored in the relative humidity range of 50-80% until equilibrium moisture content is reached. Equilibrium moisture content was also found to increase with increasing storage temperature from 5 to 25 °C within the same relative humidity.

Hemp seeds of Ukrainian varieties are placed and stored in grain warehouses in accordance with applicable sanitary regulations and storage conditions. Transportation and storage of hemp seeds should take into account their conditions in terms of moisture and dockage. Reasonable conditions for storing hemp seeds for their subsequent complex processing are seeds moisture content of 8-11%, temperature of 14–18 °C and relative humidity of 50–55%. It is advisable that hemp seeds be stored in an anaerobic environment with minimal exposure to light (Oseyko et al., 2020).

Nataša with co-authors (2020) tested various chemical agents to obtain microbiologically safe industrial hemp seeds. Such seeds can be used for further use in various food technologies (with a reduced total microbial count, total yeast and mold counts). Reasonable storage conditions were different for different microorganisms. For hemp seeds produced in 2018, the room temperature storage was the most optimal. Storing seeds in hermetically sealed bags at refrigerator / freezer temperatures revealed a suppressed yeast and mold growth. For hemp seeds produced in 2019, storage in the refrigerator (to reduce the number of enterobacteria) and in the freezer (to reduce the total microbial count) were the reasonable storage conditions. For reduced total yeast and mold counts, room temperature storage was the reasonable storage conditions. Ethanol (75 vol%) was found to be the most effective disinfectant among the chemicals tested (ethanol, sodium bicarbonate, and sodium hypochlorite).

According to (Oseyko et al., 2020), the long-term storage of industrial hemp seeds of Ukrainian varieties was found to yield the moisture content ranging from 8.2 to 10.1%, seed purity of 97.5–99.8%, and seed oil content of 31.9–34.3%. The decrease in the seed oil content observed from the second half of the storage period till the end can be attributed to the biochemical processes taking place in it throughout the long-term storage. That said, 1000 kernel weight ranged from 17.7 to 19.2 g, with the bulk weight of the hemp seeds ranging from 503.8 to 530 g/l.

Summarizing the findings, researchers and producers should give special attention to the post-harvest handling conditions, as well as to the methods and conditions of storage of industrial hemp seeds for food purposes.

3. Peculiarities of oil extraction from hemp seeds

Mechanical pressing is the principal method for hemp oil extraction (Figure 2).

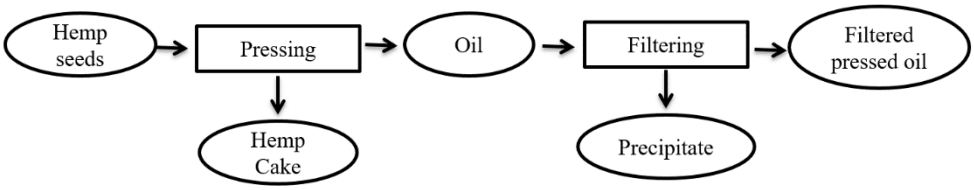

Figure 2. Cold-pressed hemp oil production scheme

Hemp oil is a rare source of nutrition through a unique ω-6/ω-3 fatty acid ratio of 3:1 (Devi et al., 2019; Leizer et al., 2000). This is beneficial for the prevention and health of the cardiovascular, ophthalmic and other human body systems (Oseyko et al., 2020; Oseyko et al., 2019). This adds value to the production of highly refined hemp oil in the food industry and related industries (Devi et al., 2019).

According to (Latif et al., 2009) the cold pressing of the material involved pretreatment of hemp seeds with enzyme preparations (Protex 7L, Viscozyme L, Kemzyme, Feedzyme, and Natuzyme). The oil content in the experimental samples (28.4-32.8%) was higher than that in the test sample (26.7%). According to the authors' data, the enzyme treatment was not affected by the content of protein, fiber and ash in seeds. No notable variations were found for the iodine value, refractive index, density and fatty acid composition of oil. The content of tocopherols in the experimental samples of hemp oil (724.4–788.8 mg/kg) was found to be higher than that in the test sample (691.2 mg/kg). When analyzing the research results, it was desirable to bring reasonable pressing temperature, dosing conditions for enzymes or mixtures of enzymes, as well as to refine additional technological operations.

Morar with co-authors (2010) determined the efficiency coefficient of cold pressing of hemp seeds (from 23.89 to 27.69% of resulting oil) with the oil content of the material from 30.89 to 33.25%. The cold pressing process was also influenced by the quality of hemp seeds, which yielded reasonable values when using a pressing nozzle with a diameter of 8 and 10 mm. The acid and peroxide values of hemp oil ranged from 0.65 to 4.45 mg KOH/g of oil and from 0.62 to 26.91 mEq O_2/g, respectively. The authors recommend using the findings as a basis for further research and launching an information campaign to raise consumer awareness of the beneficial and therapeutic health effects of hemp oil.

Da Porto with co-authors (2012) applied an experimental design methodology to optimize the process of in-vitro extraction of oil from hemp seeds using supercritical carbon dioxide. The independent variables were operating temperature (40, 50 and 60 °C), pressure (250, 300 and 350 bar) and particle size of the material (0.59, 0.71 and 0.83 mm). A second order polynomial equation was used to express oil yield and oxidation stability as a function of independent variables. The responses and variables were made consistent with each other using multiple regression equations. The maximum oil yield of 21.50% was obtained by extraction with supercritical carbon dioxide at a temperature of 40 °C, pressure of 300 bar and particle size of 0.71 mm. The highest oil oxidation stability (2.35 Eq α toc/ml of oil) was obtained at a temperature of 60 °C, pressure of 250 bar and particle size of 0.83 mm.

It was found in (Aladić et al., 2014) that a reasonable condition for obtaining an oil yield of 23.34% during cold pressing with subsequent extraction using supercritical CO_2 was a temperature of 60 °C, frequency of 20 Hz, and a 6 mm diameter nozzle. Oil (10.33%) was extracted from the press cake completely with supercritical CO_2. According to the authors, oregano essential oil has served as the best antioxidant in protecting hemp oil from oxidative breakdown.

Aladić with co-authors (2015) compared the yield and composition of hemp oil extracted with supercritical CO_2, n-hexane using a Soxhlet apparatus, and expeller pressing. Supercritical CO_2 extraction yielded extracts with a higher tocopherol content. The amount of α-tocopherol in supercritical extracts ranged from 37.09 to 110.61 mg/kg, depending on the applied technological conditions. The content of γ-tocopherol was 2-3 times higher. The content of pigments in hemp oil obtained by extraction with supercritical CO_2 ranged throughout the extraction process from 9.79 to 178.76 mg/kg of chlorophyll and from 8.15 to 57.66 mg/kg of carotene.

Devi with co-authors (2019) investigated various processes of hemp oil extraction (supercritical fluid extraction, Soxhlet, and ultrasonic treatment). Comparison was made in terms of economic assessment of an industrial scale, hemp oil yield and composition as well as physical and chemical properties of hemp oil. The maximum oil yield of 37.3% was obtained from material pretreated with ultrasound using the Soxhlet method.

Da Porto with co-authors (2015) described the pretreatment of hemp seeds without solvent exposure for 10, 20, and 40 minutes prior to oil extraction with supercritical CO_2 at

a temperature of 40 °C, pressure of 300 bar, and CO_2 consumption of 45 kg CO_2/kg of seeds. The maximum oil yield of 24.03% was obtained after a 10-minute ultrasonic pretreatment.

According to (Crimaldi et al., 2017), a combination of a high pressing temperature (70 °C) and a low screw speed (22 rpm) was found to positively affect the oil yield for the Italian variety hemp seeds under experimental conditions when using pretreatment (heating for an hour at 50 °C).

The authors in (Subratti et al., 2019) recommended using liquefied dimethyl ether as the most effective in a comparative study on the application of organic solvents in the extraction of hemp seed oil.

Esmaeilzadeh Kenari with co-authors (2020) established reasonable conditions for the use of solvent mixtures of hexane and isopropanol (0:100, 50:50, 100:0), extraction temperatures (30, 45 and 60 °C) and sonication time (30, 60 and 90 min). Reasonable conditions were obtained at a hexane-to-isopropanol ratio of 60:40, a temperature of 40.26 °C, and a sonication time of 54.4 minutes.

Summarizing the studied data, scientists and manufacturers should place special emphasis on:

- The material pretreatment methods prior to oil extraction and their effect not only on the yield of the finished product, but also on its quality and safety performance;
- Conditions for obtaining hemp oil;
- Biological, ecological and economic efficiency of production.

3.1 Peculiarities of the composition of hemp oils

Hemp seed oil is distinguished not only by its decent taste, but also by its unique fatty acid profile and the content of associated biologically valuable substances. So, hemp oil contains fatty acids, five of which are polyunsaturated. Linoleic, linolenic and γ-linolenic fatty acids are the most biologically valuable. γ-linolenic acid promotes the formation of γ-globulin, which is instrumental in human immunity. In addition, hemp oil contains tocopherols (vitamin E), which act as antioxidants in both food and other foods. Hemp oil is known to have healing properties and is recommended for use in cataracts, glaucoma, diabetes mellitus, asthma, sclerosis, epilepsy, as well as for cancer prevention (Orhan et al., 2000; Virovets et al., 2014; Laiko Iet al., 2014).

Information on the fatty acid composition of oils obtained in various ways from hemp seeds found in different parts of the world is given in Table 4.

As can be seen from Table 4,

- Oil obtained from experimental varieties of Russian regions (Shelenga et al., 2010; Shelenga et al., 2012; Iurchenko et al., 2019) was found to have the highest (17.4%) and the lowest (7.5%) content of saturated fatty acids, in particular palmitic and stearic acids;
- Oil obtained from Ukrainian varieties (Oseyko et al., 2020) was found to have the highest (19.4%) content of monounsaturated fatty acids, in particular oleic acid, with that from Russian varieties (Grigorev, 2019) having the lowest;
- Oil obtained from experimental varieties of Russian regions was found to have the highest (96.4%) and the lowest (48.8%) total content of polyunsaturated fatty acids, in particular linoleic, α-linolenic, γ-linolenic and arachidonic acids;
- Oil obtained from Iranian varieties (Abdollahi et al., 2020) was found to have the highest ω-6-to-ω-3 ratio of 7.6:1, with that from Russian varieties (Iurchenko et al., 2019) having the lowest;

Table 4

Fatty acid composition of hemp oils

Hemp oil described in	Fatty acid content, %							
	Linoleic	α-linolenic	Oleic	Palmitic	Stearic	γ-linolenic	Arachidonic	ω-6:ω-3
Anwar et al., 2006	56.5–60.5	16.8–20.0	10.2–14.0	5.7–8.3	2.2–2.8	0.6–1.6	-	2.8:1–3.2:1
Shelenga et al., 2010	42.6–57.4	10.6–22.3	8.9–15.0	6.6–14.3	1.7–3.1	1.4–7.8	0.3–2.1	2.4:1–4.6:1
Serkov et al., 2011	58.4–59.1	19.5–20.1	12.1–12.8	-	-	-	-	2.9:1–3:1
Vyrovets et al., 2011	36.0–57.0	12.0–19.0	11.9–18.8	5.8–9.9	2.5–3.5	0.7–3.8	0.1–1.1	2.5:1–2.8:1
Da Porto et al., 2012	59.6	18.0	-	-	-	3.4	-	2.8:1
Shelenga et al., 2012	42.6–57.4	10.6–22.3	8.9–15.7	6.6–14.3	1.7–3.1	1.4–7.8	0.1–2.6	2.6:1–3.1:1
Montserrat-de la Paz et al., 2014	55.0	16.0	11.0	-	-	-	-	3.4:1
Shashkarov et al., 2016	50.0–70.0	15.0–25.0	-	-	-	-	-	2.8:1–3.3:1
Mikulcova et al., 2017	55.3–57.3	16.7–20.3	9.0–12.1	5.9–6.2	2.2–2.4	3.0–4.4	1.0–1.7	2.8:1
Sova et al., 2018	54.8–55.0	14.6–14.8	16.1–16.2	6.0	3.0–3.1	2.3	1.0	3.6:1
Baibekov et al., 2019	55.8	15.2–17.8	13.4–13.5	5.8–10.7	2.6–2.8	-	-	3.1:1–3.8:1
Iurchenko et al., 2019	36.0–50.0	15.0–28.0	6.0–16.0	5.8–9.9	1.7–5.6	-	-	1.8:1–2.9:1
Grigorev, 2019	53.4–64.2	12.6–27.1	5.9–14.0	-	-	0.6–5.1	-	2:1–4:1
Oseyko, 2019	54.8–56.9	16.0–18.5	13.3–13.6	5.7–6.3	3.0–3.2	1.3–2.8	0.8–2.4	3:1–3.7:1
Abdollahi et al., 2020	57.5–64.0	7.6–22.9	-	-	-	-	-	2.8:1–7.6:1
Serkov et al., 2020	57.0	16.0	12.0	-	-	3.3	-	3:1
Oseyko et al., 2020	53.4–56.6	11.3–16.2	14.9–19.4	5.6–6.6	3.3–3.5	1.6–2.6	-	3.4:1–5:1

Comparative characteristics of the tocopherol content in hemp oils from different parts of the world are given in Table 5.

Table 5

Comparative characteristics of the tocopherol content in hemp oils

Hemp oil described in	Tocopherol content, mg/kg			
	α-	δ-	γ-	β-
Anwar et al., 2006	54.0–60.4	35.0–45.6	600.0–745.0	-
Vonapartis et al., 2015	-	7.74	24.81	-
Kriese et al., 2005	18±0.5	12±0.4	217±3.2	2±0.04
Montserrat-de la Paz et al., 2014	73.4±2.86	-	-	-
Oseyko et al., 2019	234.0–246.2	12.8–14.0	316.0–322.0	

As can be seen from Table 5,

- Oil samples from Pakistan (Anwar et al., 2006) and Canada (Vonapartis et al., 2015) were found to have the highest (745 mg/kg) and the lowest (24.81 mg/kg) γ-tocopherol content, respectively;
- Oil samples from Ukraine (Oseyko et al., 2019) and Germany (Kriese et al., 2005) were found to have the highest (246.2 mg/kg) and the lowest (18 mg/kg) α-tocopherol content, respectively;
- Oil samples from Pakistan (Anwar et al., 2006) and Canada (Vonapartis et al., 2015) were found to have the highest (45.6 mg/kg) and the lowest (7.74 mg/kg) δ-tocopherol content, respectively.

Summarizing the data of section 3.1, it should be noted that the reviewed publications give insufficient attention to the relationship between the factors of material treatment, parameters of hemp oil production, conditions of hemp oil purification and long-term storage in terms of the content of fatty acids and tocopherols.

3.2 Oxidation stability of hemp oil during storage

Thanks to its high content of ω-6 and ω-3 fatty acids and minor biologically active components with antioxidant activity, hemp oil is now generally recognized by consumers as health-promoting. Although tocopherols, polyphenols, and phytosterols prevent the oxidative degradation of hemp oils, high levels of chlorophyll can adversely affect the quality of oil (Liang et al., 2015).

Sapino and co-authors (2005) provided a comparison of hemp oils and olive oil in terms of some physicochemical quality indicators and oxidation stability assessment. The peroxide value of hemp oil (1998 and 1999 samples) was 1.57 and 5.15 ½ O mmol/kg. Hemp oil was less resistant to peroxidation than olive oil. Chlorophyll found in extra virgin olive oil had a higher photostability than that found in hemp seed oil, possibly due to higher antioxidant content in olive oil. Hemp oil was found to contain a certain amount of vitamin E (0.08 and 0.25 mg/l).

According to Abuzaytoun with co-authors (2006), the oxidative stability of flax oil and hemp oil, as well as their compositions devoid of minor components, were evaluated in the dark at 60 °C and under fluorescent light at 27 °C. According to the authors, the biologically active constituents of these edible oils are instrumental in their oxidative stability. However,

their stability is contributed by the composition of phenolic antioxidants and total tocopherols in oil, as well as the type of pigments. Untreated flax oil and hemp oil compositions showed higher stability. In addition, non-fibrous hemp oil had a higher oxidative stability than untreated flax oil. This was evidenced by the purification of the 1,1-diphenyl-2-picrylhydrazyl radical and the data on total phenols.

Raikos with co-authors (2015) investigated the effect of heating, storage and light exposure on the oxidative stability of the dispersed phase of the emulsion – hemp oil. The lipid oxidation rate increased following heat treatment and exposure to light, while oxidation markers remained relatively unchanged during storage of the emulsion at 4 °C for 10 days. The induction period of the emulsions was reduced to 26%. The concentration of substances reacting with thiobarbituric acid increased 4.5 times, depending on the processing conditions.

It was found in (Lamotkin et al., 2016) that during the determination of resistance of the composition of rape oil and hemp oils to oxidation by air oxygen (64:36) with a ω-6-to-ω-3 ratio of 10:1, after 5 days of bubbling, the acid, peroxide and anisidine values of the composition were 2.99 mg KOH/g, 22.62 mmol (SO)/kg and 2.98 U/g, respectively. Based on the findings, the authors concluded that the composition of rape oil and hemp oils is not stable during storage.

In (Liang et al., 2018), ultrasonic treatment of cold-pressed hemp oil combined with bleaching clays (sepiolite, activated bentonite and industrial clay) proves very effective in reducing chlorophyll content from 56.3 to 14.8, 9.9 and 7.8 μg/kg, respectively. The method is not only rapid and clean but requires significantly less bleaching clay. Hemp oil treated in this way exhibits greater oxidative stability making it more attractive for industrial and consumer use. The results of ultrasonic bleaching suggest its potential for prolonging the shelf-life of oil. According to the authors, utilizing the ultrasonic bleaching technique as an alternative to conventional bleaching would be beneficial to the edible oil industry.

Hamidioglu1with co-authors (2019) investigated the stability of hemp oil using natural plant extracts such as rosemary, pomegranate, and green tea, together with vitamin E. The concentration of each plant extract was 30 mg/l and 50 mg/l. Vitamin E was mixed with oil in an amount of 2 g/l. The value of the induction period of oil with additional plant extracts was significantly higher than that of the test samples. The vitamin E oil sample exhibited the longest induction period (4.12±0.04 hours at 120 °C) during the Rancimat test. The authors attribute this to the strong antioxidant ability of the tocopherol content in vitamin E. The induction period of the hemp oil sample with added extracts in the amount of 30 mg/L was 3.56±0.06 hours for pomegranate, 3.67±0.05 hours for green tea, 3.69±0.03 hours for rosemary; in the amount of 50 mg/L: 3.6±0.03 hours for pomegranate, 3.7±0.01 hours for green tea, 3.89±0.02 hours for rosemary. Herbal extracts and vitamin E had a positive effect on the peroxide value of hemp oil as opposed to the test sample. The peroxide value of the test sample was 19.4±0.12 mEq/kg, of the sample with added vitamin E – 12.14±0.17 mEq/kg, with added extracts in the amount of 30 mg/l: 14.43±0.06 mEq/kg for rosemary, 15.23±0.05 mEq/kg for green tea, 16.1±0.09 mEq/kg for pomegranate; in the amount of 50 mg/l: 13.12±0.17 mEq/kg for rosemary, 14.55±0.08 mEq/kg for green tea, 15.76±0.13 mEq/kg for pomegranate.

Moczkowska with co-authors (2020) characterized the effectiveness of antioxidants of rosemary extract obtained using various solvents (ethanol, methanol, acetone, and ethyl acetate) on the quality of hemp oil and its storage stability. The effectiveness of antioxidants was compared with hydroxytoluene butyl ether. Rosemary methanolic and ethyl acetate extracts showed the highest and lowest total phenols and antioxidant capacity, respectively. The lowest value of reaction substances with thiobarbituric acid following 14 days of storage was established for the rosemary methanolic and acetone extracts, 1.01 and 1.18 μmol/kg,

respectively. Utilization of rosemary extract indicates a greater antioxidant effect on certain fatty acids, such as α-linoleic acid, compared to the reference. According to the authors, rosemary extract can provide a natural alternative to synthetic antioxidants.

According to (Babiker et al., 2021), reasonable heat treatment of hemp seeds (14 min at 160 °C) brought about an increase in phenolic acids, polyphenols, and glycosylated flavonoids. Roasting of seeds had little effect on the fatty acid content. The amount of phosphorus and magnesium in hemp seeds dropped significantly, but the amount of calcium, iron, copper, manganese and zinc increased over time of roasting.

Summarizing the studied data, scientists and manufacturers should place significant emphasis on:

- The utilization of plant extracts as antioxidants instead of synthetic ones;
- The conditions for obtaining and storing hemp oil in order to stabilize the composition and quality indicators.

4. Peculiarities of production of hemp food products

4.1. Aspects of obtaining a hemp kernel

Petrachenko with co-authors (2019) characterized one of the promising directions of processing non-narcotic hemp seeds – obtaining a hulled seed kernel. Profound insight into structures of mechanisms for hulling with different operation principle allowed determining the peculiarities, pros and cons of the single-blow and multiple-blow techniques. The effect of the shape of the working body (impeller or disk) of the hulling mechanism on the ability to destroy the seed coat has been clarified. The oriented single-blow technique, which is implemented in the design of a centrifugal dehuller, was found to be more effective in terms of dehulling hemp seeds. The impeller of a closed sectoral type has been found to have the prospect of further utilization and requires in-depth research.

The hemp kernel production scheme is shown in Figure 3.

Prior to the hemp kernel production, hemp seeds are tested for purity and moisture. Industrial hemp seeds are loaded into a dehulling system, where the fruit coat is destroyed and the kernel is released. The resulting mixture, which consists of a ready-made hemp kernel, semi-crushed and whole industrial hemp seeds, coats and chaff, is divided into fractions. The hemp kernel yield ranges from 33.2 to 41.4%, chaff – from 0.6 to 5.2%, substandard seeds from 1.3 to 4.8%, intermediate products – from 53.3 to 61.2% (Oseyko et al., 2020).

Some manufacturers split the mixture in two stages. The first stage provides for separation into 4 fractions on a sieve cleaner: coats (waste following the air part of the separator); whole and semi-crushed seeds – rejects from the upper sieve; ready kernel – rejects from the lower sieve; chaff – outsiftings from the lower sieve. After that, the ready kernel ends up in another air cleaner that yields two fractions – ready kernel and chaff. The waste products of this technology are coats and two chaff alternatives.

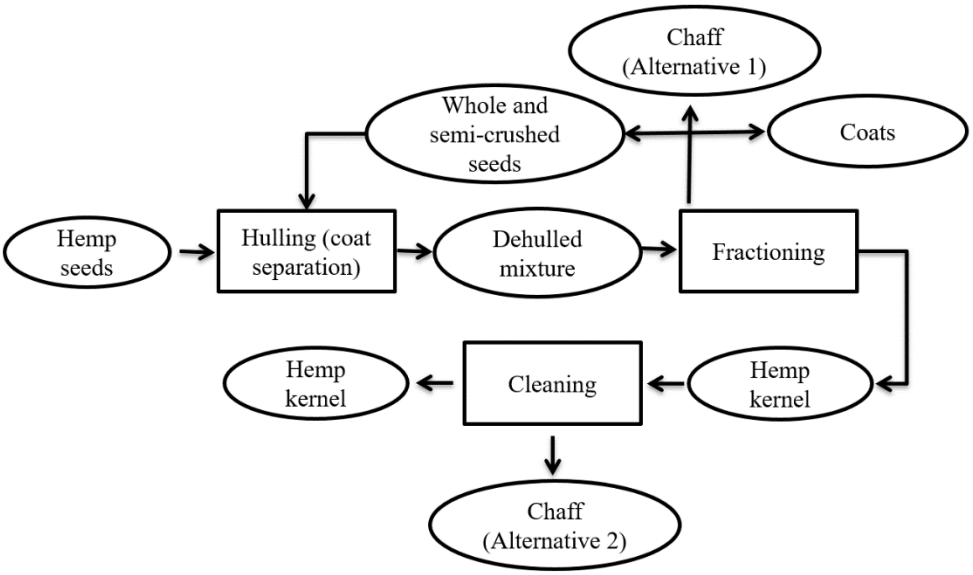

Figure 3. Hemp kernel production scheme

Physicochemical indicators of quality and amino acid composition of hemp kernel in comparison with the raw material input are given in Tables 6 and 7 (Oseyko et al., 2019).

Table 6

Physicochemical indicators of quality of hemp kernel (Oseyko M. et al., 2019)

Component	Content		
	Hemp seed findings		"Hemp-Flax"**
	Hulled	Whole	
Moisture content, %	7.0±0.02	8.4±0.02	≤7.0
Mass fraction of impurities, %	0.4±0.02	3.3±0.15	-
Acid value, mg KOH/g	3.1±0.1	3.3±0.1	-
Mass fraction of oil, *%	54.0±1	33.3±0.5	48.0
Mass fraction of protein, *%	32.8±0.2	22.5±0.15	34.0
Mass fraction of fiber *, %	5.5±0.03	32.3±0.2	6.0
Mass fraction of ash, *%	6.5±0.03	5.91±0.03	-
Mass fraction of minerals*:			
Phosphorus, g/kg	13.5	8.9	13.8
Calcium, g/kg	0.5	0.9	0.4
Magnesium, g/kg	2.7	2.4	5.6
Iron, mg/kg	94.1	74.7	76.0
Zinc, mg/kg	111.8	56.1	85.0
Cobalt, mg/kg	1.0	0.5	-
Manganese, mg/kg	38.3	59.4	57.0
Copper, mg/kg	12.6	not determined	9.0

Note: * – on a dry basis; ** – organization specialized in processing hemp and flax in the Netherlands and Romania.

Table 6 shows the improved performance of the hemp kernel compared to the whole seed. The content of oil and protein can be seen to have increased by 1.5 times, and the content of macro- and microelements (except for calcium and manganese) can be seen to have increased by 1.5 times for phosphorus, 1.25 times for iron and 2 times for zinc and cobalt.

Table 7

Amino acid composition of hemp kernel (Oseyko M. et al., 2019)

Amino acid	"n" or "e"*	Content					
		Hemp kernel		Whole hemp seeds		"Hemp-flax"	
		mg/100 g	%	mg/100 g	%	mg/100 g	%
Alanine	"n"	1624	5.4	642	5.5	1760	5.6
Arginine	"n"	4149	13.7	1409	12.1	3420	11.0
Aspartic acid	"n"	2616	8.6	1100	9.4	1870	5.9
Valine	"e"	946	3.1	351	3.0	1880	6.0
Histidine	"n"	936	3.1	326	2.8	860	2.8
Glycine	"n"	1546	5.1	644	5.5	1420	4.6
Glutamic acid	"n"	5546	18.4	2370	20.4	6340	20.3
Isoleucine	"e"	833	2.8	323	2.8	1320	4.2
Leucine	"e"	2023	6.7	791	6.8	2000	6.4
Lysine	"e"	1538	5.1	661	5.7	960	3.1
Methionine	"e"	877	2.9	263	2.3	770	2.4
Proline	"n"	1410	4.7	593	5.1	-	-
Serine	"n"	1888	6.2	656	5.6	1850	5.9
Tyrosine	"n"	1200	4.0	383	3.3	1670	5.4
Threonine	"e"	1091	3.6	438	3.8	1580	5.1
Tryptophan	"e"	not determined				210	0.7
Phenylalanine	"e"	1396	4.6	525	4.5	1740	5.6
Cysteine	"n"	604	2.0	163	1.4	1570	5.0
Total		30223	100	11638	100	31220	100

Note: * "n" is nonessential amino acid; "e" is essential amino acid.

Table 7 shows that the hemp kernel in Ukrainian variety seeds is rich in essential amino acids. The content of isoleucine, leucine, lysine, methionine, threonine, phenylalanine in the hemp kernel significantly exceeds that in the whole hemp seeds. The hemp kernel is found to have an increased content of lysine, which is usually deficient. Additional processing of hemp seeds to produce flour or protein concentrate can significantly improve data on increasing the biological value of products. In particular, thanks to additional processing, the protein content in hemp flour on adry basis can be as high as 44.0% and in protein concentrate – 52.1–75% (Oseyko et al., 2019).

Almost all Ukrainian businesses use hemp kernel waste as bedding in animal husbandry, and some in the production of fuel briquettes or pellets. But, in our opinion, it is unacceptable to incinerate or dispose of hemp kernel waste (as intermediate products). Table 8 shows the composition of intermediate products derived from the production of hemp kernel, on a dry basis (Sova. et al., 2021).

Table 8

Characterization of the composition of intermediate products derived from the production of hemp kernel (Sova et al., 2021)

Component	Content, %		
	Coats	Chaff (Alternative 1)	Chaff (Alternative 2)
moisture	7.90±0.02	12.00±0.02	8.84±0.02
protein	10.75±0.15	26.90±0.2	26.90±0.2
oil	7.81±0.2	41.23±0.5	39.13±0.5
fiber	60.23±0.5	22.20±0.2	32.45±0.2

Table 8 shows that both chaff alternatives are rich in protein (26.9±0.2%) and oil (39.13±0.5% and 41.23±0.5%). Although seed coats have significantly lower content of protein and oil, they have been found to be rich in fiber (60.23±0.5%).

The mineral composition of intermediate products derived from the production of hemp kernel, on a dry basis, is given in Table 9 (Sova et al., 2021).

Table 9

Mineral content of intermediate products derived from the production of hemp kernel (Sova et al., 2021)

Mineral	Content		
	Coats	Chaff (Alternative 1)	Chaff (Alternative 2)
Calcium, g/kg	2.10	1.72	2.06
Phosphorus, g/kg	2.37	13.00	12.72
Magnesium, g/kg	1.13	4.40	4.91
Iron, mg/kg	138.39	147.97	195.45
Zinc, mg/kg	20.52	98.31	102.23
Copper, mg/kg	13.31	14.79	15.36
Manganese, mg/kg	99.54	137.13	185.28

It can be seen from data in Table 9 that intermediate products derived from the production of hemp kernel contain significant amounts of minerals. However, the content of phosphorus, magnesium, iron, zinc and manganese differs significantly, with seed coats containing less of them in comparison with chaffs.

Further research should expediently be devoted to the utilization of intermediate products derived from the production of hemp kernel in the technology of functional food products, dietary supplements, and feed products (Sova et al., 2021).

Shen and co-authors (2020) revealed that the use of hemp kernel in the hemp protein isolate production technology increased the yield and quality of the isolate (purity, Arginine vs Lysine ratio, color). Utilization of hemp kernel as a raw material input does not affect protein composition and structure.

4.2 Aspects of hemp flour and protein (protein concentrate) production

Hemp meal contains 30-35% of protein, more than 10% of oil and 25% of fiber. 100 g of hemp cake corresponds to 73 feed units. Phytin (phytic acid calcium magnesium salt), being a major component of hemp cake, sees heavy medical use in stimulating hematopoiesis, enhancing growth and development of bone tissue, as well as in managing some diseases of the nervous system (Shashkarov et al., 2016).

Hemp cake is a unique source of protein, natural carotene, phytosterols and phospholipids, which are helpful in preventing anemia, with K, Zn, S and Mg strengthening the heart muscle and nervous system. Hemp cake contains fiber, which is essential for the normal functioning of the gastrointestinal tract; improves motor skills, eliminates toxins from the body; positively affects the respiratory system; enhances treatment of cardiovascular diseases and obesity; improves kidney and liver function. Hemp cake is unique in that it contains adequate amounts of complete protein. Bulk hemp products such as flour, fiber and protein concentrate are produced by crushing the cake and fractioning the resulting mass. The resulting fractions have a different size, with the smallest one, commonly referred to as "hemp protein", having the highest protein content. This fraction also contains considerable amounts of fat, ash and fiber. A production scheme of hemp flour, "protein" and fiber is shown in Figure 4.

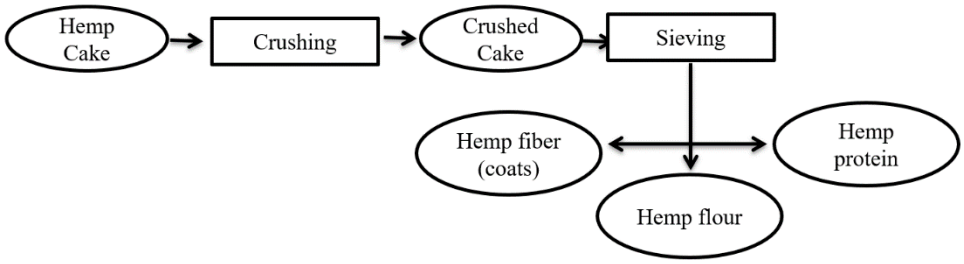

Figure 4. Bulk hemp products production scheme

Physicochemical indicators of quality and amino acid composition of bulk hemp products are given in Tables 10 and 11 (Sova et al., 2018).

Table 10

Physicochemical indicators of quality of bulk hemp products (Sova et al., 2018)

Component	Content, %, in			
	Hemp seeds	"Protein"	Flour	Fiber
moisture content, %	8.36	7.00	6.50	7.17
protein	24.70	52.14	44.01	22.65
oil*	33.62	15.68	11.65	10.62
ash*	4.99	9.55	8.84	5.05
fiber*	36.85	5.51	13.88	44.94
pest contamination	N/D			

* on a dry basis

According to data in Table 10, the hemp "protein" fraction is rich in protein, oil, minerals, which makes it the most valuable for consumption among the presented bulk hemp products. The biological value of hemp "protein" is 81.7% (Sova et al., 2018).

Table 11

Amino acid composition of bulk hemp products, mg/100 g (Sova et al., 2018)

Amino acid	Hemp seeds	"Protein"	Flour	Fiber
Alanine	735	1556	1462	671
Arginine	1647	3589	3411	1336
Aspartic acid	1359	2263	2224	1286
Valine	445	885	910	371
Histidine	413	870	806	335
Glycine	740	1272	1319	717
Glutamic acid	2870	4445	4625	2593
Isoleucine	374	782	813	331
Leucine	913	1951	1877	813
Lysine	788	1458	1300	843
Methionine	302	686	630	184
Proline	673	1358	1305	604
Serine	824	1597	1514	725
Tyrosine	469	1078	955	376
Threonine	555	1056	1029	485
Phenylalanine	653	1350	1271	570
Cysteine	197	594	545	160

Pojić with co-authors (2014) showed how the value of hemp seed flour can be increased. Chemically, two fractions that contained cotyledons (>180 and <180 μm) had a notably higher content of protein (41.2±0.04% and 44.4±0.02%, respectively), lipids (15.1±0.02% and 18.6±0.04%, respectively), and carbohydrates (4.96±0.11% and 3.46±0.08%, respectively) than fractions that contained coats (>350 and >250 μm), which, in turn, had a higher content of crude fiber (29.5±0.04% and 21.3±0.03%, respectively). All fractions were found to have balanced ω-6/ω-3 fatty acids (3:1). Antinutrients (trypsin inhibitors, phytic acid, glucosinolates and condensed tannins) are mainly located in the cotyledon fractions. The data obtained by the authors show that fractioning of hemp seed flour can be useful in concentrating valuable target compounds. Therefore, this facilitates their extraction.

4.3 Aspects of production of hemp protein concentrates and isolates

Hemp seeds contain a broad spectrum of biologically active chemical compounds. In particular, increasingly greater attention is given to proteins and biologically active peptides as an alternative source of nutraceuticals. Hemp seeds contain the salt-soluble globulins or edestin (~75%) and the water-soluble albumin (~25%) as the main storage proteins. Hemp seed proteins have a high level of arginine and a sulfur-rich protein fraction, two unique

features that impart high nutritional values. Hemp protein has antioxidant and anti-inflammatory properties. It is a by-product of hemp oil technology (Stefan et al., 2018).

Hemp protein hydrolysates generated by enzymatic hydrolysis are composed of polypeptides, oligopeptides, and free amino acids displaying high availability of biological activity. Hemp protein hydrolysates have showed different biological activities as antihypertensive, hypocholesterolemic, antioxidant, antithrombotic, and immunomodulatory effects (Zanoni et al., 2017). Hemp protein is a complete protein source containing all essential amino acids. Plant-based sources of protein are often considered inferior to animal-based ones, but hemp protein is an exception. It contains all 9 essential amino acids found in other complete protein sources such as meat or dairy products. Hemp protein is also classified as a source of high-quality protein comparable to that of soybean or egg white (Fountoulakis et al., 2008). (James et al., 2010) notes that lysine is the first limiting amino acid in hemp protein. Removing seed coats improves lysine digestibility.

Wang with co-authors (2018) suggested that hemp seed protein isolates have a higher nutritional value in terms of amino acid composition and are easier to digest than soy protein isolate. They can be used as the primary source of proteins in human nutrition.

Yin with co-authors (2009) revealed the effects of succinylation and acetylation on some functional, structural properties and trypsin digestibility of hemp protein isolate. Succinylation leads to gradual increase in hemp protein solubility from 30 to 85–90%, while in the acetylation case, the protein solubility is improved only at low anhydride levels, increasing from 30 to about 50%. Differential scanning calorimetry and intrinsic fluorescence spectrum analysis indicated gradual structural unfolding of proteins, or exposure of hydrophobic clusters to the solvent, especially at higher anhydride levels. Additionally, trypsin digestibility was significantly improved by the succinylation. The results indicated that succinylation could be applied to modify some selected functional properties of hemp proteins.

Malomo with co-authors (2014) identified the effects of pH and protein concentration on some structural and functional properties of hemp seed protein isolate (84.15% protein content) and defatted hemp seed flour (44.32% protein content). The protein isolate was characterized by a minimum protein solubility at pH 4.0, which increased as pH decreased or increased. In contrast, the hemp seed flour had minimum protein solubility at pH 3.0, which increased at higher pH values. Intrinsic fluorescence and circular dichroism data indicated that the hemp protein isolates had a well-defined structure at pH 3.0, which was lost as pH value increased. The differences in structural conformation of hemp protein isolates at different pH values were reflected as better foaming capacity at pH 3.0 when compared to pH 5.0, 7.0, and 9.0. Therefore, the functional properties of hemp seed protein products are dependent upon structural conformations as well as protein content and pH.

In (Teh et al., 2016), hemp protein isolates were hydrolyzed using proteases (AFP, HT, ProG, actinidin, and zingibain). Physical properties of hydrolysates were evaluated by particle size, zeta potential and surface hydrophobicity. HT protease had the highest rate of caseinolytic activity at the lowest concentration, 0.1 mg/ml, compared to other proteases that required concentration of 100 mg/ml to achieve their maximum rate of caseinolytic activity. This led to the highest degree of hydrolysis of hemp protein isolate as affected by HT protease in the SDS-PAGE profiles. Among all proteases and substrates, HT resulted in the highest biological activity generated from alkali extracted hemp protein isolate in the shortest time (2 hours) compared to the other protease preparations.

In (Girgih et al., 2013), hemp seed protein hydrolysate was produced through simulated gastrointestinal tract digestion of hemp protein isolate, followed by partial purification and separation into eight peptide fractions. The peptide fractions exhibited higher oxygen radical

absorbance capacity as well as scavenging of 2,2-diphenyl-1-picrylhydrazyl, superoxide and hydroxyl radicals when compared to hemp seed protein hydrolysate. Radical scavenging activities of the fractionated peptides increased as content of hydrophobic amino acids or elution time was increased, with the exception of hydroxyl radical scavenging that showed decreased trend. Although glutathione, hemp seed protein hydrolysate and peptide fractions possessed low ferric ion reducing ability, all of them had strong (>60%) metal chelating activities. Inhibition of linoleic acid oxidation using some of the hemp seed protein hydrolysate peptide fractions was higher at 1 mg/ml when compared to that observed at 0.1 mg/ml peptide concentration. Peptide separation resulted in higher concentration of some hydrophobic amino acids (especially proline, leucine and isoleucine) in the fractions when compared to hemp seed protein hydrolysate.

It was found in (Mamone et al., 2019) that hemp-based food products were considered less allergenic than those based on other edible seeds. High purity grade hemp flour and hemp protein isolate were derived from defatted hemp cakes, residues of hemp oil extract. The resulting hemp protein isolate contained almost 86% protein, represented mainly by the storage protein edestin (which accounted for 70% of the total protein). In vitro hemp protein digestibility was determined using a static model of gastrointestinal digestion. Hemp flour and hemp protein isolate showed a high degree of digestibility. The survival of potential biologically active and/or allergenic peptide sequences in digests was investigated by peptidomic analysis. Only a limited number of sequences survived gastrointestinal digestion. All known hemp allergens, including the major thaumatin-like protein, were entirely eliminated by the hemp protein isolate production process. These data support the use of hemp protein isolate as an ingredient for hypoallergenic foods.

According to (Potin et al., 2020), hemp seeds were found to contain considerable amounts of nutrients in the hemp kernel and in its derivative products 26% of protein and 36% of oil, respectively. The authors presented the current state of knowledge about the hemp kernel in terms of its composition, nutritional value, extraction, physicochemical, functional and biological properties. Various extraction methods have been proposed to extract major hemp protein fractions from the hemp cake. The protein obtained from hemp flour is classified as globulins and albumins and contains highly digestible (about 90%) essential amino acids. The authors emphasize that hemp protein hydrolysates have a wide range of health-promoting biological activities, such as antioxidant properties, metal chelation, antihypertensive, hypoglycemic properties, etc.

Raikos with co-authors (2015) investigated the effect of heat treatments on the denaturation and oxidative stability of hemp seed protein during simulated gastrointestinal digestion. Heat-denatured hemp protein isolate solutions were prepared by heating hemp protein isolate (2 mg/ml, pH 6.8) to 40, 60, 80 and 100 °C for 10 min. Heat-induced denaturation of the protein isolates was monitored by polyacrylamide gel electrophoresis. Heating hemp protein isolate at temperatures above 80 °C significantly reduced solubility and led to the formation of large protein aggregates. Additionally, the oxidative stability of the resulting peptides was investigated. Heating did not significantly affect the formation of oxidation products. The results suggest that heat treatments should ideally unfold below 80 °C in order to preserve heat stability and solubility of hemp protein isolate.

In (Pojić et al., 2014), hemp proteins have been found to form high-quality emulsions similar to those of milk-based emulsions. A novel hemp protein concentrate has been shown to have >70% solubility at pH 4.0–6.0, whereas most plant proteins are typically insoluble. Addition of hemp protein to diet led to reduced pathological intensity of renal disease and cardiovascular diseases. Moreover, hemp seed enzymatic hydrolysates exhibited antioxidant

and antihypertensive properties. According to the authors, hemp proteins and hydrolysates have the potential to be used as ingredients to formulate functional foods.

Dapčević-Hadnađev with co-authors (2020) identified the ability of hemp protein to act as a functional agent in a variety of foods. The role of hemp protein as an emulsifier, foaming, film-forming and gelling agent creates the potential for replacing synthetic agents with natural ones. Studies have revealed a biological functionality of hemp proteins, i.e. application of enzymatic hydrolysis for the production of biologically active peptides.

Summarizing information in section 4, it should be noted that the reviewed publications deal superficially with the relationship between the factors of material preparation, production process variables of hemp foods, storage conditions and time in terms of the content of functional and biologically active components.

5. Aspects of using hemp seeds and its derivative products

Foods containing hemp seeds and oil are currently marketed worldwide for both animal and human nutrition. It was estimated that the global market for hemp consists of more than 25,000 products (Cerino et al., 2020). Hemp seeds are widely used in the production of kernel, oil, flour, protein, milk, animal feed, etc. (Serkov et al., 2011; Pojić et al., 2014; Karus et al., 2004; Leson, 2006; Kolodziejczyk et al., 2012; Cherney et al., 2016; Fike, 2016; Schluttenhofer et al., 2017; Klir et al., 2019; Williams, 2019; Leonard et al., 2020; Xu et al., 2020; Della Rocca et al., 2020; Crini et al., 2020; Farinon et al., 2020). Hemp seeds or their ingredients are added to beverages, for example, in the brewing and wine industry, as well as to neutral products (Cerino et al., 2020). In Latvia (Ivanovs et al., 2017), for example, crushed hemp seeds see heavy use in the manufacture of butter-based delicacy paste. Hemp oil is used for cosmetics and personal care items, paints, printing inks, detergents and solvents. In addition to food products, hemp flour is used in animal and poultry husbandry (Silversides et al., 2005). Hemp seeds and their derivative products have been scientifically proven to have a curative, health-improving and rehabilitative effect on the human body (Noelia et al., 2019; Metwally et al., 2021; Valizadehderakhshan et al., 2021).

Table 12 shows example uses of hemp seeds and their derivative products in technologies of functional, dietary and specialty products.

Summarizing the data in Table 12, the following should be noted:

- Utilization of hemp seeds and their derivatives in various food technologies enhances the biological and nutritional value, functional and sensory properties of finished products;
- Adding hemp derivative products to the formulation of baked goods increases their shelf life;
- Utilization of hemp derivative products in bakery technologies ensures decreased amounts of gluten in finished products, which is relevant in modern conditions.

Table 12

Utilization of hemp seeds and their derivative products as a functional component

Product or semi-finished product	Hemp supplement content	Efficiency	Reference
Mixed rye-wheat bread	10% of wheat flour replaced with hemp flour	Fermentation property increased by 42%, specific volume by 26.3% and finished product porosity by 10.9%. Dough, proofing and baking time reduced by 30%. 150 g of this product meets the daily requirement for polyunsaturated fatty acids.	Samofalova et al., 2004
Wheat bread	Hemp/wheat flour ratio 10/90	Increased nutritional value. Increased content of proteins, macro- and microelements, especially iron. Decreased gluten content.	Pojić M. et al., 2015
Wheat bread	15% of hemp flour, 4% of hemp kernel and 8% of hemp oil	Increased content of proteins, essential fatty acids, dietary fiber. Decreased gluten content. Increased shelf life of bread.	Bădărău Carmen et al., 2018
Wheat bread	50% of hemp flour	Increased protein content (13.38-19.29 g/100 g). Change in the hardness of bread crust due to a decrease in bread stability index from 1.12 to 0.05. Increase in crumb browning index from 29.69 to 46.26. Increase in total polyphenols from 256.43 to 673.59 mg GAE/kg. Formation of furan derivatives (furfuryl alcohol, furfuryl aldehyde, hydroxymethylfurfural).	Mikulec et al., 2019
Wheat bread	10% of hemp flour	Intensification of dough maturation. Production time reduced by 8–20 minutes. Reduced calorie content due to low starch content. Product consumption compensates the daily requirement of the human body for proteins by 9.5%, fats by 5.5%, fiber by 13.6%, ω-3 and ω-6 fatty acids by 37 and 29%, respectively. Increased content of B vitamins and minerals (phosphorus, magnesium, calcium, iron).	Falendysh et al., 2019
Wheat bread and bakery products	10% of hemp kernel or 5% of hemp protein	Reduced baking, convexity losses of baked goods. Increased nutritional value of the finished product.	Ruban et al., 2016
Gluten-free bread	20% of hemp protein	Increase in fiber levels from 15.2 to 61.0 g/kg and dietary fiber from 29.3 to 90.0 g/kg. Increase in bread volume	Korus et al., 2017

	concentrate and flour	from 633 to 878 ml. Improved organoleptic characteristics (color and taste). Limited product staling through reduced rate of amylopectin recrystallization during storage.	
Gluten-free bread	Non-conventional starter culture using hemp, chia and quinoa flour	Reduced pH, specific volume and staling rate of the finished product. Increased bread porosity.	Jagelaviciute et al., 2021
Whole-wheat bread	5, 7, 10% of hemp flour	Product crumb and crust browning. More uniform crumb structure. Providing the finished product with a light nutty aroma and pleasing savor. Protein content increased by 4–4.4%. Content of natural food sorbents in the product increased by 17.2%. Reduced bread brittleness. Soft product for 24–48 hours.	Bazhai-Zhezherun et al., 2020
Bread sticks	15% hemp cake flour	Water absorption capacity of the resulting bake mix increased by 6%. Dough balls running increased by 7.4%. Carbon dioxide generation during dough fermentation (180 min) reduced by 16%. Titratable acidity increased by 16%.	Iorgacheva et al., 2020
Pasta	5% of semolina flour replaced with hemp flour	Increased protein, fat and crude fiber content. Improved functional properties (antioxidant activity, increased content of macro-, microelements and phenolic compounds). Consistent cooking performance of pasta. Reduced cooking time, product surface stickiness.	Pojić et al., 2014
Gluten-free crackers	20% of hemp flour	Enriched with minerals, fiber (39–249%) and polyunsaturated fatty acids. Decreased carbohydrate content (8.4–42.3%). Increased antioxidant properties.	Radočaj et al., 2014
Gluten-free sugar cookies	Hemp/cornmeal ratio 80:20	Improved organoleptic properties (texture and physicochemical properties).	Lukin et al., 2017
Shortbread cookies	20% of hemp cake	Increased cookie strength and porosity, moisture content increased by 0.5–0.8%, wetness index by 10–15%. Enriched with complete protein, chlorophyll, vitamins and minerals. Developed cookies have functional properties. 100 g of cookies covers the daily human need for dietary fiber by 11–16%.	Holia et al., 2018

Cookies	20% of hemp flour (raw and roasted)	Increased percentage of protein, oil, ash, phenols and antioxidant activity. Decreased hardness of products.	Nilgün et al., 2020
Konoplyana Nasoloda (Hemp Delight) cupcake	34% of hemp flour	Increased nutritional value of finished products. High organoleptic quality indicators.	Tkachenko, 2018; 2020
Semi-finished minced meat products	10% of hemp flour	Increased content of lipids (by 2.2%), magnesium (2.4 times) and iron (1.5 times).	Perekhodova et al., 2017
Chopped beef liver products	15% of hemp flour and emulsified hemp oil	Increased water-binding and water-holding capacity of the product. Improved fat absorbing, emulsifying capacity and emulsion stability.	Stoporeva et al., 2018
Liver pate	17% of hemp seeds	Increased fat content and nutritional value of the product. Improved fatty acid composition and sensory properties (hardness, softness and stickiness).	Zając et al., 2018
Milk drink	Hemp seeds	Increased prebiotic activity. The content of biologically active compounds is increased due to the inhibited enteropathogen growth and high levels of acetate, propionate and butyrate produced during fermentation.	Nissen et al., 2020

In addition to the foods listed in Table 12, hemp seeds are also used in the production of hemp oil softgel capsules, hemp gummies (Canada), roasted hemp seeds with sea salt, hemp jelly beans, energy drinks, hemp tea, hemp chewing gums, hemp honey, coffee beans and hemp kernel (USA), hemp lager beer, hemp protein bars (UK), hemp candies, hemp chocolate and hemp pads (Netherlands) (Sova et al., 2020).

It is essential that the technology of composite food products provide antimicrobial and antioxidant properties throughout the guaranteed shelf life (Oseyko et al., 2019).

Bartkiene with co-authors (2020) proposes fermentation with *Pediococcus acidilactici*, *P. pentosaceus*, *Lactobacillus casei* and *L. uvarum* strains, as well as ultrasonic treatment of hemp kernel paste. It includes an assessment of the content of biogenic amines and antimicrobial properties of the derivative products. Combined fermentation and ultrasonic treatment helps lower the total bacteria count in the hemp kernel paste. The treated hemp kernel was found to be rich in biogenic amines, 639.87 mg/kg. Pure lactic acid bacteria showed a reduction in a broad spectrum of pathogens. However, the hemp kernel paste exhibited a very low antimicrobial activity and formulated emulsion did not exhibit any antimicrobial properties. Treatment with selected LAB can be recommended for preparation of stable emulsions, and the most acceptable beverages can be obtained using *L. uvarum* strain.

Frassinetti and co-authors (2018) evaluated the antioxidant effect of hemp seeds and sprouts after 3 and 5 days of germination. Total polyphenols, flavonoids and flavonols content expressed on a dry basis were highest in sprouts. A number of analyses including

cellular antioxidant activity in red blood cells and hemolysis test showed a higher antioxidant activity in sprouts than in seeds. Main polyphenol (caffeoyltyramine) was identified in hemp seeds and of ω-6 (linoleic acid) was identified in sprouts. Therefore, hemp seeds and sprouts can have beneficial effects on human body and should be investigated as a potential functional food.

Siano with co-authors (2019) determined chemical and biochemical characteristics including phytosterol composition, total phenolics, antioxidant activity, and content of macro- and microelements of edible hemp resources such as seeds, oil, and flour. Hemp seeds, flour, and oil contained 767±41, 744±29, and 21±5 mg GAE/kg of total polyphenols, respectively. The antioxidant potential of hemp flour and seeds was higher than that of oil. K and Mg were the most abundant macroelements, particularly in flour, 5064.45 and 2310.54 mg/kg, respectively.

Conclusion

Hemp seeds and their derivative products are still insufficiently used in food technologies such as cereals, pasta, confectionery, food concentrate, meat and dairy and fermentation. In the short term, the theoretical, scientific and practical insights presented in this review should be used in integrated solutions for the processing of environmentally sound industrial and medical hemp seeds.

It is essential that further research be conducted on the use of drugs to regulate the antimicrobial and antioxidant properties of functional, dietary and specialty products is of paramount importance.

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Physical, chemical and sensory properties of sour grape based beverages and monitoring of their quality changes during storage

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Abstract

Introduction. The aim of the research is to determine the usability of the sour grape concentrate in beverages and monitoring of their quality changes under different storage conditions.

Materials and methods. Sultani Çekirdeksiz (*V. vinifera* L.) sour grapes were used as material to obtain the concentrate. Total phenolic contents were determined according to Folin-Ciocalteu methods. Antioxidant properties of the samples were analysed with FRAP, CUPRAC, ABTS and DPPH methods. Contents of sugars and individual phenolic compounds were determined by HPLC, and contents of minerals were determined by Atomic Absorption Spectroscopy methods.

Results and discussion. Total phenolic content, antioxidant capacity, individual phenolic compounds and mineral amounts increased depending on used sour grape concentrate rising ratios in manufactured carbonated drinks (CD), sherbet (SH) and iced tea (IT) samples. Total phenolic contents were ranged from 32.7 to 40.82 mg/L in CD, from 27.3 to 35.0 mg/L in SH and from 357.08 to 365.64 mg/L in IT samples. Antioxidant capacity values were between 66 and 4319 μM TE/L for ABTS method, 214 and 4893 μM TE/L for CUPRAC and between 186 and 4319 μM TE/L for FRAP methods. IT samples had more antioxidant capacity than SH and CD samples.

The most abundant minerals in the beverages were Na (77.41–692.28 μg/mL), Ca (159.03–358.25 μg/mL) and Fe (8.74–22.84 μg/mL). Total sugar contents of the beverages varied from 6.29 to 10.97 g/100 mL. They ranked as CD>SH>IT according to the sugar contents. Gallic, vanilic, and caffeic acids, (+)-catechin and (-)-epigallocatechin gallate were determined in all beverages whereas p-coumaric, ferulic and sinapic acids, myricetin, quercetin, (-)-epigallocatechin and (-)-epicatechin gallate could no detected in CD and SH. The phenolic compound contents in IT were more than in CD and SH for all investigated compounds. The storage conditions caused to alterations in beverage pH, acidities, TP contents and DPPH inhibitions.

Conclusions. Minerals, total phenolic contents, antioxidant capacities and individual phenolic compounds in the beverages increased depending on used sour grape concentrate rising ratios. Gallic acid was the most abundant phenolic acid in the beverages and (-)-epicatechin for IT samples was the most abundant flavonol. The beverages pH, acidity, total phenolic content and DPPH inhibition can change depending on storage temperatures and duration.

Introduction

Beverage sector, is a sub-sector in food industry, has an important place in the world food production and trade (Guimaraes et al., 2012). Alcohol free, carbonated or non-carbonated drinks are generally called as soft drinks. In recent, manufacturing of fruit drinks and some iced drinks, various fruits and their flavors are often used according to consumer demands for competition in sector. In addition, studies on innovative drinks and additives have been performing by many researchers (Balaswamy et al., 2011; Gonzalez-Molina et al., 2012; Guimaraes et al., 2012; Jori et al., 2013; Verma et al., 2014; Jooyandeh 2015). Sour grape concentrate based beverages can also be in these innovative drink groups.

In recent years, consumer demands for beverages that contain some components positively affecting health as well as basic nutrition have increased. Especially, beverages having high functional properties are more preferred by consumer. Nanasombat et al. (2015) stated that functional foods and beverages are products offering functional health benefits. The functional beverages have a wide variety such as performance and energy beverages, whey beverages, fruit and vegetable smoothies, ready to drinks, vitamin and ingredient fortified drinks, and innovative fruit juices. As people's awareness and educational level increases, demand to functional beverages also rises. The reason for this increase is that these beverages have minerals, vitamins and antioxidant compounds, which may help to defend against oxidative stress and some other health problems.

Grape is rich fruit in terms of polyphenols. Polyphenols have important effect on product quality and have antioxidant properties. Moreover, the polyphenols show preservative effects against oxidation and microbial spoilage in foods (Singleton et al., 1978). The phenolic compounds primarily distribute in the skin, stem, leaf and seed of grape, rather than pulp (Pastrana-Bonillaet al., 2003; Makris et al., 2008; Xia et al., 2010). Proanthocyanidins, anthocyanins, flavonols, flavanols, resveratrols and phenolic acids are among the grape phenolic compounds. Flavonoids include flavan-3-ols, flavonols and anthocyanins. The flavanoids have many biological activities such as inhibition of plasma platelet aggregation, radical scaving activity and exhibiting antibacterial, antiviral, antiallergenic effects (Cook et al., 1996; Yang et al., 2009). The phenolic compounds are also in the sour grape such as ripened grape, but their amounts vary depending on maturity, variety, soil and environmental conditions (Sabir et al., 2010).

Sour grapes have been used to produce verjuice and fresh beverages as traditional in Turkey and other viticulture countries for a long time. Although they have high potential for beverages production, there is very limited knowledge about their usability in nonalcoholic beverages. In addition, using of the sour grapes in the beverages are almost nonexistent and it has been no encountered their using in carbonated and iced tea beverages up to now.

Besides, no study could be found regarding physiochemical properties, antioxidant activities, phenolic profiles, sugars and minerals in the sour grape based beverages and their quality alterations during storage. Monitoring of changes on quality during the storage period in each developed product is very important in terms of both shelf life and nutrient loss since storage time and temperature can be effect on food and beverage quality.

This study investigates possibility to use of the sour grapes in preparation of nonalcoholic beverages; physicochemical properties, content of phenolic compounds, sugars, minerals and antioxidant capacity of the new manufactured beverages; changes of some physical and chemical parameters of during their storage.

Materials and methods

Materials

Sultani Çekirdeksiz (*V. vinifera* L.) grapes were used to obtain sour grape concentrate. The sour grapes were harvested in Manisa Viticulture Research Institute vineyards. In addition, beverages manufacturing were performed at the same Institute pilot grape products processing unit.

Beverage manufacturing

Sour grape concentrate (SGC) using for beverages manufacturing was firstly produced. To produce this concentrate, sour Sultani Çekirdeksiz (*V. vinifera* L.) grapes were harvested and then, stalks discarded and immediately crushed. The mash was pressed, and juice was clarified by using pectolytic enzyme, bentonite, gelatin and kieselsol. Then, sour grape juice was concentrated under vacuum. In the SGC production, the traditional wellknown clarification and vacuum evaporation processes were performed. The concentrate was stored at -18 °C until using for beverage processing. The beverages manufacturing flow diagrams are shown in Figure 1.

The sugar syrup (50 °brix) was prepared and mixed with SGC that was added at three different ratios for manufacturing carbonated drinks (CD). Then, these mixtures were diluted to 10 °Brix by using carbonated water and following that filling/sealing process was carried out. The bottles were closed with crown caps. As preservative, 250 mg/L sorbic acid and 150 mg/L benzoic acid were added. The sugar/acid (taste) balance were set as 35, 40 and 45 in the final CD beverages, respectively.

In the manufacturing of the sherbets (SH), 10 °Brix sugar syrup and SGC were mixed to produce beverages that were 20, 25 and 30 sugar/acid taste balance. 250-mL bottles were filled with sherbets and closed with crown caps. Pasteurization was done at 85 ºC for 15 min at once.

The 750 g black tea (brand Çaykur Kamelya) was brewed for 15 minutes, and then it was diluted 2 times with boiled water for preparing ice tea (IT). Then, by adding sugar syrup to the diluted brewed tea mixing, ice tea beverages that were 6, 7 and 8 °Brix were obtained. SGC was added as equal amounts to these beverages. The taste balance in final ice tea beverages were adjusted 30, 35 and 40, respectively. Filled and bottled samples were pasteurized at 85 ºC for 15 min at once. The beverages descriptions were indicated in Table 1.

Table 1

Beverage descriptions

Beverages	Soluble Solids, °Brix	Taste Balance, °Brix/Acidity
CD1	10.0	45.0
CD2	10.0	40.0
CD3	10.0	35.0
SH1	10.0	20.0
SH2	10.0	25.0
SH3	10.0	30.0
IT1	6.0	30.0
IT2	7.0	35.0
IT3	8.0	40.0

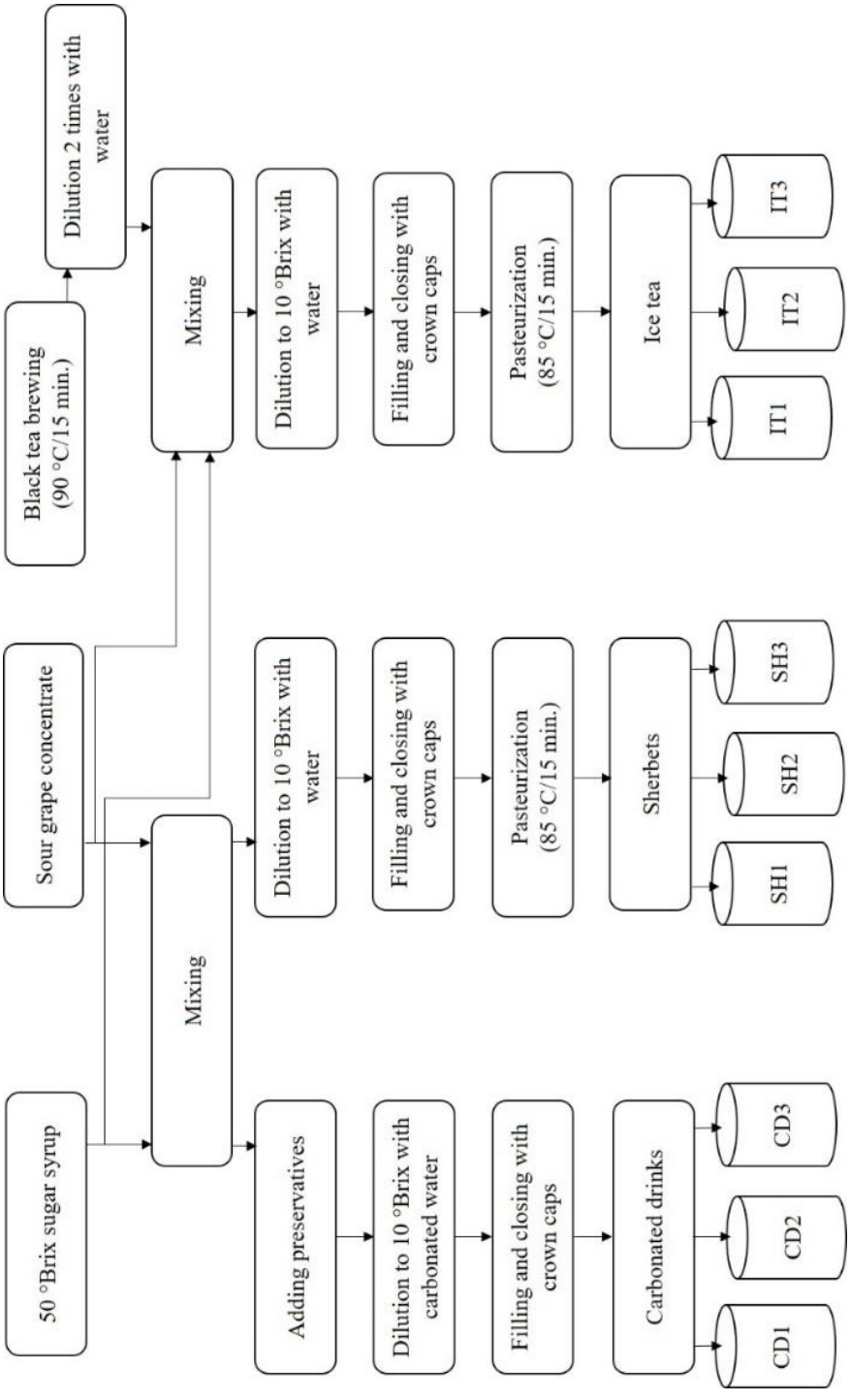

Figure 1. Beverage manufacturing flow diagrams

Determination of the physicochemical parameters, transmittances and colors

The pH values were measured by using a pH meter (Hanna 211). The titratable acidity was determined by titrating of 10 mL of sample with 0.1 N NaOH to pH 8.1 and expressed as tartaric acid percent (Ough et al., 1998).

The transmittance values were calculated by using the samples absorbance that were measured at 400 nm according to below formula. Distilled water was used as blank (Artık et al., 1996).

$$logT = 2 - A \quad (1)$$

Spectrophotometric method was used to determine colors of samples (AOCS, 1999). Briefly, 350 µl sample was filled to micro-plate cell and absorbance was read using by Multiskan FC Microplate Spectrophotometer (Thermo Scientific, Multiskango, Finland) at 420, 520 and 620 nm. Distilled water was used as blank. Color intensity (CI) and optic density (OD) at 420, 520 and 620 nm were calculated according to below formulas:

$$CI = A_{420} + A_{520} + A_{620} \quad (2)$$
$$OD_{420} = (A_{420}) / CI \times 100 \quad (3)$$
$$OD_{520} = (A_{520}) / CI \times 100 \quad (4)$$
$$OD_{620} = (A_{620}) / CI \times 100 \quad (5)$$

where OD_{420} represent yellowness, OD_{520} redness and OD_{620} blueness.

Determination of total polyphenolic content

Total polyphenolic contents (TPC) of the samples were determined according to Folin-Ciocalteu colorimetric method (Singleton et al., 1965). At first, 100 µL of reagent solution was added to each samples and the volume were completed to 4 mL and then 500 µL of 20% saturated sodium carbonate (Na_2CO_3) was added to final solution after 3 min and all of them was shaken. Then, the samples were incubated at room temperature (24±1) °C for 30 min. At the end of the duration, 350 µL of samples were transferred in a 96 well of microplate and absorbance was measured at 760 nm. 25, 50, 100, 200 and 400 mg/L of standard concentrations were used for calibration curve. Results were expressed as Gallic acid equivalent in L (mgGAE/L).

Antioxidant capacity

FRAP, CUPRAC and ABTS assays (Benzie et al., 1999; Re et al., 1999; Apak et al., 2004; Callaghan et al., 013; Wern et al., 2016) were used to determine antioxidant capacity of the beverages. In addition, DPPH assay (Brand-Williams et al., 1995) was performed to measure radical scavenging activity of beverages during storage.

ABTS•+ method

2,2′-azinobis-(3-ethylbenzothiazoline-6- sulfonic acid (ABTS•+) method that was described by Re et al. (1999) was used. At first, 7 mM ABTS and 2.45 mM potassium persulfate were mixed and incubated at room temperature 12-16 h. ABTS•+ solution was diluted by adding ethanol till 0.700 (± 0.02) absorbance at 734 nm. Then, diluted sample (60 µL) was added to 940 µL ABTS•+ reagent. The absorbance of these solution was read (t:0 and 6 min) by using spectrophotometer (Thermo scientific, Multiskango, Finland). A

calibration graphic was used to calculate results and they was expressed as μM Trolox equivalent (TE) in liter beverage.

FRAP method

The ferric reducing antioxidant power (FRAP) of the samples were determined according to the protocol that described by Benzie and Strain (1999) and modified by Wern et al. (2016). At first, 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ were prepared for the FRAP reagent. Then, 25 mL acetate buffer (3.6 pH), 2.5 mL TPTZ, and 2.5 mL $FeCl_3 \cdot 6H_2O$ were mixed to obtain a fresh reagent. The FRAP reagent, distilled water, and the samples were warmed to 37 °C. Then, 50 μL of sample and FRAP reagent were added to 2 mL distilled water at 37 °C, and the mixture was incubated for 4 min at the same temperature in dark. The mixture absorbance was read by a Uv-vis spectrophotometer at 593 nm for eight min. 50-1,000 μM standard Trolox concentrations were used for the calibration graphic, and the results were expressed as μM TE in liter beverage.

CUPRAC assay

Cupric reducing antioxidant capacity (CUPRAC) assay that was described by Apak et al. (2004) and modified for adaptation to grape products by Callaghan et al. (2013) was utilized. 150 μL of 1 M ammonium acetate, 7.5 mM neocuproine, and 10 mM copper (II) chloride dehydrate were added to 150 μL samples that were diluted by 0.05 M Tris buffer (pH 7.6). Then, they were incubated at room temperature during 30 min. After incubation, their absorbance values were measured by using a Uv-vis spectrophotometer at 450 nm. Blank was Tris buffer. Trolox concentration ranged 50 to 1,000 μM to obtain the calibration graphic. The results were given as μM TE in liter drinks.

DPPH radical scavenging assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) method analysis was performed according to Brand-Williams et al. (1995). The principle of the method is regarding the measurement of the reduction ability of the DPPH• radical on samples. 3 ml of the 1 mM DPPH• solution was transferred and 200, 400, 600, 800 and 1000 μl of diluted samples were added and standardized to 4 ml solution with methanol and incubated at room conditions (24±1°C) in dark. Methanol was used as blank solvent. Then, the absorbance was measured at 517 nm wavelength by spectrophotometer. Percent inhibition values were calculated according to blank absorbance as described the formula as shown below:

$$\text{Inhibition, \%} = ((A_{DPPH} - A_{SAMPLE})/A_{DPPH}) \times 100 \quad (6)$$

Determination of mineral content

Atomic Absorption Spectroscopy (Perkin Elmer, Analiyst 400, USA) method was used to determine mineral compositions of the samples (AOCS, 1999). At first, samples were kept at 500-600 °C to obtain ash. Then, they were solved with HNO_3 and HCl and diluted by distilled water to 100 mL. Potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), mangane (Mn) and copper (Cu) amounts of the samples were determined and given as μg in mL sample.

Determination of sugars

The sugars of the beverages were found with slight modifications to the method described by Castellari et al. (2000). At first, beverage samples were diluted distilled water and passed through a PTFE 0.45 μm syringe filter. Then, they were injected into high performance liquid chromatography (HPLC) system (Agilent 1260) for measuring. The detector was selected as the refractive index (RID), and the column was NH_2 250 x 4.6 mm, 5μm (Inertsil). Temperature of the column was set to 30 °C and injection volume was used as 20 μL. The elution time was 18 min, flow rate was 1.5 mL/min and flow was isocratic. The mobile phase consisted of acetonitrile and distilled water (80:20; v: v). The sugars of the samples were identified by comparing their retention times and spectra with those of analytical standards. The concentration of the sugars was calculated using the calibration curves and expressed as g in the 100 mL.

Determination of phenolic compounds

HPLC phenolic compound analysis were performed on Agilent 1260 system. The system is equipped with a diode-array UV detector (DAD). It consists of a quart pump, a degasser, an auto sampler and a column oven. Software program is Agilent lab advisor chemstation. C18 ODS column (250 x 4.6 mm, 5μm) was selected for the separation (Agilent).

In the analyzing of the flavanols, isocratic elution was performed using methanol, distilled water and formic acid (19.5:80.2:0.3) as mobile phase during 35 minutes at 280 wavelength. The column temperature was set to 40 °C and flow rate was 1 mL/min. The beverage samples were diluted to certain ratio by mobile phase and passed through a 0.45μm PTFE syringe filter and then injected to system as 5 μL.

To determine the phenolic acids and flavonols, HPLC analysis was performed with a gradient elution with little modifications according to Porgalı et al. (2012) and Natividade et al. (2013). The column temperature was set to 30 °C and flow rate was 1 mL/min. The injection volume and flow rate were 5 μL and 1 ml/min, respectively. The detection wavelength was 280 nm for vanilic acid, 320 nm for p-coumaric acid, caffeic acid, ferulic acid and sinapic acid, 360 nm for myricetin and quercetin. The mobile phases consisted of A: formic acid and distilled water (99.8:0.2) and B: acetonitrile and formic acid (99.8:0.2). The gradient elution was as follows: the initial elution 0% B, followed 3 min by linear gradient from 0% to 10% B, 19 min linear gradient to 13.5% B, 4 min linear gradient to 18.5% B, 10 min elution to 30% B, 9 min elution to 40% B, 7 min gradient elution to 5% B and 6 min isocratic elution step 5% B. Then, 2 min% 100 A was performed for returning to initial condition.

The phenolic compounds were identified by comparing their retention times and spectra with those of analytical standards. The concentration of phenolic compounds in the samples was calculated using by the calibration curves and expressed as μg/mL. Chromatographic analyses were performed in triplicate.

Storage

Manufactured CD, SH and IT beverages were stored at three different storage temperatures (4, 20 and 24 °C) during six months. They were analysed to determine the changing of some quality parameters initially and each two months during storage time. The common drink storage conditions were preferred monitoring of the alterations in the beverages.

Statistical analysis

The variance analysis (ANOVA) was applied to the obtained results. Duncan multiple comparison test was performed to determine the differences between the mean values ($p<0.05$ significance level was used for comparisons). The statistical analysis was performed to Granato et al. (2014).

Sensorial Analysis

A sensorial scoring test was used to determine the beverages sensorial properties (Altuğ et al., 2005). All samples were evaluated in terms of color, taste and general appreciate by seven panellists who had experiences. The samples were scored 5 (best) from to 0 (worst).

Results and discussion

Physical parameters of the beverages

The drinks physical properties such like color, clarity and appearance effect on general appreciation and being preference. The color and transmittance values of produced beverages were shown in Table 2.

There were no statistical differences between OD_{420}, OD_{520} and OD_{620} values in CD and SH. The abundant color characteristic was yellowness in CD. In SH beverages, the yellowness, redness and blueness varied from 61.85 to 63.23, 25.95 to 26.74 and 10.83 to 11.4, respectively. SGC ratios caused to these color variations. The significant statistical differences were found between IT OD_{420}, OD_{520} and OD_{620} values ($p<0.05$). IT beverages had more redness than CD and SH, and this was caused by using black tea. On the other hand, yellowness and blueness in IT beverages were lower.

Table 2

Color and transmittance values of the beverages

Beverages	OD_{420}	OD_{520}	OD_{620}	Transmittances
CD1	94.79	5.21	-	92.54^{a}
CD2	94.60	5.40	-	91.39^{ab}
CD3	100.00	-	-	90.08^{b}
SH1	61.85	25.95	11.41	79.17^{a}
SH2	62.69	26.30	11.01	74.34^{b}
SH3	63.23	26.74	10.83	66.86^{c}
IT1	22.37^{a}	67.91^{b}	9.72^{a}	0.80
IT2	22.15^{b}	68.95^{a}	8.90^{b}	0.77
IT3	22.48^{a}	67.94^{b}	9.58^{a}	0.70

* The values indicating with different letters are statistically different for each beverage group in the each column ($p<0.05$).

The transmittance percentages were calculated by measuring absorbance at 410 nm for determination of the beverage clarity. In CD and SH, the significant statistical differences were observed in terms of transmittance percentages. The best clarity values were in CD samples. The SH transmittance values were lower than CD. The used concentrate adding

amounts affected the beverage clarity. The using of black tea and concentrate caused the increase of turbidity in IT samples. For this reason, the lower clarity values were found in them. The used ingredients in the production process affected the color and clarity of the beverages.

Minerals in the beverages

Table 3 shows the mineral composition of the beverages. Four major (K, Mg, Ca, Na) and minor (Fe, Zn, Mn, Cu) mineral are investigated. The significant statistical differences were found for K, Ca, Mg and Na contents in CD samples and for K, Mg, Na, Zn and Cu contents in SH samples ($p<0.05$), but there were no differences in the other minerals. The K contents in the beverages varied between 2.59 and 45.25 μg/mL. IT samples had more K content than CD and SH. The K is one of the most important mineral for human health and World Health Organization suggests 3510 mg K intake of at least daily for adult (WHO, 2021). So, the sour grape based beverages, especially IT, can be considered as K source in daily diet. The Ca contents ranged from 159.03 to 358.25 μg/mL in these beverages and it was higher than in others. This difference was due to the using of different water sources. The same water source was used for SH and IT, but the CD was made using carbonated water from a local factory.

Table 3

Mineral compositions of the beverages (μg/mL)

Beverages	K	Ca	Mg	Na	Fe	Zn	Mn	Cu
CD1	2.59^{b}	297.28^{b}	41.65^{c}	77.41^{b}	10.26	1.39	3.09	2.96
CD2	3.98^{b}	349.50^{a}	55.73^{b}	86.74^{b}	8.74	1.18	3.87	2.78
CD3	7.59^{a}	358.25^{a}	64.12^{a}	120.80^{a}	16.69	1.58	2.07	5.47
SH1	13.90^{a}	178.33	34.16^{a}	465.13^{a}	19.61	3.01^{a}	2.02	3.40^{a}
SH2	10.45^{b}	163.50	30.45^{ab}	246.95^{b}	18.43	1.67^{b}	1.84	1.76^{b}
SH3	6.87^{c}	177.63	26.55^{b}	199.38^{b}	22.84	2.12^{b}	1.75	1.73^{b}
IT1	45.25	159.03	16.63	692.28	10.15	1.59	4.77	0.93
IT2	33.82	190.58	19.73	481.7	8.74	2.69	3.49	1.18
IT3	36.31	173.95	17.52	428.78	14.5	2.05	4.53	0.93

* The values indicating with different letters are statistically different for each beverage group in the each column ($p<0.05$).

The Mg contents of CD samples were higher than in other beverages. The Mg contents increased depending on SGC adding ratios. There was lower Mg amount in IT beverages because of this. Na contents varied from 77.41 to 692.28 μg/mL. SH and IT samples had more Na than CD beverages, and this may have caused from d used dilution water. Fe contents ranged from 8.74 to 22.84 μg/mL in beverages and it was the most abundant mineral in the micro minerals. The Mn, Zn and Cu contents were lower than other minerals in the beverages. Their contents changed 1.755-4.77, 1.18-3.01 and 0.93-3.40 μg/mL, respectively. In the beverage formulation, the ingredient choosing is an important that it affected the mineral composition. In a previous study, Silva et al. (2019) studied to determine macro and micronutrient elements contents from soft drinks. While our K results are similar with their findings, the other mineral findings are little higher. The beverages mineral contents extremely depend on used the base plant material in production process. In addition, manufacturing

process stages, packaging or bottling and using additives have less extent on the mineral concentration (Silva et al.; 2019). These differences may be caused by using of different raw materials.

Total phenolic contents and antioxidant properties of the beverages

TP and antioxidant capacities of the CD and SH beverages are shown in Figure 2 and 3, respectively. The significant statistical differences were determined in terms of TP ($p<0.05$) in CD and SH beverages, but no observed in IT ($p>0.05$). TP content in IT was approximately tenfold higher than in CD and SH due to using of black tea in formulation. The TP amounts were lower than in previous literature (Lugasi et al., 2003; Brenna et al., 2009; Wu et al., 2011).

Antioxidant capacities of the CD and SH samples increased depending on the used concentrate amounts in all 3 methods. However, these results no changed in the IT samples because of used the same concentrate ratios. The CD antioxidant capacities ranged from 186 to 228 μM TE/L, 124 to 155 μM TE/L and 66 to 77 μM TE/L for FRAP, CUPRAC and ABTS assays, respectively. Brenna et al. (2009) studied on antioxidant capacity of some caramel-containing soft drinks and they reported FRAP assay had lower sensitivity than DPPH assay in cola drinks. The FRAP, CUPRAC and ABTS assays had the similar sensitivity according to our findings in CD beverages. On the other hand, many researchers (Nikfardjam, 2008; Piva et al., 2008; Gollücke et al., 2009; Hayoglu et al., 2009; Öncül et al., 2015; Turkmen et al., 2017; Guler et al., 2018) have expressed that grape and sour grape juice or concentrate had high antioxidant activity. For this reason, the using of SGC increased the phenolic content and antioxidant capacity of CD samples. The SH samples antioxidant capacities varied between 213 and 298 μM TE/L, 149 and 214 μM TE/L and 94 and 138 μM TE/L for FRAP, CUPRAC and ABTS assays, respectively. These values changed depending on SGC in formulation. Moreover, it can be expressed there are a strong correlation between antioxidant capacity and SGC ratio.

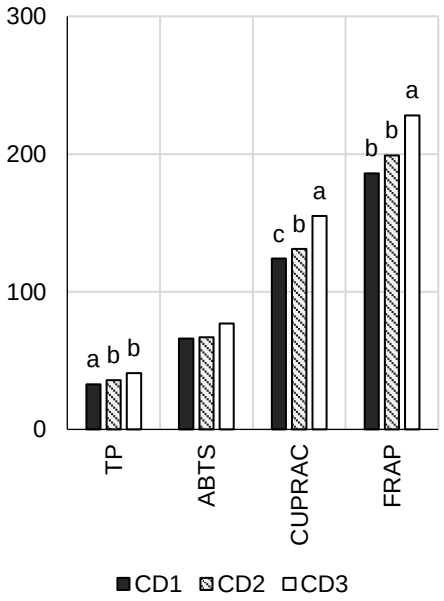

Figure 2.
TP and antioxidant capacities in CDs

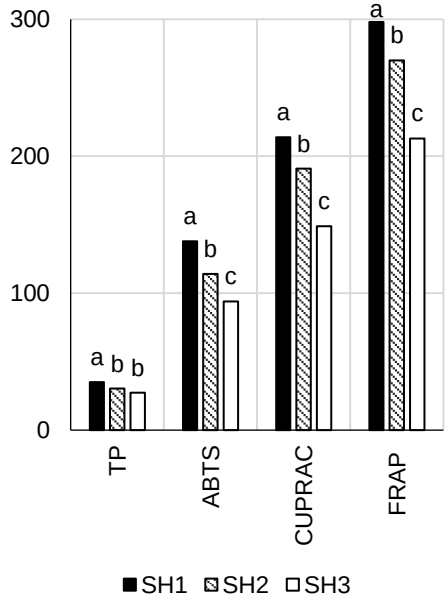

Figure 3.
TP and antioxidant capacities in SHs

Antioxidant capacities of the IT samples ranged from 3868 to 4319 μM TE/L, 4436 to 4893 μM TE/L and 2620 to 2800 μM TE/L for FRAP, CUPRAC and ABTS assays, respectively. There was no observed significant statistical differences among samples because of using the same SGC ratios in IT formulation. In IT production, only the sugar ratios were changed. Pekal et al. (2011) studied on antioxidant properties of fruit, flavoured black teas, and expressed that flavoured teas had higher antioxidant properties than fruit teas for DPPH assay and aromatized teas exhibit the highest antioxidant properties in CUPRAC assay. These findings support our results and study.

Sugar compositions of the beverages

Sugar is an important food component. It is naturally found in many foods, and used widely in industry (Zaitoun et al., 2018). It primary provides sweetness and energy in food products, and has a very important role in formation the texture and color, as well as in fermentation and preservation (Rosa et al., 2009). The sugar compositions of beverages are presented in Figure 4.

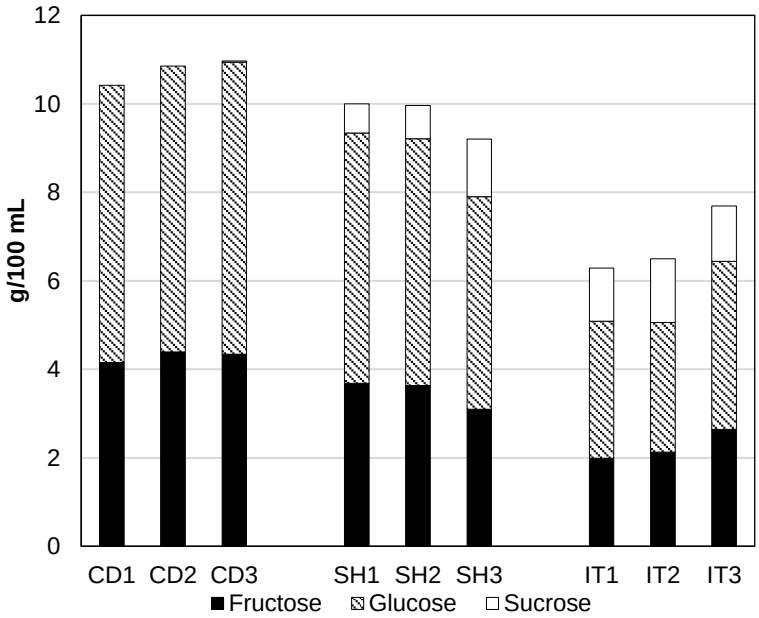

Figure 4. Sugar compositions of the beverages

The total sugar contents of CD, SH and IT beverages varied from 10.42 to 10.97, 9.20 to 10.00 and 6.29 to 7.69 g/100 mL, and the glucose-fructose ratios were ranged between 1.47 and 1.52, 1.53 and 1.54 and 1.37 and 1.55 in the CD, SH and IT, respectively. According to the ratios, the content of glucose is higher than fructose in the beverages, it can be thought that this is caused by both used of sour grape concentrate and sucrose inversion to have being during the preparation of syrup. Sabir et al. (2010) announced that grape has higher content of glucose than fructose in unripe stage. Sugar content of samples were set according to beverage group and market products. It has been tried to use the low sugar amount in the

produced beverages since the increase of consuming sugar leads to several diseases. The sugar content was kept constant to reach the target taste balance in CD and SH beverages, but they were changed in IT samples because of SGC ratios constant. In this study, it was used unripe sour grapes to obtain the concentrate that was added to provide flavour and acid to the beverages. Our findings compatible with the previous literature.

Individual phenolic compounds in the beverages

The phenolic acid, flavanol and flavonol compounds were determined in SGC based beverages and their results were shown in Table 4.

Table 4

Phenolic compounds in the beverages (µg/mL)

Compounds	CD1	CD2	CD3	SH1	SH2	SH3	IT1	IT2	IT3
Gallic acid	1.15^{c}	1.80^{b}	2.52^{a}	0.66	0.22	0.17	76.40	77.13	76.10
Vanilic acid	0.30^{b}	0.34^{b}	0.45^{a}	0.34^{a}	0.32^{a}	0.25^{b}	1.63^{a}	1.34^{b}	1.64^{a}
Caffeic acid	0.15^{b}	0.16^{b}	0.22^{a}	0.45^{a}	0.26^{b}	0.24^{b}	2.09^{a}	2.04^{b}	2.06^{ab}
p-Coumaric acid	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	0.64	0.69	0.66
Ferulic acid	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	2.60^{a}	2.20^{b}	2.42^{ab}
Sinapic acid	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	5.74^{c}	5.93^{a}	5.84^{b}
Myricetin	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	1.95	1.86	1.89
Quarcetin	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	1.31^{b}	1.55^{a}	1.46^{a}
(-)-Epigallo-catechin	*nd.*	*nd.*	*nd.*	*nd.*	*nd.*	6.80^{c}	55.57^{a}	52.49^{b}	55.91^{a}
(+)-Catechin	1.15^{c}	1.46^{a}	1.39^{a}	0.82^{a}	0.66^{b}	0.47^{c}	32.28	31.39	32.30
(-)-Epigallo-catechin- gallate	0.76^{b}	0.90^{a}	1.01^{a}	1.90^{a}	1.51^{b}	1.48^{b}	30.98^{a}	31.25^{a}	29.46^{b}
(-)-Epicatechin	*nd.*	*nd.*	*nd.*	3.79	*nd.*	*nd.*	75.14^{b}	86.14^{a}	74.01^{b}
(-)-Epicatechin-gallate	*nd.*	*nd.*	*nd.*	nd.	*nd.*	*nd.*	16.43^{a}	16.95^{a}	11.05^{b}

*The values indicating with different letters are statistically different for each beverage group in the each row ($p<0.05$).
**nd.: not detected

The gallic, vanilic and caffeic acids, (+)-catechin and (-)-epigallocatechin gallate were determined all beverages, but p-coumaric, ferulic and sinapic acids, myricetin, quercetin, (-)-epigallocatechin and (-)-epicatechin gallate could no detected in the CD and SH beverages. In addition, (-)-epicatechin was only found SH1 and IT beverage samples. The phenolic compound quantities in IT samples were more than CD and SH beverages owing to using of black tea in the formulation. The most abundant phenolic acid was gallic acid that varied from 1.15 to 25.52, 0.17 to 0.66 and 76.1 to 77.13 µg/mL in the CD, SH and IT beverages, respectively. Nikfardjam (2008) reported that Iran sour grape juice had between 36.6 and 70.6 mg/L gallic acid. According to another study results, gallic acid content ranged from 1.05 to 1.85 mg/100 g DW during sour grape juice processing (Guler et al., 2018). The current study gallic acid findings in CD and SH beverages are lower than the previous literatures because of limited use of SGC in the formulation. On the other hand, the gallic acid content of black tea watery extract was determined as 0.216 mg/100 mL by Agca et al. (2020). Cabrera et al. (2003) expressed that black tea samples included between 2.5 and 4.5 mg/g gallic acid. In another study, it was presented that in Turkish black tea gallic acid contents

were between 2.38 and 2.58 mg/g (Atalay et al., 2017). Furthermore, the fermentation and infusion conditions in tea production effect the phenolic compound contents such like gallic acid ((Fernandez et al., 2002; Zuo et al., 2002; Cabrera et al., 2003; Atalay et al., 2017). In addition, the preferred tea and fruit concentrate ratios in the formulation, dilution of infused tea and processing conditions can effect on phenolic content of the beverages. Especially, the infusion and dilution of the tea for iced tea manufacturing are one of the important processing stages.

In IT beverages, the primarily flavonol was (-)-epicatechin with 74.01–86.14 μg/mL. The (-)-epigallocatechin, (+)-catechin, (-)-epigallocatechin gallate and (-)-epicatechin gallate followed to (-)-epicatechin in terms of quantity, respectively. The tea and SGC in the formulation increased the flavonols in IT beverages as expected. It is already known tea, grape and their derivative products are among the richest products in terms of polyphenols (Fernandez et al., 2002; Zuo et al., 2002; Cabrera et al., 2003; Nikfardjam, 2008; Sabir et al., 2010; Atalay et al., 2017; Turkmen et al., 2017; Guler et al., 2018; Guler et al., 2019; Agca et al., 2020).

(-)-Epigallocatechin gallate has higher antioxidant capacity than other catechins and is an important substance for human health and it has beneficial properties to prevent metabolic syndrome (Rice-Evans, 1999; Legeay et al., 2015). The presence of the (-)-epigallocatechin gallate in all produced beverages, less CD and SH, is one of the most important findings of this study.

Sensory properties of the beverages

The sensorial scoring results are shown in the Figure 5.

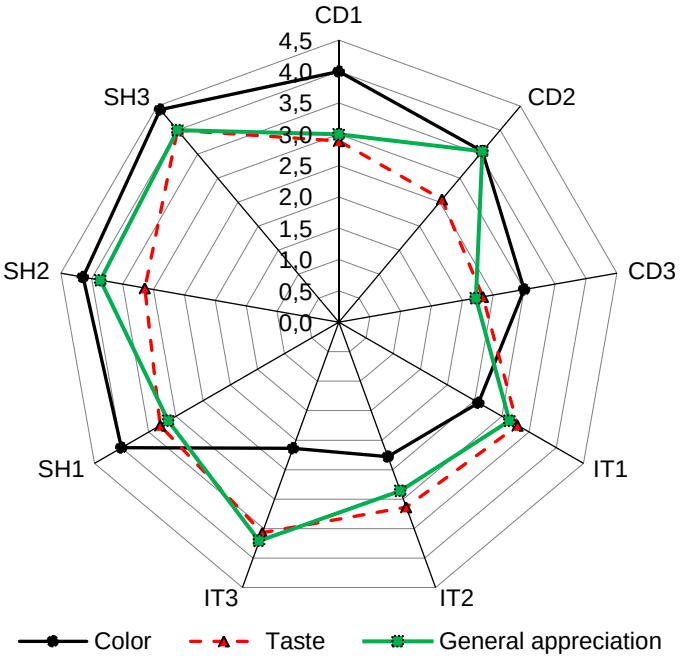

Figure 5. Sensorial scoring test results of the beverages: CD: Carbonated drinks, SH: Sherbets, IT: Iced tea drinks

Each sample was evaluated within itself beverage group. As CD beverages were evaluated, it is observed that CD1 for color and taste and CD2 for general appreciation had the highest score. In the CD samples, the varying taste balance ratios from 45 to 35 caused to reducing of the sensorial color and taste scores. As similar, the highest color, taste and general appreciation scores were observed in SH3 that had higher taste balance than others among the sherbets. The increasing SGC ratios and carbon dioxide in the CD were created an unpleasant mouth feeling together. As result, it can be stated the best taste balance ratios in terms of sensorial quality are 40–45 for CD and 30-35 for SH beverages. At the same time, it can be considered being effective of formulation, carbon dioxide using and preservation technique also. In the IT beverage formulations, sour grape concentrate ratios were kept as constant, but sugar contents were changed. The taste and general appreciation scores raised correspondingly, but color scores declined. It is found that the most acceptable taste balance is 35 for sensorial quality in the IT beverage formulation. However, the using of flavour and acidify compounds can cause the changing of that ratio.

Effects of storage on beverages qualities

The formulated beverages were stored for monitoring of pH, acidity, TP and DPPH inhibitions during six months and the changes were observed in every two months.

The changes of beverage pH values are shown in Figure 6.

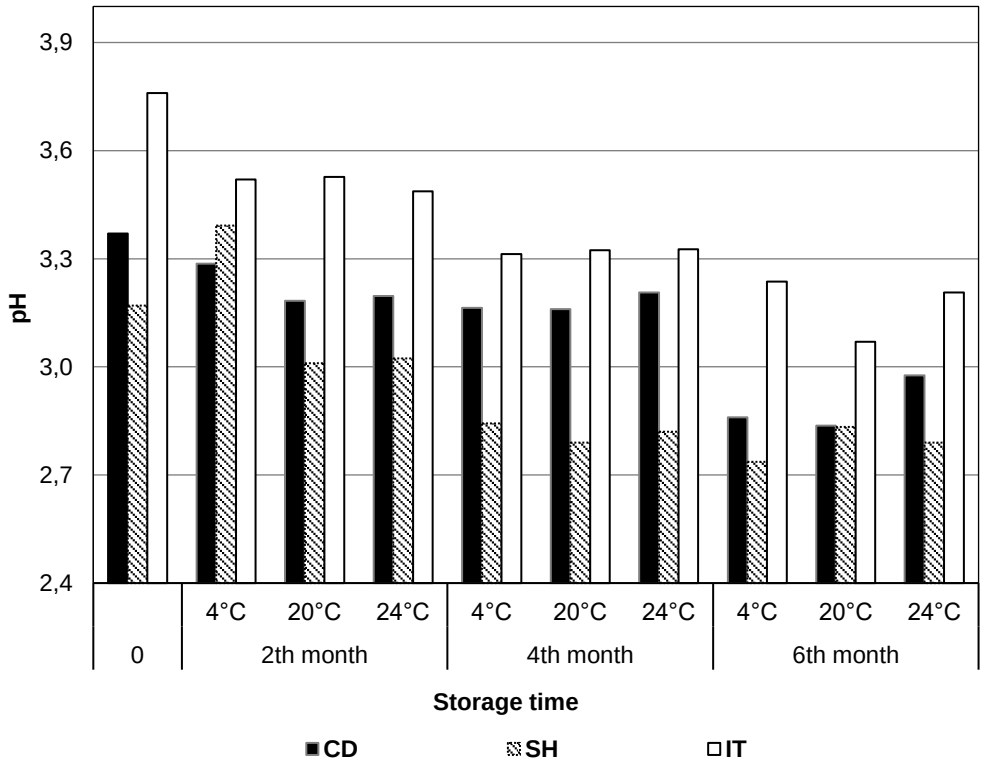

Figure 6. pH changes of the beverages during storage

The significant statistical differences were observed in the pH values, and SGC ratio and storage temperature together were important as statistically ($p<0.05$). The pH values decreased by 10.99-11.67% in CD, by 11.99-15.02% in SH, and by 12.46-17.17% in IT beverages to the end of the storage at 24°C. The decreasing of the pH values were 9.95-15.83% in CD, 10.62-13.21% in SH, and 17.26-18.06% in IT samples stored at 20°C, and 11.02-15.03% in CD, 12.50-14.40% in SH and 13.26-14.23% in IT beverages stored at 4°C. The least pH values were found in the IT and SH beverages at 20°C and 24°C the storage temperatures, respectively. There were the similar results in CD samples pH values in except CD3 ($p<0.05$). The highest pH was observed in CD1 stored at 24°C, while the least value was found in CD3 at 20°C. In the SH beverages, the highest and lowest pH values were measured SH3 and SH1 stored at 4°C, respectively. In the IT samples, IT2 and IT3 had the highest and lowest pH values stored at 20°C ($p<0.05$).

Gonzalez-Molina et al. (2012) reported that pH and titratable acidity of fruit juice mixture (lemon, elderberry and grape juice concentrate) and control juices over the 56 day of storage no changed as statistically. Balaswamy et al. (2011) found that changes in the pH were negligible as statistically after 6 months of storage at room temperature in all studied sour grape based ready to serve beverages. On the other hand, Jooyandeh (2015) stated that pH values of naturally carbonated beverages decreased from 3.2 to 2.9 in red plum beverages and from 3.4 to 3.2 in yellow plum beverages to the end of the 90 days of storage. Moreover, the pH values of carbonated pineapple juice reduced from 4.10 to 2.98 after 9 weeks storage (Jori et al., 2015). The pH values in our study were in accordance with these previous reported in literature.

The SGC ratios and storage temperatures effects on TP content in CD beverages were found as statistically significant, but this was no for SH and IT samples ($p<0.05$). TP contents were similar at 20 and 24 ºC at the end of the storage, but less of the storage at 4 ºC. The TP contents of CD samples decreased between 26 and 32% comparing to the storage beginning. On the other hand, there were no significant changes of the SH and IT beverages TP contents. Gollücke et al. (2009) studied on TP content of grape juice concentrate during process and storage period. They stated that retention of TP was 90% and 81% for Concord and Isabella grape juice during storage, respectively. In the another study, TP content of pomegranate juice decreased from 1858 to 476 mg/L stored at 4 ºC and 458 mg/L stored at 20 ºC after 6 months (Tastan et al., 2015).

The changes of DPPH inhibitions during storage can be seen in Figure 7. The inhibitions in SH decreased between 5.2 and 68.9% according to initial after 6 months storage. The furthest declining in inhibition was observed in SH beverages that were stored at 24 ºC. The inhibitions in ice tea samples decreased 5.5–41.7% end of the storage. The decreases in IT samples having stored at 20 ºC and 24 ºC was higher than stored at 4 ºC.

On the other hand, there were variations in CD samples results in terms of DPPH inhibitions according to SH and IT. The inhibitions increased between 3.9 and 25.11% in CD samples after 6 months storage. These increases in CD beverages stored at 20 and 24 ºC were higher than stored at 4 ºC.

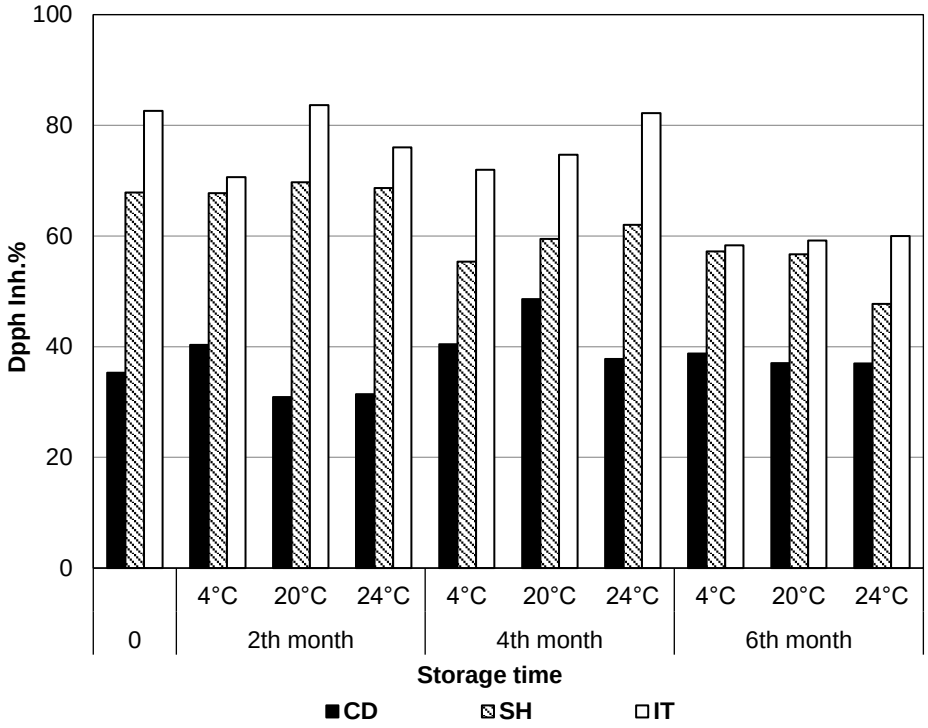

Figure 7. The changes of DPPH inhibitions during storage

Conclusion

1. This study revealed usability of the sour grape juice in beverages such like carbonated drinks, sherbets and iced teas, and identified their physicochemical properties, color and clarity, mineral and sugar compositions, total phenolic content, antioxidant capacity and individual polyphenols. The sugar-acid balance set between 20 and 45 for beverages by using ingredients. Minerals, total phenolic contents, antioxidant capacities and individual phenolic compounds in the beverages increased depending on used SGC rising ratios. The preferred concentrate ratios and used ingredients changed color and clarity also.
2. In the beverages, Ca and Na were the most abundant minerals among major minerals and Fe in minors. Total phenolic content in the iced tea was approximately in ten fold higher than in carbonated drinks and sherbets. There were also the similar situation in the contents of flavonoids, polyphenol compounds and antioxidant capacities.
3. Gallic acid was the most abundant phenolic acid in the beverages and (-)-epicatechin for IT samples the most abundant flavonols. (-)-Epigallocatechin gallate has higher antioxidant capacity than other catechins and is an important substance for human health. The presence of the (-)-epigallocatechin gallate in all produced beverages, less CD and SH, is one of the most important findings of this study.
4. The acceptable taste balance values in terms of the sensorial general appreciation were 40–45 for CD, 30–35 for SH and 35 for IT.

5. The beverages pH, acidity, total phenolic content and DPPH inhibition can be changed based on storage temperatures and duration. At the end of the storage, beverage pH values slightly decreased and acidities increased. Total phenolic contents were similar at 20 and 24 ºC after storage, but less at 4 ºC. In addition, these values decreased between 26 and 32% comparing to the storage beginning in carbonated drinks.

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Detection of raspberry honey adulterated with agave, maple, rice, corn and inverted sugar syrups using instrumental techniques

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Abstract

Introduction. Honey adulteration has negative effects from a financial and health point of view. In this work, the usefulness of the electronic tongue consisting of copper working electrode, in correlation with physico-chemical properties in the detection of adulterated honey with agave, maple, inverted sugar, cornand rice syrups is presented.

Materials and methods. Honey samples adulteration was done by adding syrup in different percentages: 5, 10, 20 and 50% (w/w). Physicochemical parameters analyzed were: color, moisture content, pH, free acidity, electrical conductivity and hiroxymethylfurfural content (HMF). The measuring system was a PGSTAT with FRA32M module coupled with three electrodes: reference electrode (Ag/AgCl), counter electrode (platinum) and working electrode (copper).

Results and discussion. Honey adulteration has significantly influenced both physicochemical and voltametric tongue parameters. There was changes depending on the adulteration agent but also depending on the degree of adulteration. Adulterated honey with inverted sugar syrup presented the highest value of L* parameter (33.14), in opposite with the lowest value in case of adulterated honey with rice syrup (27.10). The moisture content increased from 16.16% in authentic honey to 19.28% in adulterated honey with 50% syrup. The magnitude of pH decrease in adulterated honey with inverted sugar syrup, due to the presence of added citric acid. The higher increase in the value of HMF content was from 58.09 mg/kg in authentic honey to 185.07 mg/kg in adulterated honey with inverted sugar syrup. As respects to voltammetric tongue, the current intensity value for authentic honey was 0.192 mA. Pure maple and rice syrups presented the highest values, while the lowest were for the other three types of syrup. Regardless of the adulteration agent used, the fluctuations of the current intensity were highlighted starting from the adulteration degree of 5% with the copper electrode. According to Principal component analysis (PCA), the first component (PC-1) represented 88% of the variance and the second component (PC-2) 11%, totaling 99% of the initial variability. Adulterated honey samples with 5, 10 and 20% agave syrup, 5 and 10% corn syrup as well as 5% maple syrup are close to authentic honey.

Conclusions. Physicochemical parameters (color, pH, moisture content, free acidity, electrical conductivity, HMF content) can be used as a preliminary analysis, but by coupling the voltammetric tongue with it have proven their usefulness in detecting adulterated honey samples with different adulteration agents even at 5%.

Introduction

Electronic tongue is a device consisting of a group of sensors used in identification and classification the tastes of chemicals in various food samples in the liquid phase but can also be used in the qualitative and quantitative characterization of multicomponent mixtures (Peris et al., 2016). The application and development of electronic tongue is based on different electrochemical techniques: potentiometry, voltammetry, impedance, conductometry, spectrophotometry etc. (Bougrini at el., 2016; Ha et al., 2015). Chemometric instruments have an essential role in recognizing the pattern of electronic tongue, being responsible for translating multivariate signals (Shimizu et el., 2020).

Electronic tongue based on potentiometry with various chemometric methods such as PLS (Partial Least Squares), LDA (Linear Discriminant Analysis), PCA (Principal Component Analysis), ANN (Artificial Neural Network) have been successfully applied in the qualitative analysis of honey, being used in botanical origin classification, but also in adulteration detection (Veloso et al., 2018; Major et al., 2011; Escriche et al., 2012). Against the electronic tongue based on potentiometry, the one based on voltammetry has various advantages such as: simplicity, low detection limits, ruggedness, long service life (Gan et al., 2016). The most used method is cyclic voltammetry, the results obtained being found in the form of a voltamogram that allows the characterization of the oxide-reduction process over a wide range of potential (Sobrino-Gregorio et al., 2018). The components of a cyclic voltammetry system are: potentiostat, electrolysis cell, current-voltage converter as well as a software for obtaining the results (Tiwari et al., 2012). Ropciuc et al. used cyclic voltammetry to detect adulterated honey by adding malt wort and inverted sugar, with gold and silver as the working electrodes. They concluded that the adulteration of honey produced significant changes by coupling electronic tongue with physico-chemical methods and PCA (Ropciuc et al., 2017).

The purpose of this paper is to detect adulterated honey samples with different types of syrups (corn, rice, inverted sugar, agave and maple) using the correlation between electronic tongue and physicochemical parameters.

Materials and methods

Materials

Raspberry honey was purchased from a beekeeper located in Suceava County, Romania. Five sugar syrups were used for honey adulteration: agave (Clarks, Mexic), maple (BioLogistic & Distribution Partener, Canada), rice (Panaisia de Hadels GMBH Importing company, Korea), corn (Daesang Europe BV Importing company, Korea) and inverted sugar (obtained in laboratory from acid hydrolysis of sucrose).

Honey samples adulteration was done by adding syrup in different percentages: 5, 10, 20 and 50 (w/w). These adulteration percentages were chosen to observe the changes of the physicochemical parameters and voltammetric tongue data that appear depending on the proportion of adulteration agent (from a percentage of 5 to 50%). ensure good homogeneity, the samples were kept at 50 °C for 24 hours.

Physicochemical analysis

The following physicochemical parameters were analyzed using Harmonised methods proposed by the International Honey Commission (Bogdanov et al, 2002): moisture content, pH, free acidity, electrical conductivity and hiroxymethylfurfural content (HMF).

The moisture content was determined on the basis of the refractive index of honey by conversion using a standard table (Chataway table). A drop of sample was placed on an Abbé refractometer (Leica Mark II Plus, Germany) which was previously calibrated with distilled water. The measurement was performed at 20 °C, the final result obtained being expressed as a percentage (%).

pH and free acidity were determined using an automatic Titroline device (SCHOTT instrument, Germany). 10 g of honey were dissolved in 75 ml of distilled water; the pH was measured directly in solution and the free acidity was determined by titration with 0.1 M sodium hydroxide solution to a pH of 8.30. The calculation was performed using the following formula, the result being expressed in milliequivalents acid/kg honey:

$$\text{Free acidity} = \text{mL of 0.1 M NaOH} \times 10 \quad (1)$$

The electrical conductivity was measured using an XL 30 conductometer (Fisher Scientific, Germany). The analysis solution was prepared by dissolving 20 g of honey in 100 ml of distilled water. The results obtained were expressed in microSiemens per centimeter ($\mu S \cdot cm^{-1}$).

The hydroxymethylfurfural (HMF) content was determined by the method proposed by White (White at el., 1979). The preparation of the samples was carried out as follows: 5 g of honey were dissolved in 25 mL of ultrapure water (Milli-Q, Merck Millipore). The solution was transferred to a 50 mL volumetric flask, over which 0.5 mL of Carrez I solution was added and mixed; then 0.5 mL of Carrez II solution was added and made up with ultrapure water. The resulting solution was filtered through filter paper and the first 10 mL of filtrate was removed. In two test tubes 5 mL of filtrate were introduced over which 5 mL of ultrapure water (in the first test tube) and 5 mL of 0.2% sodium bisulphite (in the second test tube representing the reference solution) were added. The absorbance of the sample solution against the reference solution at 284 and 336 nm was read using a UV-3600 spectrophotometer (Schimadzu Corporation, Japan). The HMF content was calculated using the following equation, the result being expressed in mg/kg:

$$\text{HMF (mg/kg)} = (A_{284} - A_{336}) \text{ x } 149.5 \text{ x } 5 \text{ x } D/W, \quad (2)$$

where: A284 – absorbance at 284 nm, A336 – absorbance at 336 nm, D – dilution factor, W – weight of the honey sample (g).

Two instruments were used to measure the honey color: a photometer Pfund (Hanna Instruments, USA) and portable chromameter (Konica Minolta, Japan).

Ciclyc voltammetry

The measuring system was a PGSTAT with FRA32M module (Metrohm, Germany) coupled with three electrodes: reference electrode (Ag/AgCl), counter electrode (platinum) and working electrode (copper) (Metrohm, Germany). Data recording was done using a NOVA 2.0 software (Metrohm, Germany). The voltage was applied from -1V to +1V, with a scan rate of 0.5 V/s.

The electrodes were immersed in honey solution (8 g honey completed to 50 mL with deionized water). The data were read 5 minutes after introducing the electrodes into the solution, to achieve electrochemical balance (White et al., 2020). The copper electrode was cleaned by polishing with filter paper and rinsing with deionized water. All analysis were made in duplicates.

Statistical analysis

In this study, Multi-factor Analysis of variance (ANOVA) using XLSTAT trial version (Microsoft, USA), was chosen as a method to highlight the differences between authentic and adulterated honey properties. Also, the results obtained were interpreted using Principal component analysis (PCA) – Unscrambler X software – version 10.1 (Camo, Norway). This statistical method was useful in clustering honey samples.

Results and discussion

Physicochemical parameters

Authentic and adulterated honey both exhibited different characteristics, all these data being useful in quality control. Adulteration agents significantly influenced almost all physicochemical parameters analyzed ($p<0.001$). Depending on the degree of adulteration, significantly changes were for the a*, ΔE* parameters ($p<0.001$) and moisture content ($p<0.01$), for the rest the influence being insignificant ($p>0.05$).The mean values of the physicochemical parameters for authentic and adulterated honey with the five types of agents as well as the means obtained depending on the degree of adulteration are shown in Tables 1 and 2.

Color of honey reported in CIE L*a*b* space is represented by the following coordinates: L* (lightness), a* (red/green) and b* (yellow/blue) (Kek et al., 2017). Both authentic honey and adulterated samples presenting the positive values for a* and b* coordinates, fit in the shades of red-orange-yellow (the first trigonometric quadrant). Adulterated honey with inverted sugar syrup presented the highest value of L* parameter (33.14), in opposite with the lowest value in case of adulterated honey with rice syrup (27.10). This increase of the lightness value of honey by adding inverted sugar was also observed by Ropciuc et al. (Ropciuc et al., 2017). Regarding the Pfund scale, the honey adulteration with agave and inverted sugar syrups led to a shade of extra light amber, while the adulterated samples with the other three types of syrups remained in the same color shade as the authentic honey (light amber), but with significant variations in values.

pH influences the texture and stability of honey having an important role during its extraction and storage (Özcan et al., 2006). It also indicates a possible microbial growth, especially for mold and yeast (at pH 4-4.5 they can survive) (Ismail et al., 2019). The pH of all the honey samples analyzed was acidic. Adulterated honey with inverted sugar syrup decrease its value due to the presence of added citric acid. The decrease of pH value was observed not only in adulterated honey with inverted sugar but also in the one adulterated with malt wort, glucose and hydrolyzed inulin syrup (Oroian et al., 2018). In the case of adulterated honey with other types of syrups studied, the pH value showed a significant increase.

Fermentation of sugars with alcohol formation, produced under the action of microorganisms, followed by oxidation and formation of carboxylic acids lead to a high free acidity (Almeida-Muradian et al., 2007). Adulterated honey samples with rice syrup were the only ones that showed a higher free acidity (12.81 meq/kg) compared to authentic honey (10.40 meq/kg). None of the samples showed free acidity higher than 50 meq/kg.

The electrical conductivity depends on the content of proteins and mineral salts, providing information about the botanical origin of honey (Stihi et al., 2016). Inverted sugar, agave and corn syrups produced a decrease of electrical conductivity, while maple and rice syrups significantly increased its value. Ropciuc et al. also, observed a decrease of the electrical conductivity of adulterated honey with inverted sugar (Ropciuc et al., 2017)

Table 1

ANOVA of physicochemical parameters of authentic and adulterated raspberry honey with agave, corn, inverted sugar, maple and rice syrups

Parameter	Adulteration agent						*F*-ratio
	Authentic	Agave	Corn	Inverted sugar	Maple	Rice	
L*	30.26b	32.83c	30.90bc	33.14c	27.48a	27.10a	13.25***
a*	6.83a	7.90a	6.72a	8.07ab	9.41b	7.48a	3.02*
b*	30.34c	23.72b	21.69b	24.27b	15.94a	14.60a	19.05***
ΔE*	0a	5.63c	2.41ab	5.26bc	6.48c	7.65c	4.40**
Pfund (mm Pfund)	57.75abc	49.75a	55.69ab	49.83a	70.46c	65.09bc	5.75***
pH	4.05a	4.09a	4.14a	3.97a	4.62b	4.18a	7.19***
Free acidity (meq/kg)	10.40a	8.86a	8.90a	9.53a	8.86a	12.81b	9.26***
Electrical conductivity (μS/cm)	233.97ab	191.19a	187.74a	203.78a	455.98c	322.61b	9.46***
Moisture content (%)	16.16a	17.56a	17.40a	16.49a	19.57b	26.40a	4.74**
HMF (mg/kg)	58.09a	70.66a	46.35a	185.07b	46.50a	51.54a	9.19***

Means followed by different letters in the same rows are significantly different ($p<0.05$)

Table 2

ANOVA of physicochemical parameters of authentic and adulterated raspberry honey in different percentages with syrup

Parameter	Percentage					*F*-ratio
	0%	5%	10%	20%	50%	
L*	30.26a	30.76a	30.15a	29.63a	30.63a	0.19ns
a*	6.83ab	9.18c	8.44bc	7.99b	6.04a	9.15***
b*	30.34b	21.24a	20.00a	19.55a	19.38a	2.18ns
ΔE*	0a	4.12ab	3.98ab	5.15b	8.68c	6.90***
Pfund (mm Pfund)	57.75a	61.58a	60.85a	59.20a	51.02a	1.19ns
pH	4.05a	4.08a	4.12a	4.19a	4.40a	1.79ns
Free acidity (meq/kg)	10.40a	10.25a	10.11a	9.83a	8.98a	0.61ns
Electrical conductivity (μS/cm)	233.97a	242.98a	251.99a	270.00a	324.06a	0.53ns
Moisture content (%)	16.16a	16.47a	16.78a	17.41a	19.28b	5.33**
HMF (mg/kg)	58.09a	63.25a	68.41a	78.74a	109.70b	0.68ns

Means followed by different letters in the same rows are significantly different ($p<0.05$)

The moisture content shows the degree of aging of the honey and its ability to remain stable throughout storage (Sudzina et al., 2009), a high content facilitating the fermentation process (Czipa et al., 2019). Depending on the degree of adulteration the moisture content increased from 16.16% in authentic honey to 19.28% in adulterated honey with 50% syrup. Depending on the agent used, maple syrup produced the highest increase (26.40%), exceeding the maximum limit of 20%. The increase of the moisture content was observed not only by the addition of corn syrup in pure honey but also in the case of high fructose corn syrup (Ribeiro et al., 2009).

Fresh honey has small amounts or even traces of HMF, the formation being slow as long as the storage period and temperature are appropriate. Temperature exposure as well as honey adulterated with inverted sugar syrup lead to the formation of a high amount of HMF (Czipa et al., 2019). The same observation can be made by us, the adulteration with inverted sugar syrup led to a high increase in the value of HMF content (from 58.09 mg/kg in authentic honey to 185.07 mg/kg in adulterated honey). Depending on the degree of adulteration, the HMF content increased with the increase of the percentage of added syrup. Agave syrup, also, produced an increase in HMF content (70.66 mg/kg). All honey samples exceeded the maximum allowed limit of 40 mg/kg.

Voltammetric tongue

Figure 1 shows the voltammograms group made for authentic honey, pure syrups and adulterated honey samples. It is clearly observed that honey adulteration has significantly influenced the parameters of voltametric tongue. There are changes in current intensity that occur depending on the adulteration agent but also depending on the degree of adulteration. The current intensity value for authentic honey was 0.192 mA. Regarding pure syrup, the highest values were for maple and rice syrups and the lowest were for the other three types of syrup. Thus, significant increases are observed in the case of adulterated honey with 50% maple syrup (0.419 mA) and 50% rice syrup (0.283 mA). At the same time, significant decreases in current intensity values compared to authentic honey were in the case of adulterated honey with 50% inverted sugar syrup (0.068 mA), 50% corn syrup (0.047 mA) and 50% agave syrup (0.045 mA). Regardless of the adulteration agent used, with the copper electrode the variation of the current intensity were highlighted starting from the adulteration degree of 5%.

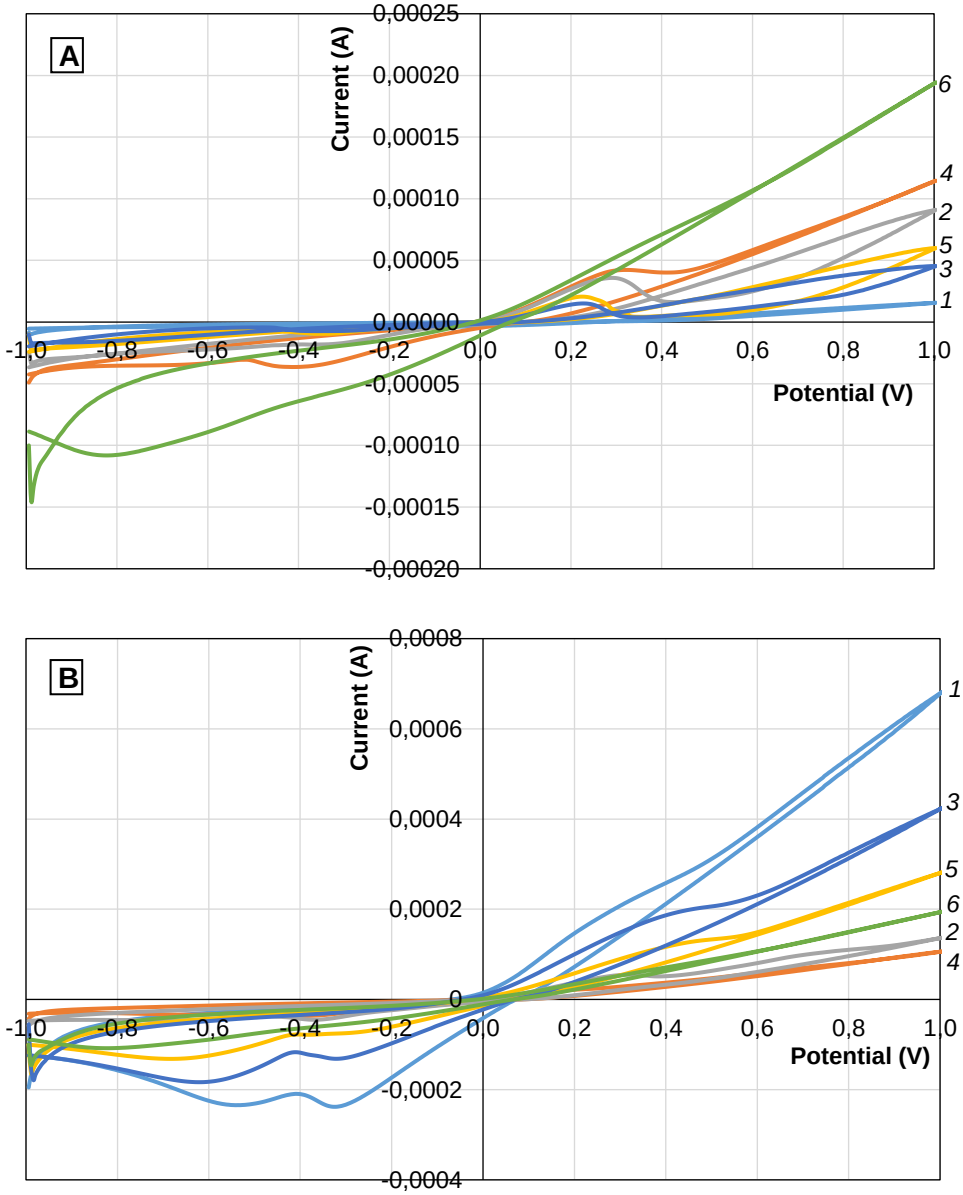

Figure 1. Cyclic voltammograms (using copper as working electrode) of authentic raspberry honey, pure syrups and honey adulterated with:
(A) – agave syrup, (B) – maple syrup, (C) – rice syrup,
(D) – corn syrup and (E) – inverted sugar syrup in 5, 10, 20 and 50

1 Agave syrup
2 Honey adulteration 10%
3 Honey adulteration 50%
4 Honey adulteration 5%
5 Honey adulteration 20%
6 Raspberry honey

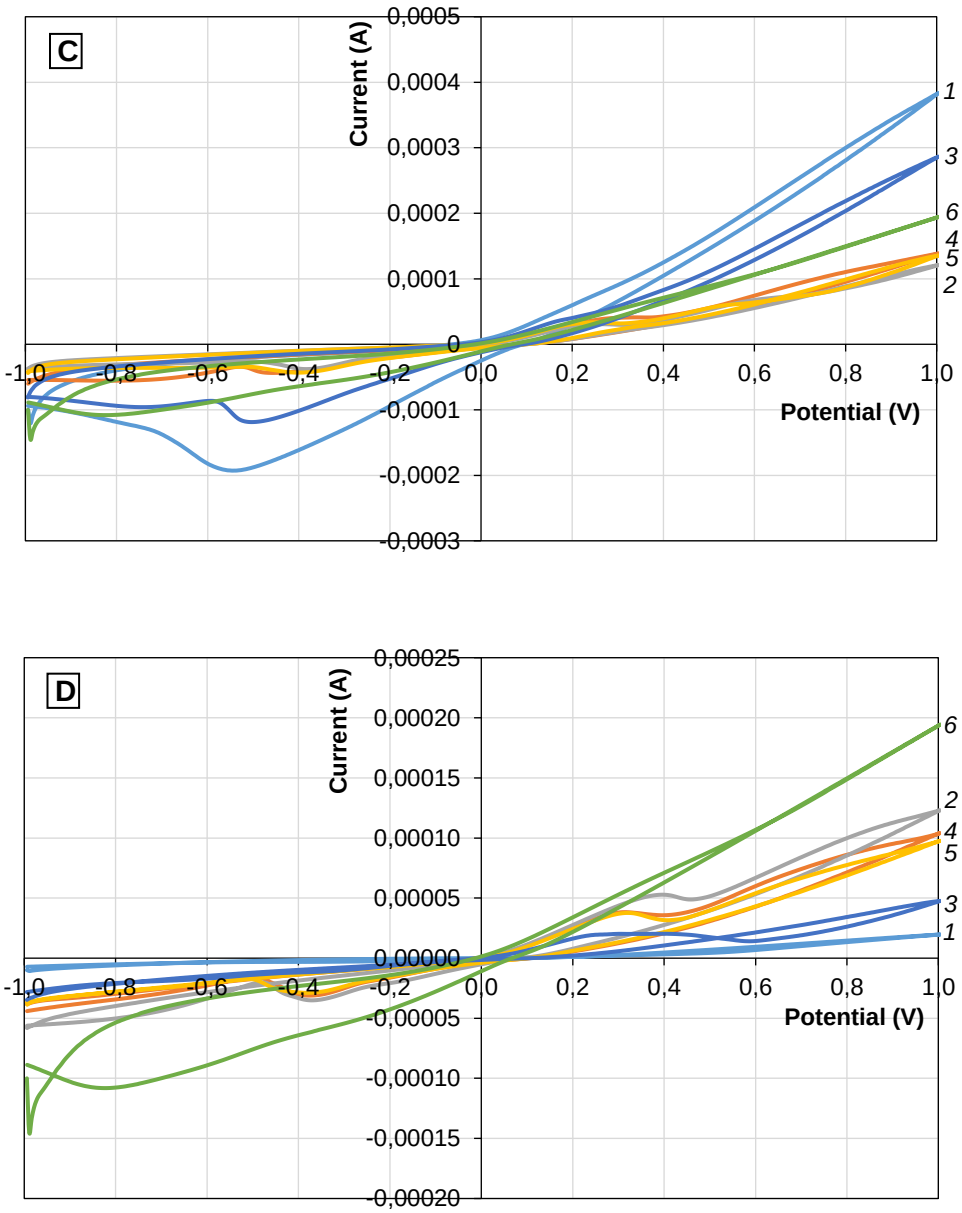

Figure 1 (*Continue*). Cyclic voltammograms (using copper as working electrode) of authentic raspberry honey, pure syrups and honey adulterated with: (A) – agave syrup, (B) – maple syrup, (C) – rice syrup, (D) – corn syrup and (E) – inverted sugar syrup in 5, 10, 20 and 50

1 Agave syrup
2 Honey adulteration 10%
3 Honey adulteration 50%
4 Honey adulteration 5%
5 Honey adulteration 20%
6 Raspberry honey

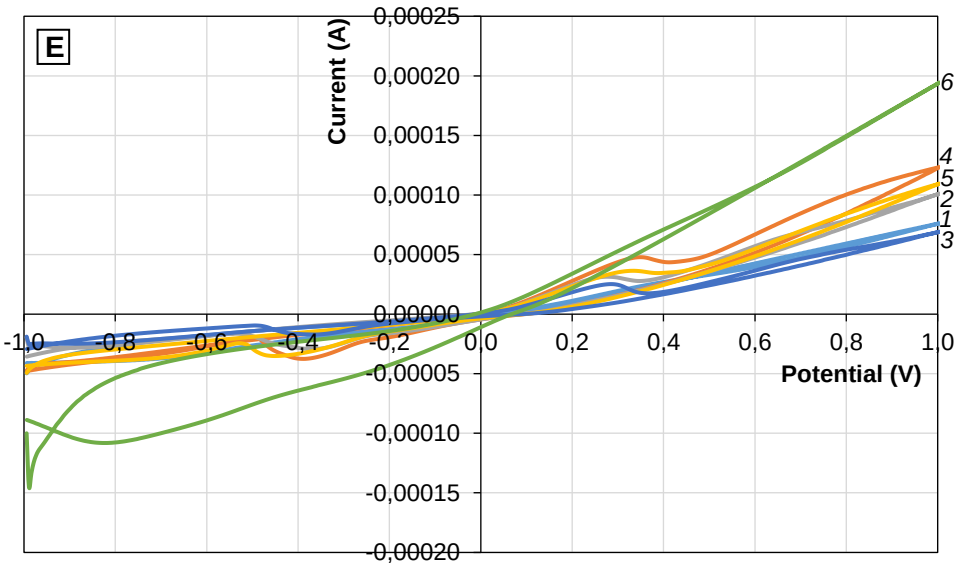

Figure 1 (*Continue*). Cyclic voltammograms (using copper as working electrode) of authentic raspberry honey, pure syrups and honey adulterated with: (A) – agave syrup, (B) – maple syrup, (C) – rice syrup, (D) – corn syrup and (E) – inverted sugar syrup in 5, 10, 20 and 50

1 Agave syrup
2 Honey adulteration 10%
3 Honey adulteration 50%
4 Honey adulteration 5%
5 Honey adulteration 20%
6 Raspberry honey

Principal component analysis

Principal component analysis (PCA) is used to reduce experimental data to two main components. Thus, the first component (PC-1) represented 88% of the variance and the second component (PC-2) 11%, totaling 99% of the initial variability. As can be seen in Figure 2, agave and corn syrups best mimics the characteristics of authentic honey. The samples of adulterated honey with 5%, 10% and 20% agave syrup, 5% and 10% corn syrup as well as 5% maple syrup are close to authentic honey. Adulterated honey samples with both inverted sugar syrup and rice syrup are found in other dials, due to their chemical composition. At the same time, there is a similarity of the chemical composition between adulterated honey samples with inverted sugar and maple syrups (5% with 10%, 10% with 20% and 20% with 50% inverted sugar syrup – maple syrup). According to Principal component analysis – loadings (Figure 3) the color parameters (L*, a*, b*), Pfund, free acidity, pH, moisture content are correlated with Cu^- and E* parameter, HMF content and electrical conductivity are correlated with Cu^+.

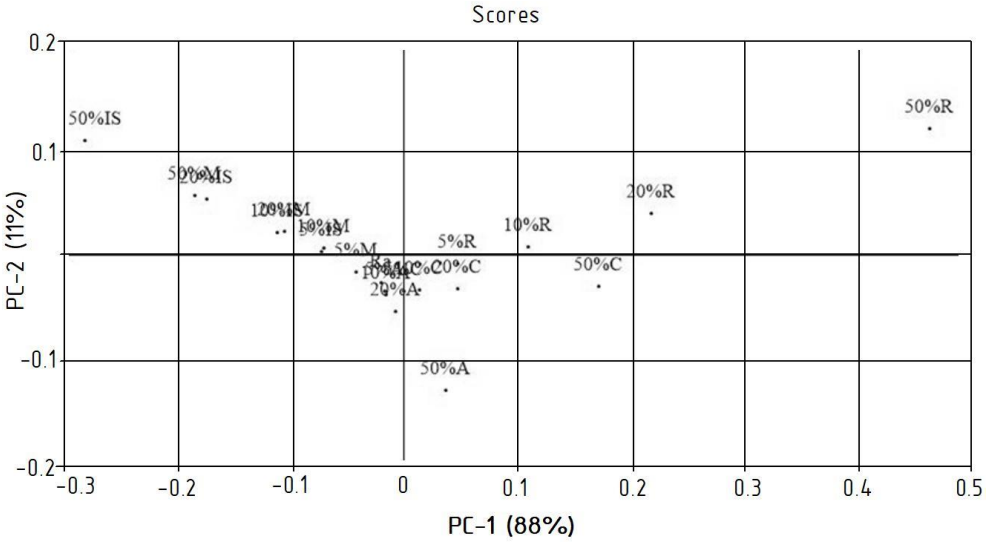

Figure 2. Principal component analysis – scores:
Ra – raspberry honey, A – agave syrup, C – corn syrup, IS – inverted sugar syrup, M – maple syrup, R – rice syrup, 5, 10, 20 and 50% – degree of adulteration

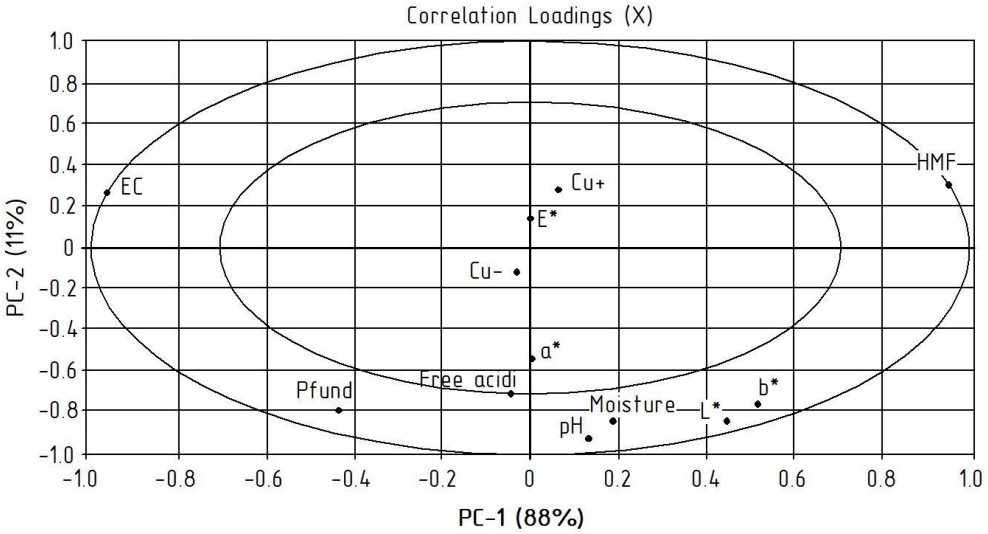

Figure 3. Principal component analysis – loadings

Other researchers have also successfully used voltammetric tongue made of various metallic electrodes both in authentication of geographical and botanical origins and in detection of adulterated honey with sugar syrups. Pauliuc et al. performed the authentication of raspberry honey with voltammetric tongue using five working electrodes (Pt, Au, Ag, TiO, ZnO and glass electrode). Using LDA, cross-validated grouped cases were in a percentage of 66.7% (Pauliuc et al., 2020). Electronic tongue system consisting of seven electrodes,

including Cu, was used to differentiate honey samples according to botanical and geographical origin but also to detect glucose and saccharose syrups in honey even in a percentage of 2%. SVMs (Support Vector Machines) and HCA (Hierarchical Cluster Analysis) showed a success rate in the recognition of adulterated honey of 100%, while PCA only 86.03% for adulterated honey with glucose syrup and 86.37% for adulterated honey with saccharose syrup (Bougrini et el., 2016). Another electrochemical system with the glassy carbon as working electrode was used to detect adulterated honey with rice syrup. The 100% recognition rate was achieved using PCA-LDA (Cai et al., 2013). Sobrino-Gregorio et al. used electronic tongue made of four electrodes (Au, Pt, Ir, Rh) to detect adulterated honey with barley, brown rice and corn syrups. The PCA analysis was able to differentiate the types of pure honey and syrups but also to discriminate the adulterated honey samples in different percentages and the PLS models were an essential tool in quantifying the level of adulteration (Sobrino-Gregorio et al., 2018). The voltammetric tongue consisting in gold and silver electrodes as working electrodes with physicochemical parameters and PCA were used in the detection of adulterated honeydew with inverted sugar syrup. The adulterated samples with 5% and 10% syrup were placed near the authentic samples, thus resulting that at lower percentages of adulteration the changes are less significant (Ropciuc et al., 2017). Likewise, the combination of the electronic tongue with physicochemical parameters led to a correct classification of 96.66% of the honey samples of different botanical origins (accacia, tillia, sunflower, honeydew, polyfloral) adulterated with glucose, fructose and invert sugar (Oroian et al., 2018).

Conclusion

1. Electronic tongue is a simple and fast method. The development of this method by testing different electrodes as well as other types of honey leads to an increase in effectiveness.
2. Physicochemical parameters (color, pH, free acidity, electrical conductivity, HMF content) can be used as a preliminary analysis, but by coupling the voltammetric tongue with it have proven their usefulness in detecting adulterated honey samples with different adulteration agents even at 5%.
3. Using Principal component analysis (PCA) in clustering honey samples it was observed that samples with 5%, 10% and 20% agave syrup, 5% and 10% corn syrup as well as 5% maple syrup are placed near to the authentic raspberry sample. Thus, we can conclude that these types of syrups added in small quantities present physicochemical and voltammetric tongue parameters closed to the authentic honey.

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Influence of spontaneous fermentation leavens from cereal flour on the indicators of the technological process of making wheat bread

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Abstract

Introduction. The aim of the research was to determine the carbohydrate-amylase complex of oatmeal and green buckwheat flour, the process of preparation of spontaneous fermentation leavens using of these types of flour, to establish the influence of spontaneous fermentation leavens on technological indicators and wheat bread quality.

Materials and methods. Oatmeal flour, green buckwheat flour, spontaneous fermentation leavens prepared using these types of flour, and wheat bread with the addition of these leavens were studied. Accelerated and express method of drying, iodometric, volumetric, autolytic test, titration and pH-metry methods, method of restoring the color of the indicator, method of displaced volume by the grain were used.

Results and discussion. Wheat flour of the first grade was the control sample in the research. The sugar-forming ability of oat and buckwheat flour was lower than in the control sample by 19.4% and 56.4%; autolytic activity by 36.9% and 43.1%; total gas formation by 31.1% and 38.6%, respectively. The decrease in enzymatic activity was due to differences in chemical composition and technology of preparation of cereals used to produce oatmeal and buckwheat flour.

In mixtures of wheat flour with oat or buckwheat flour, the sugar-forming ability was higher than in the control sample by 15.1% and 10.4%; gas-forming capacity by 12.7% and 7.3%, respectively. This was due to the effect of the active β-amylase of wheat flour on the smaller starch grains of cereal flour.

It was established that after the fifth renewal of leaven from oat and buckwheat flour it could be used for bread making, as the quality of buckwheat and oat leavens was stabilized due to physicochemical parameters (acidity – 16.0-18.0 degrees, lactic acid bacteria activity – 45-60 min).

The addition of the studied leavens in the amount up to 12% by weight of flour in the dough allowed obtaining products with organoleptic and physicochemical parameters close to the control sample.

Conclusions. Technological properties of oatmeal and green buckwheat flour allowed using them as a nutrient medium for spontaneous fermentation leavens in order to intensify technological processes and improve the nutritional value of wheat bread.

Introduction

It is necessary to expand the range and increase the nutritional value of wheat bread as it is unbalanced in terms of basic nutrients. The impetus for improving technology is also to increase public awareness of health and nutrition as a factor, which ensures the body's resistance to the negative effects of the environment and emotional stress (Ivanov et al., 2021; Kaprelyants et al., 2019; Sharma et al., 2020).

It is advisable to replace wheat flour with non-traditional types, in particular, cereal flour, which has higher nutritional value (Coelho and de Salas-Mellado, 2015; Drobot et al., 2014). Most types of cereal flour should be used in a mixture with wheat flour, because the underdeveloped protein-proteinase complex causes the inability to form gluten when kneading the dough. Other technological indicators of flour (in particular, carbohydrate-amylase complex), which directly affect the course of physicochemical, biochemical, microbiological processes in semi-finished products (leaven, dough) and the quality of finished products also requires studies (Flander et al., 2007; Hadnađev et al., 2011).

The purpose of research was the establishment of carbohydrate-amylase complex of oatmeal and green buckwheat flour, study of the process of preparation of spontaneous fermentation leaven based on these types of flour, study of the influence of spontaneous fermentation leaven on the technological process and quality indicators of wheat bread.

The main objectives of the research:

- Determination of the carbohydrate-amylase complex of cereal flour, namely oatmeal and green buckwheat flour, and mixtures of these types of flour with wheat flour;
- Development of schemes for dilution of spontaneous fermentation leavens based on green buckwheat flour and oatmeal;
- Carrying out of trial laboratory baking of wheat bread with the investigated leavens with the aim of proving efficiency of their use.

Literature analysis

Oatmeal and green buckwheat flour are promising products for processing in baking.

Oatmeal contains about 10% protein, up to 6.5% fat with a small amount of sugar (up to 1%) and starch (about 65%). The flour contains vitamins B1, B2, B6, B9, E, PP, trace elements (iron, chromium, and zinc), macroelements (potassium, magnesium, phosphorus, and sodium), and dietary fiber. Oatmeal protein is better balanced in amino acid composition than wheat protein (Berski et al., 2011; Flander, et al., 2007).

Features of its carbohydrate composition are the presence of soluble polysaccharides: pentosans (up to 14.0%), levulezan (up to 2.0%), as well as immunostimulants and prebiotics β-glucan (Keying et al., 2009; Rasane et al., 2015).

Absence of heat treatment operation in the production of green buckwheat flour allows to preserve the full range of vitamins, macro- and micronutrients, enzyme complex and powerful antioxidant properties (Dziadeka et al., 2016).

The protein contents in green buckwheat flour vary from 13 to 15%. This protein is well digested and is rich in valuable amino acids. Carbohydrate contents are 62-68% with a glycemic index of about 15, which gives the flour dietary properties. Fiber contents in buckwheat flour are about 6-12%. Fiber promotes the excretion of toxins from the body and improves the functioning of the gastrointestinal tract (Bonafaccia et al., 2003; Sakač et al., 2011).

Green buckwheat flour is rich in B vitamins (B1, B2, B6, B9), E, PP, macronutrients – potassium, magnesium, phosphorus; trace elements – iron, copper, zinc, chromium, molybdenum, manganese (Bondarenko et al., 2019).

The main functions of the use of leavens in wheat bread technology are antimicrobial, ie, ensuring microbiological purity in case of *Escherichia coli*, especially in summer, accelerating ripening and improving the rheological characteristics of the dough (Lebedenko et al., 2013).

Biological leavens of spontaneous fermentation have a number of advantages:

- Simplification of the leaven production process;
- Saving of yeast and pure cultures of lactic acid bacteria (LAB);
- Saving production space;
- Prompt response to market needs, increase or decrease in volume and range (Banu et al., 2010; Sylchuk et al., 2017; De Vuyst et al., 2005).

Information on the technologies of spontaneous fermentation leavens differs, in addition, there is a lack of research and clear schemes for the production of leavens in case of discrete production.

Most research concerns the technology of rye-wheat bread on rye leaven (Sylchuk et al., 2017), and cases when non-traditional types of flour such as buckwheat, oat and corn replace part of the rye flour (Pshenychnyuk et al., 2013; Chelyabiyeva and Sosedova, 2018). The influence of hop extract on microbiological stability of spontaneous wheat leavens and finished products with the addition of these leavens was investigated; the efficiency of using hop spontaneous leavens in wheat bread technology was proved (Yurchak et al., 2009).

However, there is no comparative characteristic of leavens using only cereal flour as a nutrient medium, without a mixture with traditional rye or wheat flour.

A large-scale study of the microflora of spontaneous fermentation leavenss from wheat and barley flour was carried out. Studies confirmed the need to control the temperature of the environment during the cultivation of leavens to ensure the stability of their microbiological state (De Vuyst, Neysens 2005; Harth et al., 2016).

Leavens from barley flour of spontaneous fermentation and with pure cultures of microorganisms were derived. It was established that at the end of fermentation the amount of soluble dietary fiber, namely β-glucan, in the leaven decreases, which proves its prebiotic properties (Andersson et al., 2004).

Insufficient attention is paid to the differences in the cycles of preparing leavens using wheat flour, both traditional and barley, as well as the influence of parameters (humidity, temperature) on the physicochemical quality of finished leavens.

Studies of the influence of spontaneous fermentation rice leaven on the rheological properties of the dough, indicators of the technological process of bread from rice flour showed that the processes of dough maturation accelerated and the intensity of acid accumulation increased (Demirkesen Mert et al., 2014; Coda et al., 2014).

Scientists have a leaven of spontaneous fermentation from corn flour was developed and the spontaneous microflora at different pH values was studied. The optimal pH value was 3.65-3.90 units of device at which strains of lactic acid bacteria and yeast of high activity and lifting power were detected (Zannini et al., 2009). Important indicators of quality for leavens are also the titrated acidity and humidity, which are insufficiently studied when using non-traditional nutrient media.

Leavens of spontaneous fermentation from buckwheat flour in different fermentation conditions were studied, species of lactic acid bacteria and yeast, more traditional for wheat and rye yeast were identified, and some species, including *Pediococcus pentosaceus, Leuconostoc holzapfelii, Lactobacalliscus Lactobacillus , Lactobacillus graminis and Weissella cibaria, Lactobacillus plantarum* were unconventional (Moroni et al., 2011; Moroni et al., 2012). The influence of leaven on the technological process and the quality of bread products remains unexplored.

The analysis of the above articles allowed concluding that the detailed study and description require features of the cultivation cycles and production cycle for each type of flour with specific parameters. It is also important to study the influence of the obtained leavens on the technological process and the quality of finished products.

Materials and methods

Materials

Preparation of cereal flour leavens

Leaven preparation consists of the breeding cycle and production cycle. To prepare the leaven, flour was mixed with water in the ratio specified in Table 1, 2.

Table 1

Sequence of derivation of spontaneous fermentation leavens from green buckwheat flour in the breeding cycle

Stage	Characteristics, biotechnological indicators
1. Mixing: Flour, kg – 0.05 Water, kg – 0.075	In 24 hours of fermentation on stage 1: Acidity, degrees – 3.2
2. In 24 hours after mixing (first renewal): Flour, kg – 0.05 Water, kg – 0.075 Pre-prepared leaven (PPL), kg – 0.125	In 24 hours of fermentation on stage 2: Acidity, degrees – 6.4 Volume increase coefficient – 1.1 Activity of LAB, min – low (115 min); Beginning of fermentation.
3. In 48 hours after mixing (second renewal): Flour, kg – 0.1 Water, kg – 0.15 PPL, kg – 0.25	In 24 hours of fermentation on stage 3: Acidity, degrees – 10.4 Volume increase coefficient – 1.21 Activity of LAB, min – low (92 min); Moderate fermentation.
4. In 72 hours after mixing (third renewal): Flour, kg – 0.2 Water, kg – 0.3 PPL, kg – 0.5	In 24 hours of fermentation on stage 4: Acidity, degrees – 14.6 Volume increase coefficient – 1.3 Activity of LAB, min – high (74 min); Intensive fermentation.
5. In 96 hours after mixing (fourth renewal): Flour, kg – 0.4 Water, kg – 0.6 PPL, kg – 1.0	In 24 hours of fermentation on stage 5: Acidity, degrees – 16.0 Volume increase coefficient – 1.35 Activity of LAB, min – high (70 min); Moderate fermentation.
6. In 120 hours after mixing (fifth renewal): Flour, kg – 0.8 Water, kg – 1.2 PPL, kg – 2.0.	In 24 hours of fermentation on stage 6: Acidity, degrees – 16.6 pH, units of device – 3.79 Humidity, % – 60.5 Volume increase coefficient – 1.3 Activity of LAB, min – high (45 min).

Table 2

Sequence of preparing of spontaneous fermentation leaven from oatmeal flour in the breeding cycle

Stage	Characteristics, biotechnological indicators
1. Mixing: Flour, kg – 0.05 Water, kg – 0.1	In 24 hours of fermentation on stage 1: Acidity, degrees – 3.8
2. In 24 hours after mixing (first renewal): Flour, kg – 0.05 Water, kg – 0.1 PPL, kg – 0.15	In 24 hours of fermentation on stage 2: Acidity, degrees – 7.8 Volume increase coefficient – 1.4 Activity of LAB, min – low (100 min); Beginning of fermentation.
3. In 48 hours after mixing (second renewal): Flour, kg – 0.1 Water, kg – 0.2 PPL, kg – 0.3	In 24 hours of fermentation on stage 3: Acidity, degrees – 11.0 Volume increase coefficient – 1.56 Activity of LAB, min – low (85 min); Intensive fermentation.
4. In 72 hours after mixing (third renewal): Flour, kg – 0.2; Water, kg – 0.4 PPL, kg – 0.6	In 24 hours of fermentation on stage 4: Acidity, degrees – 15.6 Volume increase coefficient – 1.63 Activity of LAB, min – high (68 min); Intensive fermentation.
5. In 96 hours after mixing (forth renewal): Flour, kg – 0.4 Water, kg – 0.8 PPL, kg – 1.2	In 24 hours of fermentation on stage 5: Acidity, degrees – 17.5 pH, units of device – 3.63 Масова частка вологи, % – 67.0 Volume increase coefficient – 1.68 Activity of LAB, min – high (48 min).

The dilution cycle lasted 120 hours at a temperature of 26-28 °C. In this cycle, every 24 hours a nutritious mixture of flour and water (temperature 28-30 ° C), in a ratio of 1:1.25 was added to the previous ripe leaven. Then the leaven can be used in the production cycle for making bread.

The production cycle involves the preparation of leaven with humidity (60 ± 5)%, in which the selection of leaven occurs every 10-12 hours. 70% of leaven is selected for the production, and a nutritious mixture of flour and water (ratio 1:1.25) is added to the remaining mass. The acidity of the leaven is 16.0-18.0 degrees, pH = 3.85-3.70 units of device, the activity of lactic acid bacteria – 55-65 min.

The dilution cycle lasted 96 hours at a temperature of 26-28 °C every 24 hours a nutritious mixture of flour and water (temperature 28-30 ° C), in a ratio of 1:1.2 was added to the previous ripe leaven. Then the leaven can be used in the production cycle for making bread.

The production cycle involves the preparation of leaven with humidity (65 ± 5)%, in which the selection of leaven occurs every 10-12 hours. 70% of leaven is selected for the production, and a nutritious mixture of flour and water (ratio 1: 2). The acidity of the leaven is 16.0-18.0 degrees, pH = 3.86-3.72 units of device, the activity of lactic acid bacteria – 45-60 minutes

Preparation of wheat bread using leavens

Laboratory baking of wheat bread with addition of 12% of leaven to the weight of flour was carried out. Sample with the addition of wheat leaven of spontaneous fermentation was a control sample. 6-8% of cereal flour is added with leaven, wheat flour was replaced with the appropriate amount of cereal flour. The dough was kneaded according to the recipe for 5-7 minutes using a kneading machine. Yeast suspension and saline solution were pre-prepared. The amount of water was calculated by subtracting water for kneading dough from the total amount. Water temperature was (37 ± 2) °C. After kneading, dough was left for fermentation for 90-100 min in proofer at a temperature of 30-32 °C and a relative humidity not less than 80%. After that, the dough was divided into dough pieces of 270-280 g, molded, put to forms and left in proofer for keeping at a temperature of 33-35 °C and a relative humidity not less than 80% for 30-35 minutes. The kept dough pieces were baked in a steam-humidified oven at a temperature of 180-190 °C for 25-30 minutes.

Methods

Mass fraction of sugars

The mass fraction of sugars was determined by the iodometric method with preliminary hydrolysis of the prepared aqueous extract (Manual of Methods of Analysis of Food, Beverages, Sugar and Confectionery Product, 2012).

Sugar-forming ability

The indicator of sugar-forming ability is the amount of mg of maltose formed in a water-flour suspension from 10 g of flour and 50 cm^3 of water in a volumetric flask per 100 cm^3 for 1 h of fermentation at a temperature of 27 °C (after pre-precipitation of proteins). In parallel, control with 50 cm^3 of distilled water is conducted.

The amount of maltose is determined in the transparent filtrate by iodometric method (Manual of Methods of Analysis of Food, Beverages, Sugar and Confectionery Product, 2012).

Autolytic activity

Autolytic activity is determined by the method of autolytic test by the accumulation of water-soluble substances in the water-flour suspension when heated in a boiling water bath.

A porcelain cup (weighing 30-40 g, 50 cm^3, 7 cm high) is weighed together with a glass rod, which remains in it until the end of the determination. A portion of flour 1 ± 0.05 g is weighed into a glass. 10 ± 0.02 cm^3 of distilled water is poured there and mixed.

Filled glasses are simultaneously immersed in a boiling water bath with uniform boiling of water in it. The bath should have the following parameters: capacity 1.5-1.8 dm^3, diameter – about 18 cm, height – 9-10 cm. The bath is covered with a lid with six holes according to the size of the glasses. All six glasses are immersed in the bath to provide the water level in it – 0.75-1.0 cm lower than the water level in the bath. The distance from the bottom of glasses to the bottom of the bath should be 2-3 cm.

If less than three determinations are performed at the same time, the bath should still be filled in such way to pour 10 cm^3 of distilled water in each of six glasses.

The heating of the samples is continued for 15 minutes, and during the first 2-3 minutes the contents of the beakers are stirred several times with a stick for uniform gelatinization. After its completion, each glass is covered with a funnel to prevent excessive evaporation. After 15 minutes of heating, the beakers are simultaneously removed and 20 ± 0.02 cm^3 of distilled water is poured into them, stirred vigorously and cooled to room temperature. The total weight of the autolysate is adjusted on the scales to 30 ± 0.05 g with an accuracy of 0.01 (adding 0.2-0.5 cm^3 of water). Then the mixture is stirred until foaming and filtered through a filter. It is advisable to leave the precipitate, which is poorly filtered, in a glass, and pour a layer of liquid on the filter.

During filtration, the first portions are discarded, and the following are applied to the prism of a precision refractometer. Using the table attached to the device, the content of dry matter (DM) in the filtrate is found and this value is multiplied by 30, taking into account the dilution.

The content of water-soluble substances in terms of DM of flour, is calculated by the formula:

$$x = \frac{a \times 100}{100 - W_f}$$

where W_f is humidity of flour, %; a is the content of water-soluble substances in flour, %. The discrepancy between two parallel definitions should not exceed 3%.

Gas-forming ability

The indicator of gas-forming ability is considered to be the amount of cm^3 of carbon dioxide (CO_2) released during 5 hours of fermentation of the dough from 100 g of flour, 60 ml of water and 10 g of yeast at a temperature of 30 °C.

This indicator was determined by volumetric method by CO_2 volume emitted under constant temperature and pressure (Munteanu et al., 2019; Verheyen et al., 2015).

Titrated and active (pH) acidity

Titrated acidity was determined in semi-finished products (leaven, dough) and finished products (Manual of Methods of Analysis of Food, Beverages, Sugar and Confectionery Product, 2012).

Measurement of the concentration of hydrogen ions (hydrogen index) in the leaven is measured by electrometric method using a laboratory pH meter. The pH value is determined by immersing the appropriate electrodes in a beaker with a prepared sample of leaven. Indicators (pH and temperature) are taken directly from the scale of the device.

Activity of lactic acid bacteria (LAB)

LAB activity is calculated by the intensity of recovery of the blue color of methylene blue. 20 g of leaven is mixed with 40 cm^3 of water heated to a temperature of 40 °C. 10 cm^3 is taken from the mixture in two tubes. 1 cm^3 of 0.05% aqueous solution of methylene blue is added to one tube. The second test tube is a control tube. The tubes are placed in a thermostat at a temperature of 40 °C. LAB activity is determined by the time required to decolorize the sample.

Humidity by the accelerated method

Determination of humidity was carried out in flour and finished products, drying is carried out by air-heat method, by dehydration of flour in an air-heat cabinet with fixed parameters of temperature and duration of drying (Manual of Methods of Analysis of Food, Beverages, Sugar and Confectionery Product, 2012).

Humidity by express method

The method is based on the drying of semi-finished products, flour on Chizhov device (Chizhov device modernized digital) according to the method (Manual of Methods of Analysis of Food, Beverages, Sugar and Confectionery Product, 2012).

Lifting force

The dough (portion is 20 g) is rolled into two balls, immersed in a glass (200-250 cm^3) with water at a temperature of (32 ± 2) °C. The glass is put to the thermostat. The time (min) from the moment of lowering the ball to its ascent characterizes the lifting force of the semi-finished product. Differences in two parallel definitions should not exceed 2 minutes.

Specific volume of bread

The volume of bread is determined using volumetric meters, which work on the principle of displacement of bread bulk filler (fine grain). The volume of the squeezed grain corresponds to the volume of the bread. Grain (sorghum, rapeseed, millet), which is sifted on metal sieves with a diameter of the upper sieve of 2.2 mm, the lower – 1.2 mm. The rest on the bottom sieve is used for work.

It is necessary to have two capacities, a ruler and two measuring cylinders (1000 cm^3). The grain is filled with the excess, which is raked with the edge of the ruler into the receiving container and removed through the hole. After that, the curtains of the main capacity with grain are opened manually and put through the hole into the bucket. This grain is used for determination. Method of determination: a small amount of grain is put into the main container, bread is put on it, carefully, without passing the grain, and the rest of grain is put in excess of the capacity. Grain is raked with the edge of the ruler and put into the receiving container, and then, after opening the latch – into the measuring cylinder. The volume of grain in a cylinder (cm^3) is equal to the volume of bread. Measurements are performed twice, deviations between parallel determinations should not exceed 5%.

The specific volume of bread is determined by dividing the volume of bread by its weight and expressed to the nearest 0.01 cm^3 / g.

Shape resistance (H/D)

Form stability is characterized by the ratio of the height of the hearth bread (H) to its diameter (D). H and D are determined using a special device or ruler with millimeter divisions. The greatest value of height of bread is taken. For diameter D, measure The largest and perpendicular diameters are measured and the average diameter is calculated. H / D is calculated to the third decimal place, the result is calculated to the second decimal place.

Porosity

The porosity of bread reflects the volume of the pores in a certain volume of the crumb, expressed as a percentage to the total volume. The porosity is determined using the Zhuravlev device according to the method (Verheyen et al., 2015).

Results and discussion

Determination of the indicators of carbohydrate-amylase complex of flour

The state of the carbohydrate-amylase complex of cereal flour was studied in comparison with varietal wheat flour, which, having its own characteristics, directly affects the course of technological processes, microbiota development and quality indicators of finished products (Drobot et al., 2014).

The results of the study of the carbohydrate-amylase complex of cereal flour compared to wheat flour are given in Table. 3.

Table 3

Indicators of carbohydrate-amylase complex of flour

Indicator	Flour		
	First grade wheat	Oatmeal	Green buckwheat
Sugar content, % on the DM of flour	0.9	0.7	1.4
Sugar-forming ability, mg of maltose per 10 g of flour	180.0	145	78.4
Autolytic activity, % on the DM of flour	26	16.4	14.8
Total gas formation, cm^3 / 100 g of flour	1401	965	860

Buckwheat flour has more own sugars than wheat flour by 35.7%, which will have a positive effect on gas formation in the dough at the beginning of fermentation. It is known that the flour's own sugars ensure the maturation of the dough only for 30-60 minutes. Further fermentation in the dough, its intensity depends on the activity of the enzymatic complex of flour and the susceptibility of starch to the action of enzymes involved in the formation of simple sugars for the fermentation process (Yurchak et al., 2009).

The activity of the enzymatic complex was determined by sugar-forming ability and autolytic activity. Thus, the sugar-forming ability of oatmeal and buckwheat flour (Table 3) is lower by 19.4 and 56.4%, respectively, than in the control sample, ie, the amount and activity of enzymes is insufficient. The study of the indicator of autolytic activity (Table 3) showed that the activity of α-amylase of oat and buckwheat flour is lower by 36.9 and 43.1%, compared to wheat flour, which correlates with the sugar-forming ability. These results are explained by the grinding technology and the process of hydrothermal treatment of cereals before grinding, as a result of which some enzymes are inactivated and their activity is reduced.

It can be predicted that in the case of making products based on yeast dough only from cereal flour without the addition of wheat flour, the amount of yeast will be insufficient for further fermentation and melanoidin formation after fermentation flour own sugars, so the recipe of such products must include sugar or molasses (Lebedenko et al., 2013).

The course of gas formation was investigated in dough samples from first grade wheat flour (control sample) and from cereal flour during 5 hours of fermentation. The data of total gas formation showed (Figure 1) that the dough from oat and buckwheat flour, compared to

wheat flour, is able to emit less carbon dioxide by 31.1% and 38.6%, respectively. This indicates a lower activity of amylolytic enzymes of these types of flour, in particular β-amylase and is confirmed by low sugar-forming ability (Table 3). In addition, this is due to the technology of grinding cereals, which produces flour with low dispersion (ie, high size), which requires further research. It can be assumed that the enzymatic activity also decreases due to the technological processes of preparation of cereals for grinding (Bondarenko et al., 2019).

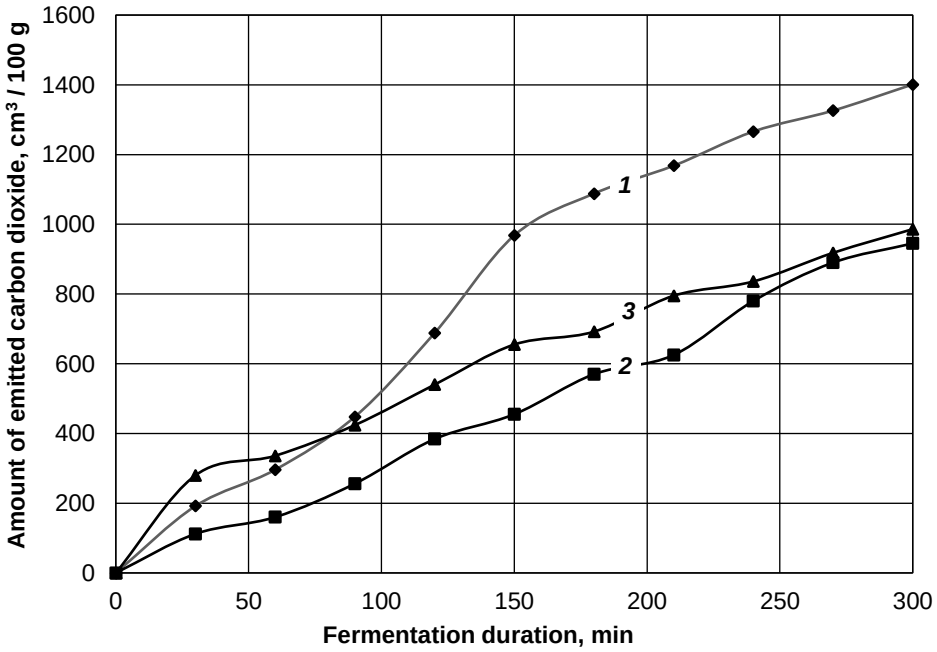

Figure 1. Total gas formation of the studied types of flour:
1 – Control sample;
2 – Green buckwheat flour;
3 – Oatmeal flour.

Determination of technological parameters of wheat flour mixed with cereal flour.

Model experiments were performed for which flour mixtures with a ratio of wheat and cereal flour of 80:20 were prepared. This ratio of flour in mixtures is due to previous studies, which found that the maximum replacement of wheat flour with cereal flour, which does not lead to a significant deterioration of the structural and mechanical properties of the dough and product quality, was 20% (Drobot et al., 2014).

In flour mixtures, as in previous samples, technological parameters were determined. The obtained results are given in Table 4.

Table 4

Technological indicators of mixtures of wheat flour with cereal flour

Indicator	Control sample (wheat flour of the first grade)	20% of cereal flour was added instead mass of wheat	
		Green buckwheat	Oatmeal
Sugar-forming ability, mg of maltose per 10 g of flour	180	201	212
Total gas formation, cm^3 / 100 g of flour	1401	1512	1604

It was found that mixtures of wheat flour with buckwheat and oat flour have a higher sugar-forming ability by 10.4 and 15.1% (table 4) and the total gas formation (Fig. 2) by 7.3 and 12.7%, respectively, than wheat flour. This is due to the action of β-amylase, which is contained in sufficient quantities in wheat flour, and, accordingly, in mixtures, as well as the size of starch grains and its high susceptibility to enzymes. Thus, the average values of the size of starch grains of wheat flour – (12.4 ± 1.9) μm, in oatmeal – (7.4 ± 0.87) microns, buckwheat – (6.6 ± 0.52) microns (Bonafaccia et al., 2003; Berski et al., 2011; Sakač et al., 2011). This correlates with the results of determining the technological parameters of flour, oatmeal had a higher sugar-forming ability and autolytic activity than buckwheat.

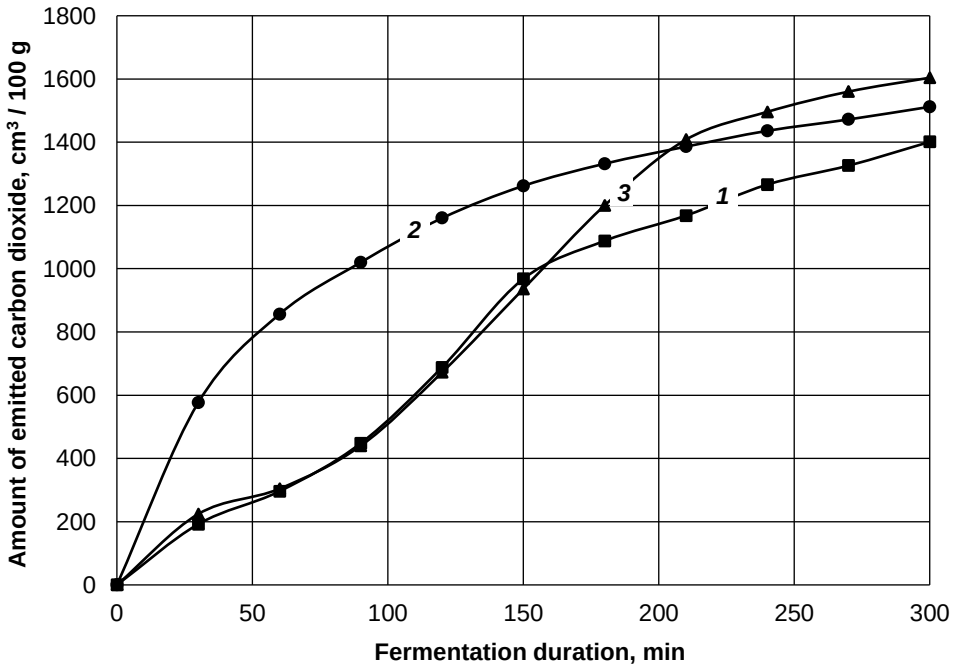

Fig. 2. Total gas formation of mixtures of wheat flour with cereal flour
1 – Control sample;
2 – Green buckwheat flour;
3 – Oatmeal flour.

Use of spontaneous fermentation leaven in the technology of wheat bread

To determine the influence of leavens on the technological process, the dough was prepared from wheat flour of the first grade with the addition of 12% of leaven to the weight of flour (6-8% of cereal flour was added with leaven, which replaced wheat flour). Dough with wheat leaven of spontaneous fermentation was a control sample. The results are shown in Table 5.

Table 5

Parameters of technological process and indicators of quality of wheat bread with addition of leaven of spontaneous fermentation

Indicator		Sample with the addition of leaven		
		wheat (control sample)	buckwheat	oatmeal
Dough				
Humidity of dough, %		43.5	44.2	44.5
Acidity of the dough, degrees	initial	2.8	3.0	2,8
	final	4.0	4.2	4,4
Lifting force, min		00:50	00:46	01:01
Duration of aging, min		40	36	35
Bread				
Specific volume, cm^3 / 100 g		240	225	220
H/D of hearth bread		0.48	0.44	0.46
Porosity, %		82.0	80.0	79.0
Humidity, %		42.8	43.5	44.0
Acidity, degrees		3.5	3.8	3.8

It was found that during the fermentation period the titrated acidity increased by 1.2–1.4 degrees in all samples, which is explained by the acidity of the introduced leavens. The addition of leaven had a positive effect on the lifting force. The value of the lifting force increases, approaching the control sample. This is due to the high fermentation activity of leavens (Pshenychnyuk et al.,2013; Mikhonik et al.,2018). A positive effect is observed due to the aging of the samples, as samples with the addition of buckwheat and oat leaven ripened slightly faster. This is due to the acidic medium in which the dough quickly acquires the desired rheological characteristics.

According to organoleptic parameters, the products differed slightly from each other. The crust of the bread had uniform color, from light yellow to light brown, bread with buckwheat leaven had gray tinge. The products had developed, uniform, fine and thin-walled porosity, baked and elastic crumb. The aroma and taste of the control sample is characteristic of wheat bread. The sample with the addition of buckwheat leaven had a faint aroma and taste characteristic of buckwheat flour.

The results of determining the physicochemical parameters indicated that a significant effect on the mass fraction of moisture was not observed. The higher acidity of the leaven caused an increase in the acidity of the finished products, but the values did not exceed the permissible limits. Replacing part of wheat flour with part of cereal flour in the composition of the leaven causes a deterioration in volume, shape stability and porosity, as these types of flour do not have a developed gluten skeleton (Pshenychnyuk et al., 2013; Oliinyk et al.,2017), but at the recommended dosage (Hetman et al., 2020) decreases slightly.

Conclusions

1. Features of the chemical composition of buckwheat and oatmeal flour allow to use for the nutrient medium of leavens in order to intensify technological processes, improve the nutritional value and expand the range of bread products.
2. Studies of carbohydrate-amylase complex showed that buckwheat flour, compared to wheat flour, has more sugars, which has a positive effect on gas formation in the dough at the beginning of fermentation, but the activity of amylolytic enzymes in oatmeal and buckwheat flour is low. Thus, the sugar-forming ability of oat and buckwheat flour was lower, compared to wheat flour, by 19.4 and 56.4%; autolytic activity – by 36.9 and 43.1%; total gas formation – by 31.1 and 38.6%, respectively. The decrease in enzymatic activity is due to differences in chemical composition and technology of preparation of cereals used to produce oatmeal and buckwheat flour.
3. Compared to the control sample, in mixtures of wheat flour with oat or buckwheat flour, the sugar-forming ability was higher by 15.1 and 10.4%; gas-forming capacity – by 12.7 and 7.3%, respectively. This is due to the effect of the active β-amylase of wheat flour on the smaller starch grains of cereal flour.
4. During the cultivation of spontaneous fermentation leavens from buckwheat and oatmeal flour, it was found that after the fifth renewal the quality of leavens in terms of organoleptic and physicochemical parameters is stabilized, with an acidity in the range of 16.0-18.0 degrees and LAB activity 45-60 min, and can be used in the production cycle for making bread. Provided that the necessary parameters and breeding scheme are observed, it is possible to obtain leavens with indicators which are able to ensure the course of the technological process and obtain high quality products.
5. As a result of trial laboratory baking it was found that the preparation of wheat bread using spontaneous fermentation leavens from oatmeal and green buckwheat flour in the amount of up to 12% allows to obtain products which are close to the control sample by organoleptic and physicochemical parameters.

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Phytic acid content and in-vitro digestibility of several cereal and legume types treated with different processes

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Abstract

Introduction. The present study aims to determine the relationship between phytic acid (PA) content/digestibility rate of the grains, which are widely consumed in daily diet, and the methods often used in preparing food for consumption.

Materials and methods. In this study, the dephytinization effect of soaking (for 12 h), boiling (at 100 °C for 1 h), autoclaving (at 121°C for 15 min), fermentation (at 25-27 °C), and germination processes on several grains were studied. PA contents, Mineral Digestibility (MD, %), and Protein Digestibility (PD, %) were determined, and their effects comparatively evaluated before and after processes.

Results and discussion. There were decreases in PA contents in legumes and cereals for all the methods applied. Fermentation (<800 mg/100 g) of the flour and germination for the other samples (<700 mg/100 g) were found to yield more effective results to PA degradation. Between 20-70% reduction by fermentation and 50-80% reduction by germination of PA contents in the samples were found. Moreover, MD level in cereals, which was especially low before germination, increased up to 2 times after the germination. PD, %, was found to increase with the effect of the processes, except for boiling and soaking. Especially germination process was more effective in tems of PD. Increasing rate of PD for ten bean samples was up to 200%. Wheras the PA contents in the cereal flour samples after their spontaneous fermentation were decreased, their digestibility values were increased statistically ($p<0.05$).

Conclusions. All processes applied such as soaking, fermentation, or heat treatments, which are widely used to preparation of the grains, provide an advantage in dephytinization at different rate. Combined use of techniques such as soaking, and boiling which are used in the preparation of various cereal and legume products for consumption, is also be effective in terms of nutritional quality and their combinations were proposed.

Introduction

The cereals and legumes have a poor nutritional value in comparison with the animal source foods, due to their amino acid compositions and lack of some essential micronutrients. Additionally, most of the cereals and legumes contain high amount of phytic acid (PA), which is considered as an antinutrient compound because of its ability to form strong chelates with metal cations, especially with Ca^{+2}, Zn^{+2}, and Fe^{+2}, to form phylate and decrease bioavailability of these important microelements in the human intenstine (Gibson et al., 2010; Özkaya et al., 2018; Perera et al., 2018). Therefore, diets that are rich in cereals and legumes having high phytate content may lead to micronutrients deficiency (Ma et al., 2013). In cereal grains such as wheat and rice, PA ($C_6H_{18}O_{24}P_6$, inositol hexaphosphates-IP6) is found in bran fraction such as aleurone layer and pericarp, and in corn it is found in endosperm (Gupta et al., 2015). PA is the primary storage form of phosphorus, comprising 1–5% by weight in cereals, nuts and legumes (Vats and Banerjee, 2004). PA exhibits high binding capacity with positively charged proteins via electrostatic interactions and affects their activity (Pakfetrat et al., 2018; Wang and Guo, 2021).

Increasing micronutrient intake and bioavailability in food through food processing is a sustainable method to prevent micronutrient malnutrition (Ertop and Bektaş, 2018). The pretreatment and processing techniques widely used in preparing the foods might yield a reduction in antinutrient compounds. Soaking, dehulling, cooking and fermentation are important traditional methods used to reduce antinutrients. Furthermore, germination and fermentation enhance the nutritional value of cereals and legumes due to significant changes in chemical composition and elimination of antinutritional factors (Abdelrahaman et al., 2005). Several studies were conducted on certain grains and seeds which examined their anti-nutrients and nutritional properties under different processes and conditions (Özkaya et al., 2017a; Özkaya et al., 2017b; Deng et al., 2015; Yagoubet al., 2008; Rehman and Salariya, 2005). However, no study comparatively analyzing industrial/domestic common methods for treatment of grains having high consumption rates was found. The present study aims to fill this gap about the relationship between phytic acid content/digestibility rate of grains, which are widely consumed, and the methods often used in preparation of food for consumption.

Materials and methods

Materials

The wheat sample named as "Ekiz wheat" (*Triticum aestivum*) was supplied from the crops harvested in July 2017 at Devrekani, Kastamonu, Turkey. The flour was milled (milling yield 70%) in a milling factory (Üçbaşak Milling, Devrekani, Kastamonu). The hulled rice (paddy) and rice sample (milling yield 68%) were supplied from a local rice producer. For preparing the rice flour, the dried rice grains were milled using a laboratory mill (EQM-402 Mixer Mill, Spain). The barley sample named "Aydan Hanım", local oat sample, and rye sample named "Black rye" were supplied from the crops harvested in 2017 from Gövdecili village of Yozgat, Turkey, and the samples were milled in traditional stone mills of a local company. Before the milling process (stone mill), the grains (barley, oat and rye) were hydrated by adding 200 g water per 10 kg of the kernel to make the seed coats less brittle and to prevent kernel breakage (Bottega et al., 2009). Dry bean, chickpea, and green lentil samples were obtained from a commercial firm in Kastamonu, Turkey.

Proximate analyses

Ash, protein, and moisture contents of the samples were determined using AACC Approved Methods (AACC, 2000) and numbered as 08-01.01, 46-12.01, and 44-15.02, respectively. The nutrient or mineral contents were expressed on a dry matter basis. pH values of the fermented samples were measured using a digital pH meter (Ohaus ST3100). Phytic acid content was determined by using the colorimetric procedure of Haug and Lantzsch (1983) and content of phytic acid was calculated accordingly.

Sample preparation

Soaking. Ten grams of cereal and legume grains were weighed and soaked with 10 mL water and kept under the room temperature for 12 h.

Germination. Fifty grams of cereal and legume grains were washed and then immersed in 100 mL of water at 20-24 ℃ for 8 h. Grain samples were drained and kept for 8 h without water to attain a homogenous moisture distribution from crumb to crust. Then, 2 more times these processes were re-performed. The grains were placed in the dishes having 40–50 mm diameter, their bottom and top surface covered with cotton paper, and they were kept at 25 ± 2 °C (Pakfetrat et al., 2018). To supply the germination moisture, a certain amount of water was sprayed onto the grains every day for a total of 4-5 days of germination. The germination process was terminated when the germinated part reached the original size of the grains.

Hydrothermal processes.

Autoclaving. Ten grams of cereal and legume grains and 10 mL water were placed into borosilicate glasses, and the heat treatment was performed at 121 ℃ for 15 min in the autoclave.

Boiling. Ten grams of cereal and legume grains were weighed and boiled in 10 mL water at 100 °C for 1 h in a water bath.

Fermentation. The spontaneous fermentation technique (back-slopping method) was used for fermentation of wheat, oats, barley, rye, and rice flour. Ten grams of wheat flour and 10 g water (dough yield: 200) (Hayta and Ertop, 2018) were mixed and fermented spontaneously in 3 stages until the dough reached pH levels lower than 4.5 at 25-27°C.

In-vitro mineral (MD) and protein digestibility (PD) determination

The sample (1 g) was incubated with 25 mL of pepsin solution (0.03 N 1 L HCl + 2 g of pepsin) at 37 °C for 3 h. Each sample was filtered using ashless filter paper. The pellet and filter paper were burned together in the furnace at 900 °C, and the ash content was calculated. The digestible mineral content was obtained together with their differences. The MD, %, values were obtained (Hayta and Ertop, 2018) by using the following equation:

$$MD,\ \% = \frac{Digestible\ Mineral\ Content}{Total\ Mineral\ Content} x100$$

The in-vitro PD values determined by the method of Rizzello et al. (2014) PD, %, values were calculated using the following equation:

$$PD,\ \% = \frac{N\ in\ supernatant - N\ in\ pepsin\ enzyme}{N\ in\ sample} x100,$$

where N is content of nitrogen.

Statistical analysis

The data were shown as the mean ± standard deviation. Variance analysis (ANOVA) (IBM-SPSS 1.0.0.781) by Tukey test ($p<0.05$) was used for comparing the results between all samples. t-test was carried out before and after the procedure in order to determine the effects of application ($p<0.05$).

Results and discussion

Effects of the soaking process

There were statistically significant differences ($p<0.05$) between the phytic acid contents of raw grains (Table 1). The highest PA content was found in wheat grain, while the lowest content was found in paddy. The contentss found in legumes (bean, chickpea, and green lentil) were similar to each other. The PA content in the grains was significantly ($p<0.05$) decreased by the soaking process< and their PA contents were very close to each other.

Phytates are concentrated in endosperm in legume seeds (Lestienne et al., 2005). The bran and husk fractions in cereals contain higher amount of PA. Therefore, during the soaking process, the water can reach phytase in the bran and husk layer of the grains more easily compared to the central endosperm layer of legume grains. The soaking process increased the MD, %, of the cereal and legume grains and the difference was found to be statistically significant in all the samples ($p<0.05$). Moreover, it was also found that the MD, %, of the legumes before and after the soaking process was higher than that of cereals. It has been shownin several studies that soaking of the cereals and beans reduced the PA content and consequently increases the MD (Perlas and Gibson, 2002; Coulibaly et al., 2011).

The soaking process decreased the protein digestibility rate of all the cereal and legume grains, and the change was found to be statistically significant, especially for rye, paddy, chickpea, and green lentil. This might be due the protease inhibition (trypsin inhibitor), which is an antinutrient compound found in raw legumes and certain cereals. It was demonstrated that protease inhibitors reduce the proteolysis, amino acid absorption, and protein bioavailability of diet proteins by suppressing the activities of trypsin, chymotrypsin, and amylase enzymes in the small intestine (Ergün et al., 2002). Oomah et al. (2011) found that trypsin inhibitors have capability to inactivate trypsin and digestive enzymes. Accordingly, despite the hydrolysis of PA in the soaking process, it can be stated that the protease inhibitor reduces the digestion of proteins in the intestinal pH in-vitro medium and thus reduces the protein digestibility rate of cereals and legumes. It was found that the heating increased protein digestibility in legumes due to the inactivation of protease inhibitors and the denaturation of the proteins (Eltayeb et al., 2007).Therefore, the use of the soaking process altogether with other treatments such as germination, heating, and fermentation colud be very useful. Phytic acid contents and digestibility rates of the samples before and after soaking process are shown in Table 1.

Table 1

Phytic acid contents (a) and digestibility rates (b and c) of the samples before and after soaking process

(a)

Sample	Content of phytic acid, mg/100 g		
	Before	**After**	***p* value***
Wheat	2471.88±0.31[a]	1487.50±2.56[a]	0.002
Barley	2328.13±2.08[b]	1356.25±0.01[g]	0.000
Rye	1715.63±1.10[g]	1390.63±0.31[e]	0.001
Oat	2050.00±2.74[c]	1312.50±0.62 [h]	0.001
Paddy	1559.38±0.22[h]	1428.13±0.13[d]	0.001
Bean	1831.25±1.59[d]	1450.00±0.09 [c]	0.000
Chickpea	1806.25±0.88[e]	1478.13±0.49 [b]	0.001
Green lentil	1790.63±0.49[f]	1368.75±0.80[f]	0.001

(b)

Sample	Mineral digestibility, %		
	Before	**After**	***p* value***
Wheat	45.38±4.60[abc]	71.91±1.75[bcd]	0.042
Barley	40.72±0.00[bcd]	65.72±1.67[de]	0.042
Rye	45.70±0.42[abc]	70.10±1.91[cd]	0.050
Oat	36.24±2.01[cd]	62.49±1.78[e]	0.043
Paddy	30.16±1.01[d]	60.61±0.45[e]	0.009
Bean	53. 83±1.89[a]	79.21±0.65[ab]	0.016
Chickpea	52.16±2.74[ab]	84.11±0.62[a]	0.012
Green lentil	52.47±0.14[ab]	77.32±1.33[abc]	0.034

(c)

Sample	Protein digestibility, %		
	Before	**After**	***p* value***
Wheat	50.66±3.30[bc]	47.07±1.97[b]	0.320
Barley	36.49±0.97[de]	33.38±1.57[c]	0.298
Rye	53.81±3.19[b]	35.33±1.16[c]	0.040
Oat	40.54±0.10[cd]	43.54±0.92[b]	0.189
Paddy	48.69±1.24[bc]	13.93±1.40[e]	0.022
Bean	25.98±0.08[e]	25.07±1.19[d]	0.584
Chickpea	77.29±2.24[a]	61.00±1.10[a]	0.043
Green lentil	71.60±2.65[a]	20.95±0.52[de]	0.007

Table 2

Phytic acid contents (a) and digestibility rates (b and c) of the samples before and after germination process

(a)

Sample	Content of phytic acid, mg/100 g		
	Before	**After**	***p* value***
Wheat	2471.87±0.31^{a}	621.68±0.67^{b}	0.000
Barley	2328.12±2.08^{b}	548.79±0.79^{e}	0.000
Rye	1715.62±1.10^{g}	488.48±0.07^{f}	0.000
Oat	2050.00±2.74^{c}	576.14±0.44^{d}	0.000
Paddy	1559.37±0.22^{h}	698.19±0.54^{a}	0.000
Bean	1831.25±1.59^{d}	463.43±0.17^{g}	0.000
Chickpea	1806.25±0.88^{e}	616.56±0.65^{c}	0.000
Green lentil	1790.63±0.49^{f}	441.36±0.80^{h}	0.000

(b)

Sample	Mineral digestibility, %		
	Before	**After**	***p* value***
Wheat	45.38± 4.60^{abc}	79.97±1.39^{b}	0.026
Barley	40.72±0.00^{bcd}	76.76±1.06^{bc}	0.019
Rye	45.70±0.42^{abc}	83.67±0.77^{ab}	0.013
Oat	36.24±2.01^{de}	79.83±1.33^{b}	0.019
Paddy	30.16±1.01^{e}	61.29±1.58^{d}	0.032
Bean	53. 83±1.89^{a}	86.03±1.06^{a}	0.021
Chickpea	52.16±2.74^{ab}	83.18±0.98^{ab}	0.020
Green lentil	52.47±0.14^{ab}	73.60±0.90^{c}	0.027

(c)

Sample	Protein digestibility, %		
	Before	**After**	***p* value***
Wheat	50.66±3.30^{bc}	68.54±1.00^{e}	0.036
Barley	36.49±0.97^{de}	74.08±1.17^{d}	0.020
Rye	53.81±3.19^{b}	85.82±0.88^{a}	0.017
Oat	40.54±0.10^{cd}	79.42±1.00^{bc}	0.016
Paddy	48.69±1.24^{bc}	64.16±1.01^{e}	0.042
Bean	25.98±0.08^{e}	79.97±1.21^{b}	0.014
Chickpea	77.29±2.24^{a}	78.07±0.01^{bcd}	0.008
Green lentil	71.60±2.65^{a}	74.16±0.77^{cd}	0.186

* (P<0.05) means that the values statistically different in the same line

**Different letters indicate significant differences (P<0.05) in the same column

Effects of germination process

A significant decrease ($p<0.05$) was obtained in the PA contents in all grains and legumes after the germination process (Table 2). In several studies (Eltayeb et al., 2007; Greiner and Konietzny, 2006; Marshall et al., 2011) it was also reported that germination is a very effective method for reducing contents of phytate acid and other antinutrient factors in grains. During the germination process, activation of the phytase enzyme accelerates, so its ability to break phytates down increases (Fayyaz et al., 2018). Moreover, it was reported that the pre-soaking process before germination caused a significant reduction from 55% to 76% of PA content in the grains (Masud et al., 2007). Therefore, germination is the most effective method to decrease the content of PA (Figure1).

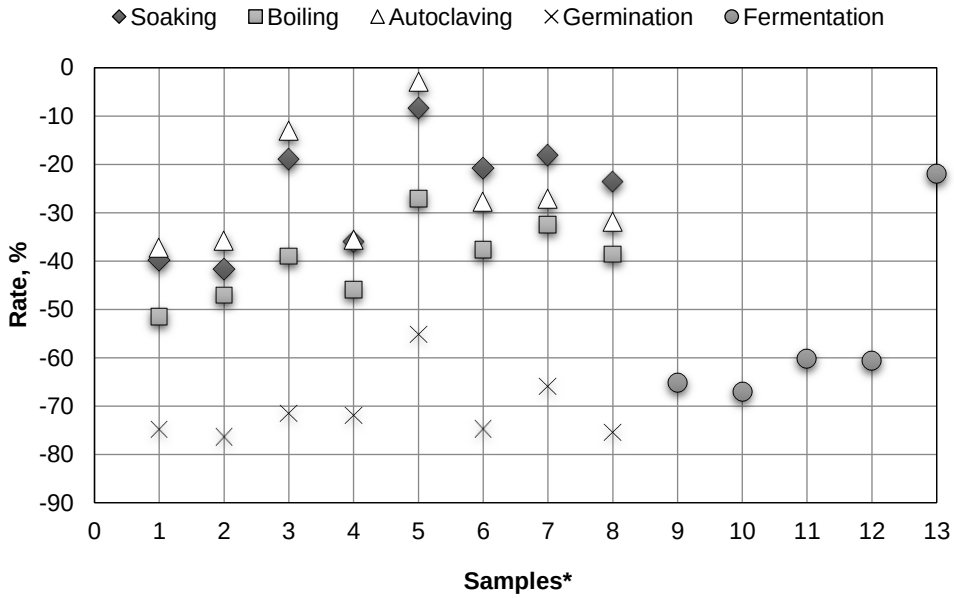

Figure 1. **Effect of the processes on the rate, %, of change of the phytic acid content**
* Number of the samples;
1 – Wheat, 2 – Barley, 3 – Rye, 4 – Oat, 5 – Paddy, 6 – Bean,
7 – Chickpea; 8 – Green lentil, 9 – Wheat flour, 10 – Barley flour,
11 – Rye flour, 12 – Oat flour, 13 – Rice flour

Application of all processing methods increased the MD level in all samples. Germination was the most effective method among them (Figure 2). The germination process significantly increased the MD rates for both grain and legume samples ($p<0.05$) (Table 2). The MD level in cereals, which was especially low before germination, increased up to 2 times after the germination. The most affected sample was the paddy. The highest increase among the legumes was found for chickpeas. Masud et al. (2007) showed that germination is a highly effective method thanks to the reduction of PA content by up to 40%. Although the non-germinated grains have a low endogenous enzyme activity (Greiner and Konietzny, 2006), the endogenous

phytase activity degrading the phytate increased during the germination of grains. Thus, PA affecting the bioavailability of major minerals such as Ca and trace ones such as Fe, Cu, Zn and Mn and binding them was degraded (Ertop and Bektaş, 2018). It was found that the total ash content in such legumes as mug beans, pea, and lentil increased after the germination process (Fayyaz et al., 2018; El-Adawy et al., 2003). Therefore, the increase of MD might be due to the increased ash (total mineral) content of the sprouted grain.

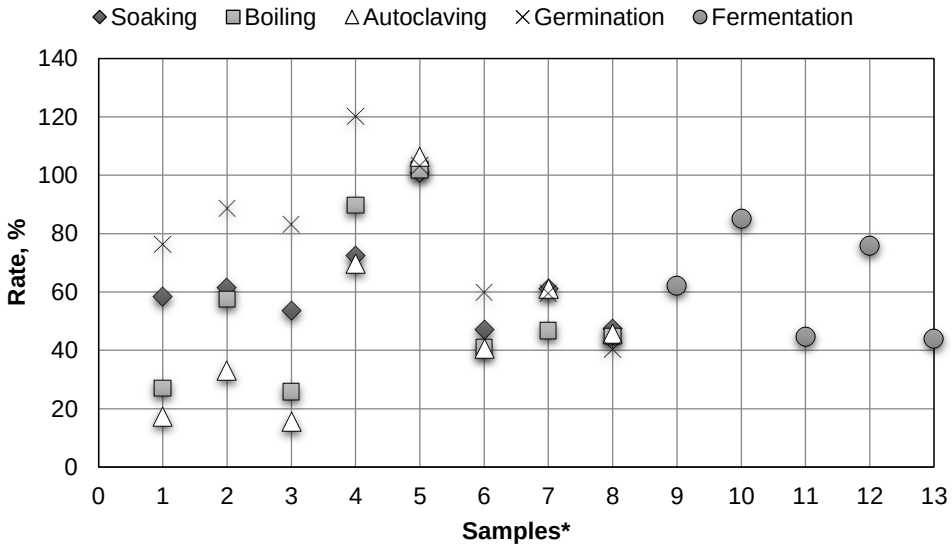

Figure 2. Effect of the processes on the rate, %, of change in the MD:
* Number of the samples;
1 – Wheat,2 – Barley, 3 – Rye, 4 – Oat, 5 – Paddy, 6 – Bean,
7 – Chickpea; 8 – Green lentil, 9 – Wheat flour, 10 – Barley flour,
11 – Rye flour, 12 – Oat flour, 13 – Rice flour

The germination method was the most effective method in increasing the PD value of cereals, especially the barley, rye, and oats (Figure 3). The germination process increased ($p<0.05$) the PD, %, for all grain and legume samples except for green lentils (Table 2). Legumes are valuable plant-based protein sources in the daily diet. However, besides the level of proteins, their digestibility rate is also important. It was determined that the PD, %, of the bean, which was rather low, 25.98%, initially, increased significantly to 79.97% after the germination (Table 2). An approximately 207% increase was served for the bean (Figure 3). The barley exhibited a result similar to the bean. The PA in grains is in the form of complexes with proteins and several metal cations. The inhibition of PA, which depends on the phytase activity during the germination, resulted in an increase in the PD. In a study carried out by Ghavidel and Prakash (2007), germination significantly improved the in-vitro bioavailability of Fe and Ca minerals, protein, and thiamine, as well as in-vitro digestibility of starch and proteins in several legumes. The germination process increased the amount of protein and particularly the amount of amino acids such as lysine and tryptophan in the Mung bean (Adil Shah et al., 2011) and oats (Peterson, 1998; Skoglund et al., 2008). Thus, the nutritional value was increased. Sharif et al. (2013) reported that, together with the increase of the protease

enzyme activity because of the germination process, the protein quality of the grain increased, lysine content and protein bioavailability enhanced, and the minerals became more useful by chelating with proteins. Increase of PD in germinated grains was due to the removal of certain antinutrients such as protease inhibitor (trypsin inhibitor) and PA (Khalil and Mansour, 1995).

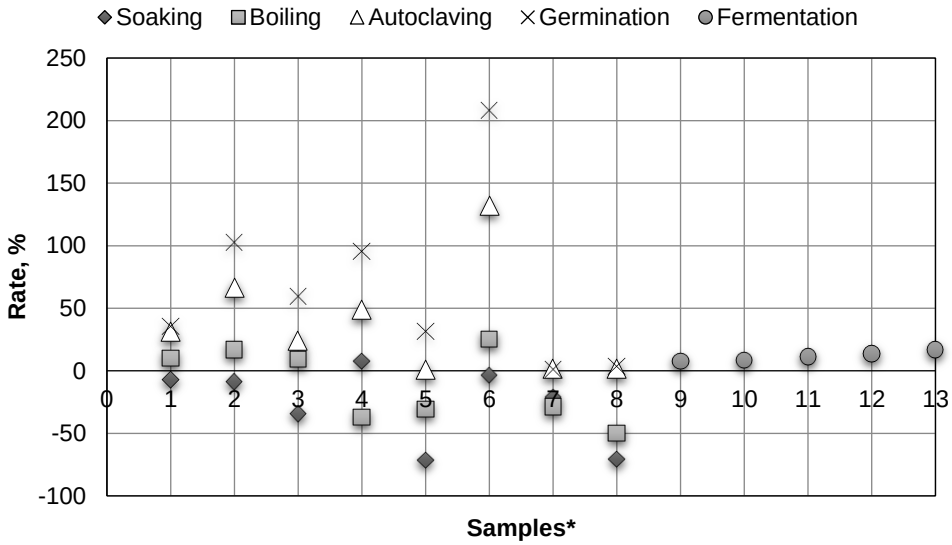

Figure 3. Effect of the processes on the rate, %, of change in the PD
* Number of the samples;
1 – Wheat, 2 – Barley, 3 – Rye, 4 – Oat, 5 – Paddy, 6 – Bean,
7 – Chickpea; 8 – Green lentil, 9 – Wheat flour, 10 – Barley flour,
11 – Rye flour, 12 – Oat flour, 13 – Rice flour

Effects of hydrothermal processes

Autoclaving

The autoclaving process yielded a significant ($p<0.05$) decrease in the PA content in all grains, as seen in Table 3. The wheat sample had the highest phytic acid content before and after the treatment. After autoclaving, the highest PA content among the grains was in wheat, whereas the lowest level was in the rye sample. Among the legume samples, PA was at the same level in beans and chickpeas and higher than in the green lentil ($p<0.05$).

An increase in MD, %, in both legumes and grains was achieved by autoclaving and the change was statistically significant ($p<0.05$) in all samples, except for rye. However, the digestibility of legumes was higher than cereals before autoclaving and this did not change after the treatment. Post-treatment digestibility rates of cereals were determined to be in the range from 52.23 to 62.19% and those of legumes was in the range from 75.50 to 83.95%.

Table 3

Phytic acid contents (a) and digestibility rates (b and c) of the samples before and after autoclaving process

(a)

Sample	Content fo phytic acid, mg/100 g		
	Before	**After**	***p* value***
Wheat	2471.87±0.31^{a}	1553.13±2.43^{a}	0.002
Barley	2328.12±2.08^{b}	1493.75±0.62^{c}	0.000
Rye	1715.62±1.10^{g}	1490.63±1.81^{c}	0.005
Oat	2050.00±2.74^{c}	1321.88±0.40^{d}	0.000
Paddy	1559.37±0.22^{h}	1512.5±0.02 ^{b}	0.000
Bean	1831.25±1.59^{d}	1321.88±0.66^{d}	0.001
Chickpea	1806.25±0.88^{e}	1315.63±0.13^{d}	0.000
Green lentil	1790.63±0.49^{f}	1221.88±1.19^{e}	0.001

(b)

Sample	Mineral digestibility, %		
	Before	**After**	***p* value***
Wheat	45.38±4.60^{abc}	53.23± 0.42^{d}	0.034
Barley	40.72±0.00^{bcd}	54.15±1.01^{d}	0.048
Rye	45.70±0.42^{abc}	52.86±1.08^{d}	0.095
Oat	36.24±2.01^{cd}	61.52±0.96^{c}	0.024
Paddy	30.16±1.01^{d}	62.19±0.31^{c}	0.006
Bean	53.83±1.89^{a}	75.75±1.55^{b}	0.045
Chickpea	52.16±2.74^{ab}	83.95±0.83^{a}	0.017
Green lentil	52.47±0.14^{ab}	76.49±0.98^{b}	0.026

(c)

Sample	Protein digestibility, %		
	Before	**After**	***p* value***
Wheat	50.66±3.30^{def}	66.37±0.14^{bc}	0.006
Barley	36.49±0.97^{de}	60.59±1.26^{d}	0.033
Rye	53.81±3.19^{b}	66.80±1.02^{bc}	0.050
Oat	40.54±0.10^{cd}	60.32±1.30^{d}	0.042
Paddy	48.69±1.24^{bc}	48.99±1.76^{e}	0.893
Bean	25.98±0.08^{e}	60.27±1.36^{d}	0.025
Chickpea	77.29±2.24^{a}	78.76±0.65^{a}	0.265
Green lentil	71.60±2.65^{a}	72.95±0.07^{ab}	0.033

PD, %, for all grain samples increased after the autoclaving. This increase was statistically significant ($p<0.05$) for all grains, except for paddy and chickpeas. A significant increase of the PD values was in barley and beans (Figure 3). The autoclaving process increased PD by 132% in the bean, but only 2% increase was observed in the other legume samples. It was reported that autoclaving at 121 °C reduced the PD in legumes compared to boiling (Rehman and Salariya, 2005). Heat treatments increased the PD of legumes due to the removal of protease inhibitors prevented the absorption of proteins in the intestine, as well as the denaturation of the proteins (Mubarak, 2005). The results achieved in the present study corroborated the finding reported by Messina (2014) showed that heat treatment increased the nutritional bioavailability of beans. The autoclaving has an important effect in terms of MD and PD, especially considering that the legumes are generally cooked under pressure while preparing the foods.

Boiling

The boiling process significantly decreased ($p<0.05$) the PA content in grains, except paddy and chickpeas (Table 4).

Table 4

Phytic acid contents (a) and digestibility rates (b and c) of the of the samples before and after boiling process

(a)

Sample	Content of Phytic acid, mg/100 g		
	Before	**After**	***p* value***
Wheat	2471.87±0.31^{a}	1200.00±2.03^{c}	0.001
Barley	2328.12±2.08^{b}	1231.25±2.56^{a}	0.001
Rye	1715.62±1.10^{g}	1046.88±0.13^{g}	0.000
Oat	2050.00±2.74^{c}	1109.38±0.75^{e}	0.000
Paddy	1559.37±0.22^{h}	1137.50±0.53^{d}	0.001
Bean	1831.25±1.59^{d}	1140.63±0.40^{d}	0.000
Chickpea	1806.25±0.88e	1218.75±2.30^{b}	0.002
Green lentil	1790.63±0.49^{f}	1100.00±2.12^{f}	0.002

(b)

Sample	Mineral digestibility, %		
	Before	**After**	***p* value***
Wheat	45.38±4.60^{abc}	57.67±1.70^{d}	0.088
Barley	40.72±0.00^{bcd}	64.12±0.90^{cd}	0.024
Rye	45.70±0.42^{abc}	57.46±1.74^{d}	0.094
Oat	36.24±2.01^{cd}	68.75±0.57^{bc}	0.011
Paddy	30.16±1.01^{d}	60.89±2.33^{d}	0.048
Bean	53.83±1.89^{a}	75.94±1.05^{ab}	0.030
Chickpea	52.16±2.74^{ab}	76.51± 1.11^{a}	0.029
Green lentil	52.47±0.14^{ab}	75.99±0.57^{ab}	0.015

(c)

Sample	Protein digestibility, %		
	Before	**After**	***p* value***
Wheat	50.66±3.30^{bc}	55.81±1.29^{a}	0.156
Barley	36.49±0.97^{de}	42.72±1.03^{b}	0.104
Rye	53.81±3.19^{b}	58.68±1.17^{a}	0.150
Oat	40.54±0.10^{cd}	25.52±1.22^{d}	0.052
Paddy	48.69±1.24^{bc}	33.83±2.66^{bcd}	0.113
Bean	25.98±0.08^{e}	32.55±2.12^{cd}	0.199
Chickpea	77.29±2.24^{a}	55.01±1.20^{a}	0.034
Green lentil	71.60±2.65^{a}	35.83±2.10^{bc}	0.037

* (P<0.05) means that the values statistically different in the same line
**Different letters indicate significant differences (P<0.05) in the same column

The highest PA content was in barley and the lowest one was in the rye. Moreover, it decreased in barley, wheat, and oats by 50%. In a study about the effects of cooking methods on the nutritional quality of some vegetables and legumes, soaking and cooking of the peas and beans were effective in removal or redudictioln of the antinutrients such as PA (Fabbri and Crosby, 2016). In the present study, the boiling process was more effective than the autoclaving in terms of PA degradation (Figure 1). The optimum temperature of the phytase enzyme is 45-60℃ (Pandey, 2001) and its activity decrease at 60℃ (Yanke, 1999). Thus, the phytase enzyme degrades at the autoclaving temperature and it was less effective in terms of PA degradation. The boiling chickpeas at 100 ℃ for 90 min decreased PA by 28.93% (Alajaji and El-Adawy, 2006). Singh et al. (2015) found that boiling for 40 min was effective in reducing the PA. The present case shows that the degradation of PA decreases due to the decrease of phytase enzyme activity if the heat treatment temperature and time applied to the grains increase.

The increase in MD of the grains, except for wheat and rye, was statistically significant ($p<0.05$).The boiling process yielded approximately a 2-fold increase in the MD values for the oat and paddy samples. As a result, MD of legume samples was higher than for the cereal grains after the boiling process. PD values decreased in paddy, oat, chickpeas, and green lentils but increased in other samples. However, these changes were statistically non-significant for other cereals, except for the increase in chickpeas and green lentils ($p>0.05$).

Effects of fermentation

The initial level of PA level of cereal flour samples and the levels after the spontaneous fermentation were statistically significantly different ($p<0.05$) (Table 5). Since the initial PA conten in rice flour was already lower than that in the other grain flours, the fermentation slightly affected it. PA was at the level of 0.004% in the endosperm layer of rice (O'Dell et al., 1972). This value is very low when compared to other grains. The fermentation of cereal flours gave high reduction rates in PA (Figure 1). The decrease in PA content was the lowest in rice flour, while the highest decrease in PA content was in barley flour (67%). Rizzello et al. (2012) reported that the addition of coarse bran to the wheat flour dough increased phytase enzyme activity by 2 times.

Table 5

Phytic acid contents (a) and digestibility rates (b and c) of the flour samples before and after fermentation process

(a)

Sample	Phytic acid, mg/100 g		
	Before	**After**	***p* value***
Wheat flour	1900.00±0.71[a]	662.50±0.18[d]	0.000
Barley flour	1940.62±0.66[b]	639.06±1.52[e]	0.000
Rye flour	1709.37±1.28[d]	678.12±0.09[c]	0.000
Oat flour	1818.75±0.44[c]	714.06±0.24[b]	0.000
Rice flour	921.87±0.49[e]	718.75±0.75[a]	0.002

(b)

Sample	Mineral digestibility, %		
	Before	**After**	***p* value***
Wheat flour	49.61±0.49[a]	80.50±1.22[a]	0.025
Barley flour	44.61±0.60[a]	82.53±1.23[a]	0.021
Rye flour	58.30±1.20[a]	84.37±1.67[a]	0.041
Oat flour	46.44±6.08[a]	81.71±0.12[a]	0.002
Rice flour	58.35±2.86[a]	83.98±1.91[a]	0.047

(c)

Sample	Protein digestibility, %		
	Before	**After**	***p* value***
Wheat flour	74.46±2.19[a]	80.22±0.37[a]	0.041
Barley flour	65.19±2.85[a]	70.51±0.14[d]	0.017
Rye flour	66.51±1.09[a]	74.13±0.46[c]	0.038
Oat flour	65.63±1.41[a]	74.64±0.44[c]	0.031
Rice flour	66.13±0.43[a]	77.39±0.32[b]	0.018

* (P<0.05) means that the values statistically different

**Different letters indicate significant differences (P<0.05) in the same column

This shows that cereal bran has a high phytase activity. In the present study, rice flour, which had lower phytase activity since it does not contain bran layer, presented less PA decrease by the fermentation. The phytase enzyme has broad substrate specificity and usually exhibits optimum activity in the range between pH 4.5 and 6.0 (Pandey et al., 2001). Leenhardt et al. (2005) showed that, due to the acidity formed by lactic acid bacteria in sourdough

fermentation, the pH dropped below 5.5 and the increased phytase activity decreased the PA content in wheat flour by 70%. In the present study, pH values were determined to be 4.5 in wheat flour, 4.6 in oat flour, 4.7 in rye flour, 4.6 in barley flour, and 4.1 in rice flour after the fermentation. Thus, the post-fermentation pH values of cereal flours reached an adequate acidity level of pH 4.0-6.0, where the phytase enzyme could show optimum activity and PA degradation occurred.

The difference between initial MD values of cereal flours was statistically non-significant ($p>0.05$). It was determined that there was a significant ($p<0.05$) increase in MD after spontaneous fermentation for all flour samples. Because of fermentation of the barley flour having the lowest MD, its digestibility increased by approximately by 2 times.

The differences between the initial PD values of flour samples were statistically non-significant. Increases in PD after fermentation were, however, statistically significant ($p<0.05$). It is known that the lactic acid bacteria had also protease activity, and they activated the endogenous enzymes naturally found in the cereals through the organic acids, which are important metabolites produced during fermentation. Because of both direct and indirect protease activities in the dough, content of proteins decreased and water-soluble nitrogenous substances and PD increased (Gobbetti et al., 2014). Furthermore, it was indicated that the free amino acid levels in the medium increased as a result of the breakdown of proteins (Hansen and Schieberle, 2005).

Conclusion

Examining all the processes applied in the present study together, it can be said that germination and fermentation processes are the most effective methods. In general, all the processes reduced the amount of phytic acid, which is an important antinutrient. Some of these methods are already used in the preparation of cereals and legumes as food. Therefore, soaking of chickpeas and beans, boiling them in water, and then cooking not only softens the grain but also reduces the level of phytic acid and increases mineral and protein digestibilities. The combination of heating, fermentation, autoclaving, and other processes used in the present study, which affect via the change of chemical structure, the formation of insoluble complexes, and degradation of phytic acid can be suggested as a treatment that resulted in the significant reduction of the content of PA.

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Main trends in application of novel natural additives for food production

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Abstract

Introduction. The review considered the major novel natural additives for food products.

Materials and methods. Analysis of scientific knowledge about novel natural additives for food products has been done.

Results and discussion. In recent years, a lot of research has been conducted to study the application of novel natural additives such as plants with pharmaceutical properties, plant materials with antioxidant activity, essential plants oils, extracts from plant materials seaweeds, seeds products, different gluten-free cereal and pseudo-cereals flours for replacement of gluten in bakery products, dietary fibers, edible coatings materials of plant origins, in preparation of different traditional food products. The main goal of this trend is to improve the health value of these products without significant change of their technological parameters and acceptability by consumers. These new trends include replacement of animal fat by low calorie materials with high content of fatty acids in meat products; use of plant materials with antioxidant properties, for example, extracts from fruits, vegetables, spices, and herbs containing phenolic substances instead of synthetic antioxidants in food preparation; replacement of gluten in bakery products with plant free gluten substitutes; use of plant essential oils as natural preservatives to prolong shelf-life of food products. Some researches concern the application of plant additives with specific pharmacological effect to produce food products useful for prevention of different diseases or supplying the consumers with needed quantities of essential for the health state substances. The present study reviews the main trends in application of novel natural additives used recently in food production.

Conclusions. Review information can be valuable for researchers and managers to prioritize the research and innovation directions in enhancement of food products.

Introduction

In recent years, a lot of research has been conducted to study novel natural additives to improve the health value of different traditional food products. These new trends include replacement of animal fat by low calorie material with high content of fatty acids in meat products (seed oils, for example); replacement of gluten in bakery products with plant free gluten substitutes; use of addition of plant material with antioxidant properties instead of chemical preservatives to prolong shelf-life of food; an application of edible coating with incorporated natural antioxidant and antimicrobial agents in different food products to protect their properties and extend time of storage. Application of plant additives with specific pharmacological properties was proposed to produce food products useful for prevention of different diseases or supplying the consumers with needed quantities of essential for the health state substances.

The aim of the present study was to review the main trends in application of novel natural additives used recently in food production.

Trend 1. Plant additives with pharmaceutical properties for food products

A new trend in food production is an addition to traditional food products of different plant additives which are used for medical purposes and have pharmacological properties. For example, a tree-shaped plant Bail fruit (*Aegle marmelos* L.), widely distributed in South East Asia, is known as one which has pharmacological properties and traditional medical usage and is also consumed as a food. It was reported that Bael fruit has cardio-protective, radio-protective, gastro-protective and hepato-protective effects, as well as antioxidant, antibacterial, antiviral and anti-diabetic properties (Mulyaningsih et al., 2020; Sarkara et al., 2020; Venthodika et al., 2021). Numerous scientific researches partially along with clinical trials are providing now to establish the ways for usage of *A. marmelos* for different food products such as bael juice, syrups, beverages, jam, toffee, and candy (Mali et al., 2020; Ullikashi et al., 2017). However, pharmacological effects of food products based on bail fruit as well as recommended dosage of its consumption should be studied in further research (Venthodika et al., 2020).

Moringa oleifera is an example of plants with pharmacological properties which are proposed for food preparation (Milla et al., 2021). This plant is considered a source of nutritional and bioactive compounds such as fibres, phenolic compounds, fatty acids, vitamins, minerals and functional peptides, and many authors proposed its application in preparation of functional food products mainly in meats, bakery, juices and sandwiches to improve their nutritional and health value. Only for bakery products, leaf powder from *Moringa oleifera* is proposed to be used for preparation of cookies (Chizoba, 2014), bread (Bolarinwa et al., 2019; Ogunsina et al., 2011; Rabie et al., 2020; Sengev et al., 2013), brownie (Castro-Lopez et al., 2017); rice crackers (Manaois et al., 2013), snack (Devisetti et al., 2015), and cakes (Kolawole et al., 2013).

Lycium plants attract attention by their high nutrition and medicinal value (Jiang et al., 2021). Different parts of *Lyceum* plants, namely berries, leaves and root barks, are used for a long time in traditional Chinese medicine in the treatment of age related diseases such as atherosclerosis and diabetes (Potterat, 2010). Meanwhile, *Lycium* fruit (Goji berries) is widely consumed as a food, for example in Chinese soups and as herbal tea (Ma et al., 2019). Berries, leaves and root barks of *Lycium* plants contain 84 different phenolics (Qian et al.,

2017) and other bioactive substances such polysaccharides and terpenes. *Lycium* fruit can be consumed as dried whole berries and as berries incorporated in meat products, flours and beverages. Addition of *Lycium* berry puree (2.5 and/or 5%) and chia seeds in beef burger increased its antioxidant ability and decreased lipid peroxidation (Antonini et al., 2020). This new product was accepted by consumers as functional burgers. Partially replacement of wheat flour up to 40% with goji berry by-product that is left after production of goji juice/concentrate resulted in significant increase of protein, free phenolic, insoluble and soluble dietary fibers contents in the muffins and cookies (Bora et al., 2019). White chocolate added with *Lycium* berry was positively evaluated by consumers (Ferreira et al., 2017). Incorporation of *Lycium barbarum* berries into yogurt improved it's the antioxidant properties and enriched biological value (Taneva and Zlatev, 2020).

Trend 2. Essential plants oils for food preservation

Application of plant essential oils as natural preservatives is a new trend in food processing. Essential oils are concentrated volatile compounds extracted from different plant materials such as leaves, fruits, flowers, roots, seeds, wood and have been extracted from such plants as sweet orange, lemon, lavender, oregano, rosemary, sage, ginger, clove, cinnamon, curcumin, spearmint, pomegranate peel, grape seed, basil, coriander, garlic cloves, eucalyptus and myrtle. A lot of essential oils are generally recognized as safe (GRAS) and are permitted for use in food products. One of the important properties of essential oils is ability to suppress microbial growth, and this antimicrobial activity could be used in food preservation instead of conventional chemical agents (Hyldgaard et al., 2012). It was shown that tested essential oils had very low minimal inhibitory concentration against different bacteria species estimated as 0.05–60 μg/mL (Aumeeruddy-Elalfi et al., 2015; Bajera et al., 2017). However, the antimicrobial effect of essential oils can be changed and depends on composition of food and used food additives (Garcia-Diez et al., 2017).

It was shown that the addition of the spice nutmeg (*Myristica fragrans*) essential oil in concentration 20 mg/L increased oxidative and microbial stability and extended shelf life of cooked sausages (Sojic et al., 2015). Essential oils as food additives are often used with combinations of other natural preservatives. For example, the addition of *Zataria multiflora* Boiss essential oil and grape extract in quantity 0.1% and 0.2%, respectively, in raw buffalo patties decreased the level of lipid oxidation and extended the shelf life of tested meat products. The presence of essential oil and grape seed extract demonstrated strong antimicrobial effect in raw buffalo patties inoculated with *Listeria monocytogenes* (Tajik et al., 2015). Combination of cinnamon essential oil and grape seed extract added to Lyoner-type sausages improved their odor and color scores, extended their shelf life during refrigerated storage and inhibit the growth of *Clostridium perfringens* in artificially contaminated samples (Aminzare et al., 2018).

Essential oils could be applied as natural antimicrobial and antioxidant agents for fish and other seafood to protect them from microbial spoilage and lipid oxidation and prolong the shelf life (Hassoun and Coban, 2017). To increase effectiveness of treatment, combinations of different essential oils could be used. However, it is necessary to study possible cytotoxicity and toxicity of used essential oils.

It was proposed to use edible microcapsules containing essential oils, especially the starch microcapsules, for prolongation of the shelf life of food instead of chemical preservatives (Ju et al., 2020). These microcapsules are environmentally friendly; ensure slow release of essential oil, and protect food products from the flavor influence.

Microencapculated essential oil from *Perilla frutescens* L. Britt had good antibacterial activity and was recommended to be used for strawberry preservation and prolongation of the shelf life (Li et al., 2018).

Trend 3. Extracts from plant materials for replacement of synthetic antioxidants

Extracts from fruits, vegetables, spices, and herbs usually contain phenolic substances which ensure their strong antioxidizing activity. So, these natural products could replace synthetic antioxidants in preparation of food (Aminzare et al., 2019).

Extracts of plant Roselle (*Hibiscus sabdariffa* L.), used in traditional folk medicine in many countries, are rich in polyphenols such as polysaccharides, anthocyanins, and organic acids (Riaz and Chopra, 2018). Compounds from these extracts have antioxidant and antibacterial properties and can be used as natural additives (Marquez-Rodríguez et al., 2020). A phenolic extract obtained from hibiscus calyces possessed antimicrobial activity against such foodborne pathogens as *Listeria monocytogenes Salmonella enterica, Escherichia coli, Staphylococcus aureus,* and *Bacillus cereus* and being using for meat products preparation showed preservation effect. Spraying of ethanolic or phenolic extracts from hibiscus on beef steaks resulted in prolongation of the meat`s shelf life (Marquez-Rodríguez et al., 2020). Generally, there are a lot of studies which propose using extracts from different fruits and vegetables as natural antioxidants for meat products. Some examples are shown in Table 1.

Table 1

Use of extracts from plant materials as natural antioxidants in meat products

Extracts from	Meat product	References
Acerola fruit	Beef patties	Realini et al., 2015
Guarana seed or Pitanga leaf	Lamb burgers	de Carvalho et al., 2019
Ginkgo leaves	Meat balls	Kobus-Cisowska et al., 2014
Hibiscus calyces	Beef steaks	Marquez-Rodriguez et al., 2020
Hyssop	Pork meat	Fernandez-Lopez et al., 2003
Lotus seed epicarp	Chinese sausage	Qi and Zhou, 2012
Mugwort and Rosemary	Pork patties	Hwang et al., 2016
Murraya koenigii berries	Meat batter	Kumar and Kumar, 2020
Red pitaya	Pork patties	Bellucci et al., 2021
Rosemary	Pork meat	Fernandez-Lopez et al., 2003
Uulam raja leaves	Beef patties	Reihani et al., 2014
Pitangueira leaves	Pork sausage	Luciano et al., 2021

The addition of extract from acerola (*Malpighia emarginata*) extended the shelf life of beef patties by improving their lipid stability (Realini et al., 2015). Extracts from ulam raja leaves (*Cosmos caudatus*) and green tea extract incorporated in beef patties demonstrated a strong lipid oxidation inhibitory effect and an improvement in cooking yield and textural properties (Realini et al., 2014). The incorporation of mugwort (*Artemisia vulgaris*) extract, rosemary (*Rosmarinus officinalis*) extract and ascorbic acid added in quantity 0.05% each

delayed oxidative deterioration and maintained quality of pork patties during their refrigerated storage (Hwang et al., 2016). Extract from less conventional plants, such as Pitangueira (*Eugenia uniflora*), was proposed to be added in fresh pork sausage to extend their shelf-life (Luciano et al., 2021). Extracts from rosemary or hyssop inhibited lipid oxidation, degradation of heme pigments, and slowed down metmyoglobin formation during cooking and storage (Fernandez-Lopez et al., 2003). Lotus (*Nelumbo nucifera* Gaertn) seed epicarp, the main by-product of lotus seed processing, is rich in polyphenols. Extracts from lotus seed epicarp being used for supplementation of pork homogenate which represented Chinese Cantonese sausage resulted in retarding of lipid oxidation (Qi and Zhou, 2012). Extract from Ginkgo (*Ginkgo biloba*) leaves added in pork meatballs inhibited lipid and cholesterol oxidation processes during 21 days of products refrigerated storage (Kobus-Cisowska et al., 2014). The addition of red pitaya extract to pork patties improved consumer acceptance of these meat products due to improving colour and extended the shelf life of parties during storage at 2°C due to retarding the oxidative processes (Bellucci et al., 2021). The addition of encapsulation *Murraya koenigii* berries extract ensured higher oxidative stability of meat batter (Kumar and Kumar, 2020). Incorporation of guarana seed or pitanga leaf extracts delayed discoloration and ensured oxidative stability of the lamb patties during their refrigerated storage, meanwhile sensorial properties were not changed (de Carvalho et al., 2019).

It is known that berries contain a lot of phenolic compounds, especially anthocyanin. Extracts of such berries as bearberry (*Arctostaphylos* sp.), blueberry (*Vaccinium* sp.), blackberry (*Rubus* sp.), cloudberry (*Rubus chamaemorus*), cranberry (*Vaccinium* sp.), blackcurrant (*Ribes nigrum*), strawberry (*Fragaria* ananassa), and grape berries (*Vitis* sp.), contained antioxidant polyphenols, so, they were recommended to be used for stabilizing meat products instead of synthetic antioxidants (Lorenzo et al., 2018). Extract from cloudberry showed strong antioxidant activity due to presence of flavonoids and was recommended to be added to pork patties to prevent lipid oxidation in the cooked product (Rey et al., 2005).

Extract from herbs could be used for stabilization of oil that replaces fat in meat products. Chia oil was enriched with rosemary obtained with ultrasound-assisted extraction to ensure oil oxidative stability. This mixture was microencapsulated and used to replace 50% fat in burgers (Heck et al., 2018). The similar effect was observed when guarana seed and pitanga leaf extracts were added as natural antioxidants to lamp patties prepared with replacement of fat by chia oil emulsion (de Carvalho et al., 2019). It was shown that addition of mentioned plant extracts delayed discoloration of the lamb patties during storage at 2°C, as well as lipid and protein oxidation, without changes of their sensorial properties. Replacement of 20% pork back with dietary fiber extracted from makgeolli lees did not decrease the quality of frankfurters with 30% fat (Choi et al., 2014). Jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg) is a Brazilian grape-like fruit. The addition of up to 0.5% of jabuticaba peel extract to bologna-type sausages improved their oxidation stability during storage due to extract antioxidant activity without affection of sensory quality (de Almeida et al., 2015).

Use of thuja (*Thuja occidentalis*) cones or peach (*Prunus armeniaca*) seeds extracts with the content of total phenolic compounds 0.8 and 0.2% by dry weight, respectively, were added in quantity 50 mL of extract to 1 kg of minced meat in raw chicken ground meat as antioxidants (Yogesh and Ali, 2014). Addition of these extracts inhibited lipid oxidation during chicken ground meat storage at 4°C, increased water holding capacity and decreased cooking loss. However, authors noticed that *Prunus* family kernels and thuja cones contain compounds which could have toxic effects being consumed more, so further studies were

needed to estimate the possibility of using these fruit extracts in meat preparation. Cinnamon (*Cinnamomum Zeylanicum*) essential oil contains phenolic and polyphenolic compounds and could be used in low concentrations (0.02% and 0.04% v/w) in preparation of Lyoner- type sausage to inhibit lipid oxidation and increase sausages chemical stability (Aminzare et al., 2015).

Natural polyphenols extracted from different plants have the potential to be used as antioxidant and antimicrobial agents instead of synthetic additives to preserve fish and fish product quality (Maqsood et al., 2013). There are also some researches concerned about their use as functional ingredients in meat and fish processing (Gokoglu, 2019; Patel, 2015).

Natural antioxidants can be added to edible oils to enhance their stability. For example, addition of essential oils from cinnamon or clove to hazelnut or poppy oils successfully prevented lipid oxidation and were recommended to be used instead of synthetic antioxidant butylated hydroxyanisole (Ozcan and Arslan, 2011).

Plant extracts could be used in the dairy products. It was shown that the extracts from such spices and herbs as cinnamon, cloves, garden cress, oregano and lemon grass demonstrated antibacterial activity against foodborne pathogens including *Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus,* and *Escherichia coli*. Being incorporated in cheeses they also enhanced taste, odor, color and total quality of the product (Tayel et al., 2015). Effectiveness of five spice and herb extracts used against development of three foodborne pathogens in cheese at room temperature 23ºC was demonstrated (Shan et al., 2011). The decrease of pathogen numbers and the inhibition of lipid oxidation were observed in cheese treated with these extracts.

Plant extract can be incorporated in dairy products replacing synthetic additives to improve their antioxidant activity. Thus, cottage cheese added with aqueous extracts of fennel (*Foeniculum vulgare* Mill) or chamomile (*Matricaria recutita* L.) microencapsulated in alginate showed higher antioxidant activity after the 7th day of storage in comparison with control prepared without plant extracts (Caleja et al., 2016). Combination of essential oils and plant extracts is an attractive alternative for synthetic preservatives in cheese production to ensure microbiological safety of the products (Gouvea et al., 2017). However, the influence of their incorporation on activity of lactic bacteria and the sensory characteristics of products should be studied as well as studies concerning more effective combinations of different essential oils and plant extracts.

Cinnamon leaf and bark essential oils added in quantity of 0.5% in strawberry shakes showed strong antimicrobial activity against *Salmonella typhimurium* and *Listeria monocytogenes* and completely inhibited both bacteria after 8 days storage at 4 °C (Brnawi et al., 2018).

Natural antioxidants were also proposed to be used in bakery products. For example, the incorporation of fennel and chamomile aqueous extracts rich in phenolic compounds in biscuits ensured similar antioxidant activity as a synthetic antioxidant, the butylated hydroxylanisole, widely used in food production (Caleja et al., 2017).

Despite evident beneficial effects on food stability and its protection from microbial spoilage and oxidation, more studies should be conducted to prove safety of essential oils or plant's extracts in food production and evaluate possible side effects (Lourenço et al., 2919; Ribeiro-Santos et al., 2017). New antioxidants to be used in food production should be approved by the European Food Safety Authority (EFSA) or by the United States Food and Drug Administration. A new food additive must be safety evaluated by (1) its chemistry and specifications; (2) existing authorizations and evaluations (data of previous risk assessments); (3) proposed uses and exposure assessment, and (4) toxicological studies (ANS, 2012).

Trend 4. Application of seaweeds in food nutrition

Edible seaweeds (macroalgae) are presently a subject of many investigations. Macroalgae have been a part of local nutrition in Asian East and Southeast countries from ancient times, and the health benefit of seaweed and seaweed extract is shown in different studies (Brownlee et al., 2012; Shannon and Abu-Ghannam, 2019). Seaweeds or seaweeds extracts were proposed to be used to increase health value and shelf life of different food products as well as for preparation of low-fat products with decreased content of calories and saturated fatty acids and improve overall quality of foods (Roohinejad et al, 2017). Edible seaweeds are considered to be a good source of dietary fibers, peptides, phlorotannins, essential amino acids, antioxidants, vitamins, unsaturated fatty acids, carotenoids and abundant minerals that can be incorporated in meat, fish, bakery and others food products as a source of nutrient as well as a source of compounds needed to be consumed by people suffering from a wide spectrum of disorders or diseases (Lordan et al., 2011; Fitzgerald et al., 2014; Cardo et al., 2014; 2015).

There is a lot of research about using macroalgae, or extracts from them to create new products which could be used by the population suffering from different cardiovascular diseases (Cardoso et al., 2015). Mainly meat-based products with addition of seaweeds were proposed and studied as functional foods for populations with cardiovascular-health problems. The main aim of seaweed incorporation in the meat products is to improve the composition of fatty acids and to reduce the content of cholesterol, salt and fat. To improve fatty acid content of such meat products as patties, frankfurters and steaks seaweeds wakame *Undaria pinnatifida*, nori *Porphyra umbilicalis*, and sea spaghetti *Himanthalia elongata* were added as a source of bioactive substances with simultaneous reduction of content of sodium and fat (5% of each seaweed to pork meat) (Cofrades et al., 2017). These meat products were tested on male rats to study their health effects. The authors indicate that every kind of algae has its own effect based on its composition, and should be subject of individual study.

Research done in Korea with volunteers showed that addition of sea tangle *Laminaria japonica* to chicken and pork patties had improved postprandial plasma glucose and lipids profiles in borderline-hyperlipidemic adults (Lim et al., 2013).

To reduce sodium content in meat products, addition of selected seaweeds (*Porphyra umbilicalis, Palmaria palmate, Himanthalia elongata* or *Undaria pinnatifida)* in quantity 1% (w/w) in reformulated frankfurters were conducted to replace salt. The most promising results were obtained when seaweed *H. elongate* was used (Vilar et al., 2020).

Edible seaweeds have been a source of selenium and iodine (Cherry et al., 2019; Circuncisao et al., 2018) which are needed to ensure optimal thyroid function (Schomburg and Kohrle, 2008). Preparation of functional meat products enriched with seaweeds *Cystoseira* or *Fucu*s (2% by dry weight to raw stuff) was proposed to supply the population with needed daily quantities of iodine and selenium (Kryzhova et al., 2021).

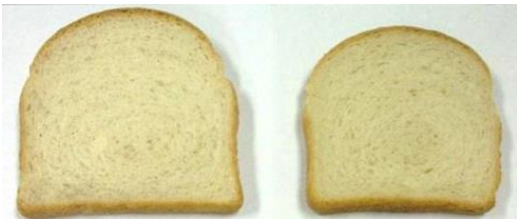
Bakery products are other subjects to be enriched with microalgae. Seaweeds *Lemna minor* or *Ulva rigida* were studied as additives in bread preparation to extend its shelf-life (Kılınc et al., 2013). A renin-inhibitory protein of edible seaweed *Palmaria palmata* was incorporated in bread at quantity 4% (Fitzgerald et al., 2011). It was shown that the bioactivity was evident after bakery procedure but no significant changes in texture or sensory properties were observed. The addition of seaweed *Ascophyllum nodosum,* 4% per loaf, reduced energy intake at a meal but not nutrient uptake (Hall et al., 2012).

Effect of addition of *Fucus vesiculosus* seaweed powder to wheat flour on dough and bread properties showed that it could be added at maximum of 4% (flour basis) without

deterioration of enriched breads (Arufe et al., 2018). Further increase of quantity of seaweed powder added to dough resulted in significantly increasing of density and crumb firmness and changing of bread crust colour.

For the last few years some beverages prepared with microalgae or extract from them were developed. These beverages were proposed to be used as health drinks. Beverage prepared from macroalgae sea trumpet possessed strong antioxidant properties correlated with the contents of polyphenols (Nagai and Yukimoto, 2003). Beverages prepared from this sea algae had angiotensin I-converting enzyme inhibitory activities: sea trumpet (0.625 mg dry matter/mL), hizikia (7.79 mg dry matter/mL), and wakame (26.4 mg dry matter/mL) and could be used not only as drinks supplying minerals, but also to suppress the hypertensive activity of angiotensin I (Nagai et al., 2016). To prepare a beverage for the prevention of hypertension, seaweed *Hizikia fusiforme* was used as a main part of composition (Kim, 2008).

So, seaweeds could have wide applications in preparation of food products with certain health benefits. However, when seaweed is added to a food product as a source of a certain component it is necessary to estimate possible effects of other substances presented in this seaweed. For example, seaweed *Laminaria* contained too much selenium and iodine to be recommended as additives for meat products in concentrations higher than 1–2% (Kryzhova et al., 2021). The content of seaweed added to products should be higher regulated because health effects could depend on the quantity of seaweed consumed. For example, consumption of parties with cooked rice containing sea tangle powder substituted, 2.5% of meat, by women volunteers improved blood glucose concentration; meanwhile patties substituted with cooked rice containing 25% or 50% sea tangle powder might ameliorate plasma lipid profiles (Oh and Lim, 2011). So, the health benefits of new products should be checked before they could be recommended for the food market. Physico-chemical and sensory quality of food product depends on the quantity of added seaweed. Addition of powder of *Fucus* or *Cystoseira* in quantity 2% to raw stuff in preparation of different meat products did not significantly change their sensory properties (Kryzhova et al., 2021). Characteristics of pork parties with sea tangle powder of *Laminaria japonica*, 1 – 3%, were similar to the control parties (Choi et al., 2012). Meanwhile, a comparison of the quality of the breakfast sausage containing 1, 2, 3, and 4% powder of *Lamina japonica* showed that sausage with 1% had the highest overall acceptability (Kim et al., 2010). The addition of 2 – 8% dried red seaweed *Kappaphycus alvarezii* powder to wheat flour increased the water absorption of the dough and decreased its stickiness properties (Mamat et al., 2014). However, no significant difference in the slickness values of the dough added with different quantities of seaweed was observed. The firmness of the bread increased over the period of storage and positively correlated with the percentage of seaweed powder added to flour. The quality of produced bread was affected by the amount of added seaweed (Figure 1). The addition of seaweed decreased the bread density, the volume of bread and increased the yellowness of the bread crumb obtained.



F1 F2

F3 F4 F5

Figure 1. Bread produced with seaweed powder, %: 0 (F1), 2% (F2), 4% (F3), 6% (F4), 8% (F5).
(Adapted from Mamat et al., 2014).

Trend 5. Plant seeds as additives to food products

Seeds of different plants have specific chemical compositions and in recent years a lot of researches studying the possibility of their usage for preparation of functional food products were published. Chia seed has been in the focus of many studies. Chia (*Salvia hispanica* L.) originally was grown in Mexico and Guatemala and the nutritional properties and health-promoting properties of chia seeds are known, especially, due to oil content (between 30% and 33%) with high percentage of unsaturated fatty acid (around 80% of total acid value) and presence of omega-3 α-linolenic acid and other polyunsaturated fatty acids. Chia seed is also a source of dietary fiber (up to 38%), minerals (4–5%), and protein (15–25%) (Kulczynski et al., 2019; da Silva et al., 2017). The seeds are characterized also by high contents of polyphenols and antioxidants (Hrncic et al., 2020). For its nutrient and health values chia seed was announced as plant material which is "The new golden seed for the 21st Century" (Orona-Tamayo et al., 2017).

The use of chia seeds to enhance the quality of food products has been studied intensively. The addition of chia seeds to beef burgers doubled or tripled the content of polyunsaturated fatty acids, increased the content of polyphenols and antioxidant activity (Antonini et al., 2020). Hedonistic tests conducted for different age groups of population showed acceptability of this food product and indicated its potential application as a functional burger.

Chia seeds were added to different bakery products to improve their nutritional value. It was shown that addition of chia seed mucilage in bread and cakes for replacement up to 50% of fat did not affect significantly technological and organoleptic characteristics of healthier bakery products (Fernandes et al., 2017).

The addition of chia flour to the biscuits improved not only the nutritional value of the product, but also increased the antioxidant capacity, content of phenolic compounds, protein,

fiber and polyunsaturated fatty acids; however, contamination process was increased, so the shelf life of the product was decreased (Mesias et al., 2016).

It was shown that reduction of the amount of hydrogenated vegetable fat with chia seeds or chia flour in breads increased the levels of fibers and polyunsaturated fatty acids, mainly omega-3, but affected the technological quality of the loaves decreasing the specific volume and the total score values. However, both types of bread obtained a high level of acceptability by consumers, meanwhile bread with the addition of chia flour had a higher index of purchase intent than the bread with chia seeds (Coelho et al., 2015).

It was proposed to use different seeds to be incorporated in the different bakery products. Pumpkin seeds could serve as a source of proteins with high content of tryptophan, carotenoids, protein, minerals, including zinc, iron, magnesium and manganese, fiber and omega 3 and omega 6 fatty acids. Pumpkin seed products were added to wheat flour to produce blends with protein levels of 15, 17, 19 and 21% for loaves of breads production (El-Soukkary, 2001); it was recommended the addition of pumpkin seeds powder to wheat flour up to 15% to improve nutrition value and sensory quality of cookies (Alshehry, 2020); 50% replacement of wheat flour with pumpkins seed flour was proposed in preparation of cupcakes (Batista et al., 2018). Pumpkin seeds flour was recommended to be added in gravy to increase its nutrition and health value (Sharma and Sarla, 2017).

Flaxseed (*Linum usitatissimum*, L.) has high contents of protein and fiber and low contents of carbohydrate and fat, contains such compounds as phenolic acids, lignans and flavonoids with antioxidant characteristics, minerals especially Ca, K, P and Mg (Khaled et al., 2019). Flaxseeds have potential health benefits including reduction of blood cholesterol levels and the risk of cardiovascular diseases. Sensory evaluation of bread and cakes showed that partial replacement of wheat flour especially in the range of 5 to 10 per cent with flaxseed meal could be conducted without significant changes of organoleptic properties but with enhancement of fiber and protein contents (Khaled et al., 2019).

Preparation of cookies with partial replacement of the wheat flour with roasted or ground flaxseed showed that cookies with 15% level of substitution and below had acceptable evolution properties (Rajiv et al., 2012). Incorporation of red mombin (*Spondias purpurea* L.) seed flour into the chocolate brownies produced using wheat flour increased dietary fiber and ash contents, physical characteristics, and antioxidant activity allowing longer preservation of the product. It was shown that red mombin seed flour could completely replace wheat flour in chocolate brownies (de Abreu et al., 2021). Microencapsulated garden cress seed oil in whey protein concentrate with oil/protein ratio of 0.4 was used in biscuits preparation to replace flour and fat (Umesha et al., 2015). It was shown that annatto (*Bixa Orellana* L.) seeds powder served as a source of antioxidants to retard lipid and protein oxidation in pork patties during their storage at 4°C and displayed also antimicrobial activity (Cuong and Chin, 2016).

Trend 6. By-products of plant processing for food preparation

Many by-products of plant processing were proposed to be used as antioxidants in preparation of meat products (Aminzare et al., 2019). Half of the nitrite in the pork luncheon roll was replaced with 1.5% of tomato (*Solanum lycopersicum*) pulp powder. Rolls with reducing content of nitrite had higher acceptability. However, the addition of bigger amount of tomato pulp powder (3%) affects the colour and texture of the rolls (Hayes et al., 2013). Extracts from tomato pomace used for spreading on lamb longissimus thoracis surface showed antioxidant activity and improved shelf-life of atmosphere-packaged meat (Andres

et al., 2017). Quinoa paste was used at quantity 5% for partially replacing fat in pork liver pate (Pellegrini et al., 2018). The addition of quinoa paste to pork liver pate retarded the lipid oxidation, increased emulsion stability, so stability of the product was increased. At the same time, the samples with quinoa pasta had higher fiber content and reduced content of fat, so the healthiness of the product was improved. The most acceptable sample was the pâté containing red quinoa at 5%.

Addition of different plant materials to dough, mainly for replacement of animal fat to improve health properties of food products, was studied. However, application of hemicellulose- containing substances was also tried to be used for replacement of sugar or flour in bakeries. Ginger powder was proposed to be added (1% and 2%) to rabbit burgers (Mancini et al., 2017). The results showed that this addition increased the content of polyunsaturated fatty acids omega-3 and omega-6 as well as antioxidant capacity of rabbit burgers while lipid peroxidation values were decreased. By-products, such pomaces or puree, from the fruit juice industry are characterized by high content of bioactive components and fiber. Addition of green banana puree in pound cakes for replacement of traditionally used butter were studied (de Souza et al., 2018). It was shown that replacing fat at 25% resulted in reduction of 20% and 40% of sugar in green banana puree low-fat cakes meanwhile sensory characteristics did not change significantly. It was shown that the sponge cakes cooked with black currant and aronia pomace for replacement of fat (30% substitution) were well accepted by consumers, meanwhile partial replacement of sugar or flour decreased the quality of sponges (Quiles et al., 2018). Replacement of up to 50% of fat (butter) by avocado puree in muffins resulted in decrease of caloric value of low-fat muffins; meanwhile their organoleptic properties were acceptable (Othman et al., 2018). Okra gum was recommended to be used for replacement of fat (100% substitution) in chocolate bar cookies (Romanchik-Cerpovicz et al., 2002).

Trend 7. Replacement of gluten in bakery products

In recent years, a lot of research was conducted to find the ways for replacement of gluten proteins in bakery products. Gluten proteins, mainly gliadins and glutenins, represent about 80% of the wheat flour protein and ensure the elastic and extensible properties of dough. However, it has been considered as a wheat grain allergen and about 1% of the world population is sensitive to gluten (Battais et al., 2008; Wang et al., 2017). So, there are a lot of propositions of the replacement of wheat flour with different gluten-free cereal flours to produce gluten-free bakery products (Peris et al., 2019). Among gluten-free cereal flours used for substitution of wheat flour there are rice flour (Bourekoua et al., 2016; Sciarini et al., 2010) corn flour (Bourekoua et al., 2016); sorghum flour (Marston et al., 2016; Rao et al., 2016), and combinations of mentioned above flours, for example, rice and soybean flours in ratio of 70 to 30 (de la Luz Guerrero-Elizarraraz, 2017), rice, corn and soy flours (Sciarini et al., 2010) and others.

There are also a lot of researches studying possibility or the replacement of wheat flour by flour of pseudo-cereals such as buckwheat (*Fagopyrum esculentum* and *Fagopyrum tartaricum*) (Jan et al., 2015; Sakac et al., 2015), legume flours (Cheng et al., 2016; de la Hera et al., 2012; Pasqualone et al., 2019a), quinoa (*Chenpodium quinoa*) (Watanabe et al., 2014), amaranth (*Amaranthus* sp.) (Chauhan et al., 2016) and their mixture (Buresova et al., 2017).

It is known that gluten is responsible for flour technological characteristics to make high quality bakery products. However, it was shown that combination of some flours with certain

properties allowed to obtain gluten-free bread with good characteristics. Thus, gluten-free breads prepared with different combinations of rice, corn and soy flours differed significantly by their quality. It was established that bread made from a mixture of rice, corn and soy flours, taken in ratio of 40:40:20, had the best qualities characteristics such as high specific volume, good crumb appearance and soft texture (Sciarini et al., 2010) (Figure 2).

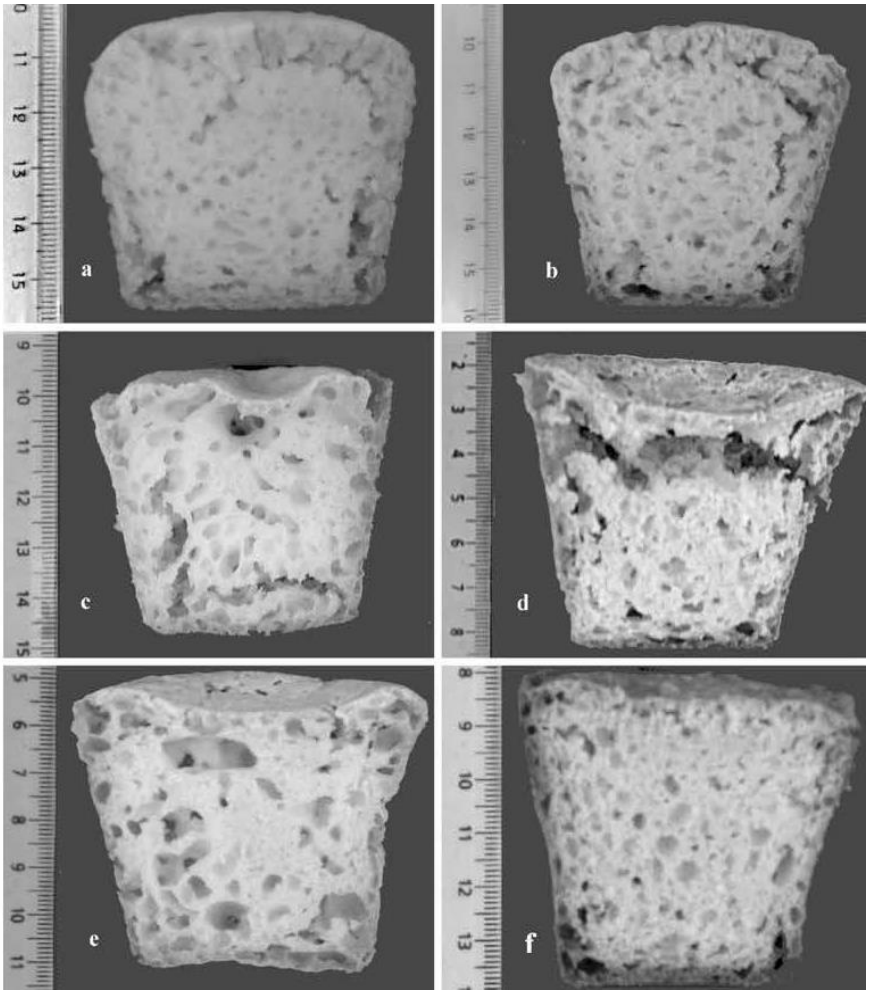


Figure 2. Images of gluten free bread slices: rice (a), rice/soy 90:10 (b), rice/corn 50:50 (c), corn/soy 80:20 (d), rice/corn/soy 45:45:10 (e), rice/corn/soy 40:40:20 (f) ((Sciarini et al., 2010).

Addition of quinoa (*Chenopodium quinoa*) flour to the cookies to substitute for 7.5% or 15% of wheat flour increased their antioxidant capacity, content of lysine and decreased the peroxide value (Watanabe et al., 2014). In spite of that, the addition of 15% of quinoa flour affected flavor and it was suggested that they were acceptable to the panelists. In another

research, the cookies added with amaranth flour up to 60% showed good acceptability (Chauhan et al., 2016).

The mixture of gluten free flours of amaranth, buckwheat, chickpea, corn, millet and quinoa was blended with rice flour to compare their effect on technological parameters of bread production (Buresova et al., 2017). Addition of buckwheat flour (30 g/100 g; 50 g/100 g of rice flour) improved bread quality, meanwhile addition of amaranth, chickpea and quinoa flours affected the aroma and taste of breads, and addition of millet and corn flour deteriorated dough and bread quality.

Recently, a lot of research was done connected with usage of acorn flour in bread preparation. Acorn flour is rich in bioactive compounds, antioxidants and can be used as a gluten free material for bakery (Martins et al., 2020; Pasqualone et al., 2019b). Acorn flour added in the quantity of 23% and 35% of the flour mixture which consisted of buckwheat and rice flours showed good technological properties and its usage could improve bread nutritional and sensory characteristics (Martins et al., 2020).

Trend 8. Seed oil in food production

Application of a mix of grape seed oil (0%, 5%, 10% and 15%) and 2% rice bran fiber was proposed for partially substituting the pork fat content in meat batter. The samples with increasing content of grape seed oil had not only reduced content of the animal fat, but were characterized with lower loss during cooking emulsion stability, and apparent viscosity (Choi et al., 2010).

Grape seed oil was also proposed to be used in preparation of meat emulsion. Grape seed oil emulsified in combination with gelatin and alginate was used for partial replacement of pork fat. This replacement improved the quality of meat emulsion and its health value (Kim et al., 2020). Grape seed oil demonstrated higher antioxidant activity than sunflower oil and olive oil. The addition of grape seed oil for 40% fat replacement in pork patties reduced the formation of heterocyclic amines during cooking without change of eating quality.

Trend 9. Dietary fibers in food production

Dietary fiber is the part of plant food that cannot be absorbed by humans and is resistant to enzymatic digestion in the human gastrointestinal tract (Yang et al., 2017). By their chemical structure dietary fibers with the exception of lignin are polysaccharides such as cellulose, hemicellulose, pectin, gums, and mucilage. All dietary fibers are divided in two groups: soluble (cellulose, hemicellulose and lignin) and dissolves in water to form gels (pectin, gums, and mucilage). The numerous studies were conducted to show positive influence of fiber intake and health benefit, mainly by the reduction of blood cholesterol, as well as improving of colonic function, and regulation of blood glucose (Dhingra et al., 2012).

Recommended daily intake of dietary fibre for adults in most countries is between 25 and 35 g (25–32 g/d for women and 30–35 g/d for men) and less for children and older adults (Stephen et al., 2017). Dietary fiber is recommended to be used in preparation of various functional foods like meat products, bakery, drinks, and beverages.

The addition of dietary fiber to meat products was proposed to increase their nutritional and healthy value as well as increase their acceptability and cooking yield (Talukder, 2015). A dietary fiber containing material recovered from the olive mill wastewater was proposed

to be added to meatball to improve their cooking properties and reduce fat content (Galanakis et al., 2010). Sausage is a very popular meat product but the content of fat in it is too high. Partial substitution of fat in sausages with dietary fiber is recommended (Ham et al., 2016; Souza et al., 2019; Yang et al., 2010). Addition of hydrated oatmeal in quantity 10% for partial replacement of fat in sausages resulted in improving of technological parameters such as cooking yield and textural properties and an increasing of low-fat sausages acceptability (Yang et al., 2010). Low-fat beef sausages were prepared with addition of water to replace fat and pineapple fibre was added in quantity of 1% to bind additional water in sausage formulations. (Henning et al., 2016). The textural parameters of low-fat sausages were lower that of the control, however, addition of pineapple fiber in combination with water is considered as a way to reduce lipid content in sausages, increase the dietary fibre component and improve the health value of product (Henning et al., 2016).

The by-product from the production of red wine from *Vitis vinifera* grapes in the form of flour with a high content of dietary fiber (40%) was used to replace 1, 2 and 3% of bacon in salami preparation (Mendes et al., 2014). This addition resulted in a significant increase in the nutritional properties of the produced salami. Some studies are referred to usage of dietary fibre in preparation of meat emulsions to improve their quality. *Makgeolli* lees, by-product of a production of traditional Korean rice and cereal wine, added as a source of dietary fiber to chicken meat emulsion system in quantity of 2%, improved its stability and viscosity and decreased cooking loss (Choi et al., 2010a).

The addition of dietary fiber to bakery products is widely used to increase their nutritional value, improve their acceptability and extend the shelf-life. The addition of soluble fiber, which was partially hydrolyzed guar gum, 3.4 g, and water, 36 mL, in 100 g flour for noodles preparation had a significant positive effect on textual properties and increased the soluble fiber content to 3.6% as compared to 1.1% in control noodles (Mudgil et al., 2016).

Banana peel is a waste which is produced in a huge quality as a by-product of the food processing industry. Meanwhile, it is a rich source of dietary fibre (43 – 50%), polyunsaturated fatty (linoleic α-linolenic) acids and micronutrients (Mohapatra et al., 2016). Banana peel powder was proposed to be added to chapati dough up to 20% (Kurhade et al., 2016). It was shown that addition of banana peel powder resulted in increase of dough stickiness and improved pliability. The substitution of wheat flour with 30% matured green banana flour increased total dietary fibre contents, content of minerals as well as antioxidant activity of noodles meanwhile the quality of noodle with banana flour was comparable to the control (Choo and Aziz, 2010). Stabilized rice bran flour with high content of dietary fiber (26%) was added at level of 5% to enrich the pizza dough (De Delahaye et al., 2005). The sensorial evolution showed acceptability of enriched pizza dough and ensured it stability during 60 days at −18 °C.

Incorporation of food grade orange fiber extracted from orange juice by-product, consisted from peels and seeds, was tested as a fat-replacer at different percentages (30, 50, and 70%) in brioches, bakery confectionery products (Caggia et al., 2021). It was shown that brioches with 50% fat substitution had lower fat content, increased content of dietary fiber and improved technological properties.

New coffee by-products, silverskin extract, have been proposed to be used as a source of dietary fibre and bioactive compounds in preparation of developed novel food products (Figure 3) (Iriondo-DeHond et al., 2020).

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A **B** **C** **D**

Figure 3. Products with coffee silverskin extract:
(A) beverages from Arabica and Robusta;
(B) wheat bread slice;
(C) gluten-free bread;
(D) biscuits
(Iriondo-DeHond et al., 2020).

There are some studies proposed additions of dietary fiber in dairy products, mainly in yogurts. Among the sources of fiber proposed to be added in yogurts there were oat fiber, wheat bran, pineapple and pina colada, fibers from apple, wheat, bamboo, asparagus, guar gum, orange fiber and date fibre (Hashim et al., 2009). It was shown that addition of fiber in yogurt may increase its health value, but can affect yogurt's technological characteristics and acceptability.

Trend 10. Novel edible coatings materials of plant origins

New trend in food production is an application of edible coating of different food product (fruits, vegetables, meat, fish, bakery, and snacks) to protect their quality, create a barrier against moisture lost and gas diffusion, prevent the loss of natural volatile flavour compounds and color components, delay enzymatic and microbial spoilage and extent shelf life (Sapper and Chiralt, 2018). Edible coating materials could be directly applied by spraying or dipping method over the food product to obtain a thin protective layer, but the preferred technique for edible coating is dipping. Materials for development of edible coating include polysaccharides such as starch, alginate, cellulose, chitosan, pectin, edible biopolymers pullulan and carrageenan, and plants gums; peptides such as gelatin, collagen, gluten, casein, and lipid-based materials, mainly different waxes (Suhag et al., 2020). Natural antioxidant and antimicrobial agents have also been incorporated in the edible film to increase the protection properties of food products and extend their shelf life.

Some examples of edible coating applications are shown below. Coating with 1% chitosan helped to maintain strawberries quality during storage (Yan et al., 2019). Arabic gum and almond gum were proposed to be used as novel edible materials for coating vegetables and fruits. The ripening process of semi-matured green tomatoes treated with aqueous solution of gum arabic, 1.5%, was delayed during their storage at 32°C and 35- 42% relative humidity for 28 days without changes in postharvest quality (Krishnadev, 2017). Coating of sweet cherries with 10% almond gum or arabic gum delayed their ripening during their storage at 2ºC and 90–95% relative humidity for 15 days and extended the shelf life (Mahfoudhi and Hamdi, 2015). Coatings with the guar gum, 0.15% (m/v), added with calcium chloride, 0.1% (m/v), glycerol, 0.1% (m/v), and ginseng extract, 1% (m/v), helped

to maintain the quality of sweet cherry and extend sweet cherries' shelf life during storage at 20 °C and 70–75% relative humidity for about 8 days (Dong and Wang, 2018).

Edible films can be used as an alternative for prolongation of the shelf life of fish. Coating with gelatin-chitosan film incorporated with 4% of oregano essential oil was proposed for fish preservation (Wu et al., 2014). Biodegradable chitosan, 2% (wt./vol.), coating incorporated with black pepper essential oil, 1.5% (wt./vol.) was used for common carp fillet coating to extend its shelf life at refrigerated storage at 4°C (Moosavi-Nasab et al., 2016). Rosemary essential and extract oil, 2000 pm and 200 ppm by total phenol basis, respectively, were entrapped in carboxyl methyl cellulose to be used as edible coating for smoked eel (Choulitoudi et al., 2017). Application of this coating retarded oxidation processes in eel and showed antimicrobial activity against bacteria *Pseudomonas* spp. and lactic acid bacteria.

It was shown that coating reduced microbial load of the samples, improved fish fillet quality with the increasing of shelf life from 8 to 16 days. However, there is no data according acceptability of this coating fish. An edible gelatin films added with carvacrol, a monoterpenoid phenol presented in essential oils in many spices, was used to wrap of prefried breaded hake medallions (Neira et al., 2019). It was shown that the medallions wrapped in film can be cooked and consumed without removing the packaging and acceptance by the consumer was good.

Application of a new edible coating of pectin containing clove essential oil was proposed for treatment of bream fillets for extension of shelf life during refrigeration improved water holding capacity, color, and texture of the fillets, decreased weight loss, lipid oxidation, number of Gram-negative bacteria, meanwhile lactic acid bacteria were not affected (Nisar et al., 2019).

Dry fermented sausages were coated with chitosan added with essential oil of oregano for protection from lipid oxidation during seven months of storage. It was shown that coating sausages had better odor and flavor at the end of the experiment (Krkic et al., 2013). Biodegradable film contained gelatin and incorporated in it 7.5% microparticles of powder papaya peel as natural antioxidant was used as packaging material for lard. It was shown that this packing material was effective as active barrier with high antioxidant activity and was recommended as environmentally friendly packaging of fat products (de Moraes Crizel et al., 2018).

Coating of potato chips was conducted using almond gum concentration 20 g/L, 75 s frying time at 160 °C frying temperature (Bouaziz et al., 2016). It was shown that coating decreased the oil uptake of potato chips by 34% and improved their sensorial qualities such as color, appearance, crispiness, taste, odor which resulted in chip's better acceptability.

It was strongly recommended to incorporate berries into edible films. Thus, blackberries particles with high content of anthocyanin incorporated in arrowroot starch ensured the presence of bioactive compounds and antioxidant capacity of protective film (Nogueira et al., 2019).

Conclusions

Application of novel natural additives, such as plants with pharmaceutical properties, plant materials with antioxidant activity, essential plants oils, extracts from plant materials seaweeds, seeds products, different gluten-free cereal and pseudo-cereals flours for replacement of gluten in bakery products, dietary fibers, edible coatings materials of plant origins, in preparation of different food products is widely studied in recent 10 years. The

main object of these researches is improving the health value of traditional products, saving or improving their quality and acceptability by consumers as well as an extension of shelf life.

Despite evident beneficial effects, careful examination of new products should be done in every case to establish an optimal dosage of additives, to prove its safety and evaluation of possible side effects on human health.

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Study on antioxidants extraction from oak bark and their use for oxidation stability of sunflower oil

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Abstract

Introduction. Food industry requires the antioxidants obtained from natural raw materials. It is important to establish the methods for incorporation of hydrophilic antioxidants to oil and fat products.

Materials and methods. Oak bark was used as the raw material for the obtaining of antioxidants. The aqueous-ethanol solutions, alkali and acid addition, and microwave treatment were used for flavonoid extraction. The dry substances content in the extracts was determined gravimetrically. The kinetics of sunflower oil initiated oxidation was studied using a volumetric method in the presence of azobisisobutyric acid dinitrile.

Results and discussion. Solvents with different ratios of water: ethanol were used to obtain extracts form oak bark. Application of higher ethanol concentrations in solvent resulted in increase of extract concentration. The extract concentration increased by 45% with an increase of ethanol concentration from 50 to 80%. Addition of 1% ascorbic acid increased the yield of extractive substances from 2.3 to 3.1%; addition of 1% citric or lactic acid increased the yield up to 4.3 and 3%, respectively.

The microwave extraction does increase the extraction rate by 8 times and the yield of extractive substances by 1.5 times (from 2.3% dry substances in extract to 3.5%).

The water-alcohol extracts added to sunflower oil together with the emulsifier dispersed it to particle sizes not more than 2–3 μm. Water and alcohol were removed by distillation under reduced pressure. After evaporation of solvent, the diameter of the hydrophilic phase particles decreased to nano-size.

The dispersion of oak bark extracts in sunflower oil resulted in a 1.8-fold increase of the period of induction of sunflower oil oxidation, determined by the kinetics of accelerated oxidation and the accumulation of peroxides during the storage.

Conclusions. The use of food acids in combination with a water-alcohol solvent and a microwave treatment effectively increased the extraction of antioxidants from oak bark.

Introduction

Fats and oils are the products that have high rate oxidative deterioration due to the presence of unsaturated fatty acids (FA) (Javidipour et al., 2016). The interaction of fats with oxygen results in formation of a number of oxidation products (peroxide and hydroperoxide compounds, aldehydes, ketones, acids, etc.), which are considered to be toxic substances and they should not be present in food products. Rate of fats oxidation depends on many factors: degree of FA unsaturation, ions of polyvalent metals content, storage temperature, oxygen access, moisture content, etc (Xu et al., 2017). The main way to inhibit the rate of fat oxidation is using of antioxidants (Großhagauer et al., 2019).

Currently there is a number of antioxidants discovered in the world (Oswell et el., 2018). However, the list of healthy and officially legalized antioxidantsis relatively short. When the antioxidants are used in medicine, pharmacy, or food technologies they have to satisfy to special requirements. Ionol, butylhydroxytoluene, propyl gallate, tocopherols, carotenoids are the most commonly used antioxidants in oil and fat industry (Gokoglu et al., 2006). Recent studies have shown that using of synthetic antioxidants can affect the human health (Morteza-Semnani et al., 2006), thereafter there is a need to search and implement the antioxidants obtained from natural raw materials (Olszowy et al., 2019). Perhaps the largest group of natural antioxidants is flavonoids, the most common group of polyphenolic compounds (Cisneros-Yupanqui et al., 2020).

Flavonoids are not synthesized in animal or human cells, and their presence in tissues depends entirely on plant foods consumption. It is proven that when the flavonoids are present in the human body, they are included in numerous processes of cell metabolism, gene expression and even protect the body against parasites and infections (Durazzo et al., 2019). They play a significant role in prevention of cardiovascular disease and cancer Durazzo et al., 2019). Currently, the antioxidant effect of flavonoids obtained from different natural sources has been proved (Huyut et al., 2017). Therefore, the task of developing a variety of products, which contains flavonoids, is actual for the food industry. Flavonoids are phenolic compounds of different structure (known about eight thousand representatives) (Huyut et al., 2017), which are characterized by various physical and chemical properties like solubility in polar and nonpolar solvents. Therefore, despite the variety of known methods for flavonoids extraction, the easiest and the safest method is to extract them with two “edible” solvents of different polarity: water and ethanol. However, the amount of solvents and the extraction time are too high in this process. In addition, the alcoholic solutions with ethanol concentration less than 50% v/v cannot avoid oxidation processes in phenolic extracts during extraction (Shi et al., 2003). Therefore, the optimization of the flavonoids extraction process from plant and other raw materials have to be developed.

On the other hand, the positive physiological effect of flavonoids, their effectiveness as antioxidants makes these compounds essential for the shelf life increase of oil and fat. But the problem is that the extracts of flavonoids or polyphenolic compounds are hydrophilic and insoluble in oils or fats, so their application is currently limited to water-soluble systems or emulsions (Belščak-Cvitanović et al., 2018). Thus, it is necessary to develop the technology of injection of such hydrophilic antioxidants to the fat.

In addition, the high prices of flavonoid extracts limit their using (Small et al., 2016). Therefore, oak bark was chosen as the cheap and common source (Skrypnik et al., 2019) for this study.

The aim of this work was to optimize the of water-alcohol extraction of flavonoids from oak bark and study the inhibition of sunflower oil oxidation by concentrates of oak bark antioxidants.

Materials and methods

Antioxidants extraction from plant raw materials

Oak bark was chosen as a raw material for antioxidants obtaining. It is a well-known source of flavonoids. Oak bark usually contains catechinic tannins (0.4%), free gallic and ellagic acids, galothannins (10–20%), quercetin, flobafen, resins, pectin substances (6%), sugars (levulosin and others), proteins, starch and minerals.

Oak bark was crushed using a laboratory mill RRH – 100 (Ukraine) at 28 000 rpm, after that the samples were sieved through a laboratory sieve with mesh size of 1 mm. The samples were stored at (-10)° C during four weeks (Ushkalova et al., 1016). Water and ethanol (96% vol.) mixtures were used to extract flavonoids. The dry oak bark in a mixture of solvents (at ratio of 1:3), respectively, was stirred at 100 rpm and (45±5) °C during 2 hr. The extracts were filtered and the dry substances content was determined gravimetrically.

Microwave extraction

For microwave extraction the microwave chamber with the 2.450 GHz frequency and 300 W power was used (Li et al., 2011). The extraction was carried out during 10–100 min in the flask attached to the backflow condenser. The extracts were filtered and the dry substances content was determined gravimetrically.

Influence of pH on the extraction process

Sodium hydroxide or edible acids (ascorbic, citric as a powder and lactic as 40% solution) were added to the aqueous-ethanol mixture to adjust alkali or acid medium.

The flavonoids extraction was carried out according to the above mentioned procedure.

Determination of sunflower oxidation rate

Fully refined and dewaxed sunflower oil was used as a model system to study the inhibitors influence on oils and fats oxidation, since it tends to oxidative deterioration due to high content of unsaturated fatty acids.

The sunflower oil oxidation rate was studied by a volumetric method (Ghosh et al., 2019) in manometric device (Varburg type). The 5 ml reaction chamber was thermostated, equipped by manometer, source of oxygen (99%) and vacuum pump. At the beginning of the measuring the air was displaced by oxygen (the operation was repeated 5–8 times). The volume of oxygen absorbed by the sample was measured according to changes of level of stained fluid in a graduated tube connected with a reaction chamber.

The research was carried out at 70 ℃ under conditions of initiated oxidation. For oxidative reaction 2 g of oil mixed with 3 ml xylene and 0.3 ml of 0.1 mol/L solution of oxidation initiator azobisisobutyric acid dinitrile (AIBN) in xylene. Reaction mixture was blew by oxygen during 1 min and thermostated at 70 °C during 10 min before measurements. Oxidation curves at 70 °C were measured as volume of absorbed oxygen and plotted in coordinates: heating time (t, min) − height of absorbed oxygen column (H, mm).

Induction periods of oil oxidation were calculated from the curve of oxidation by the graphical method (Ghosh et al., 2019) as a segment of the abscissa axis cut by a perpendicular from the point of intersection of the tangents to the kinetic curve.

Periods of induction of sunflower oil oxidation without the addition of antioxidants and with antioxidant extract from oak bark were calculated.

The antioxidant activity was estimated as efficiency of the oxidation inhibition of sunflower oil and calculated according to:

$$\mathrm{AOA} = \frac{\tau_{\mathrm{i}}}{\tau_{\mathrm{s}}},$$

where $\tau_{\mathrm{i}}, \tau_{\mathrm{s}}$ – the duration of induction period of sunflower oil with and without antioxidants.

In addition, the degree of sunflower oil oxidation rate was determined by changes of peroxide values (Xu et al., 2017). Two samples of refined, deodorized and winterized sunflower oil with and without addition of 2% of aqueous alcohol solution of oak bark extracts were stored in opened flasks for 50 days at 25 ℃ with free access of air and light. The peroxide value of oil was determined every 2 days. The kinetic curves of samples oxidation were plotted and the duration of the induction period of hydroperoxide accumulation was determined (Ghosh et al., 2019).

Determination of peroxide value

Determination of peroxide value of the extracted oil was carried out according to the standard IUPAC methods (IUPAC, 1987).

Particles size determination

The particles size of the obtained mixture "water-soluble antioxidant − oil" were determined under light microscope. The hydrophilic part was dyed with a water-soluble dye (methyl-orange). The maximum resolution of the microscope was adjusted as 0.2 microns.

Statistical analysis

Samples were analyzed in triplicate. Statistical analysis was performed using Microsoft Excel 2007 (Microsoft, City of Redmond, USA). The results were reported as mean±SD. Differences were considered to be significant at validity of α=0.95.

Results and discussion

Effect of ethanol concentration on the extraction of oak bark antioxidants

The extraction of oak bark under different water:ethanol ratio demonstrated that higher ethanol content in solvent had resulted in increase of extractive substances concentration (Table 1). The extract concentration had increased by 45% under increasing of ethanol concentration from 50 to 70%. Simultaneously, the induction period of sunflower oil oxidation with addition of 2% of extract had increased (Table 1).

Table 1

Influence of water: ethanol ratio on the extracts concentration and their antioxidant activity

№ of sample	Water:ethanol (96% v/v) ratio	Extract concentration, % of dry substances	Sunflower oil induction period of oxidation with addition of 2% of extract, min
1	50:50	1,57 ±0.014	46 ±2.8
2	40:60	1,95 ±0.042	50 ±3.5
3	30:70	2,30 ±0.035	57 ±1.4
4	20:80	2,27 ±0.021	52 ±1.4

Thus, the optimal parameters of antioxidants extraction from oak bark (Table 1): water: ethanol ratio 30:70, solids-to-solvent ratio 1:3, temperature – (45±5) ºC, duration – 2 hours. These conditions were chosen for the next experiments.

It was shown (Piccand et al., 2019) that there is a significant correlation between the composition of oak bark extract and its antioxidant activity. We have investigated the antioxidant activity of the sunflower oil under addition of the obtained oak bark extracts. The duration of induction period of oil initiated oxidation is commonly used as the measure of oil oxidation stability (18). The oxidation curve of sunflower oil with (sample 2) and without (sample 1) addition of 2% of oak bark extract is shown on the Figure 1. The induction period of sunflower oil initiated oxidation had been increased from 31 to 57 minutes under addition of extract, that proved the high antioxidant properties of obtained extracts (Table 1). The value of antioxidant activity with the addition of extracted from oak bark substances was AOA = 1.8. This result indicates the effectiveness of the extracted from oak bark antioxidants (Li et al., 2012).

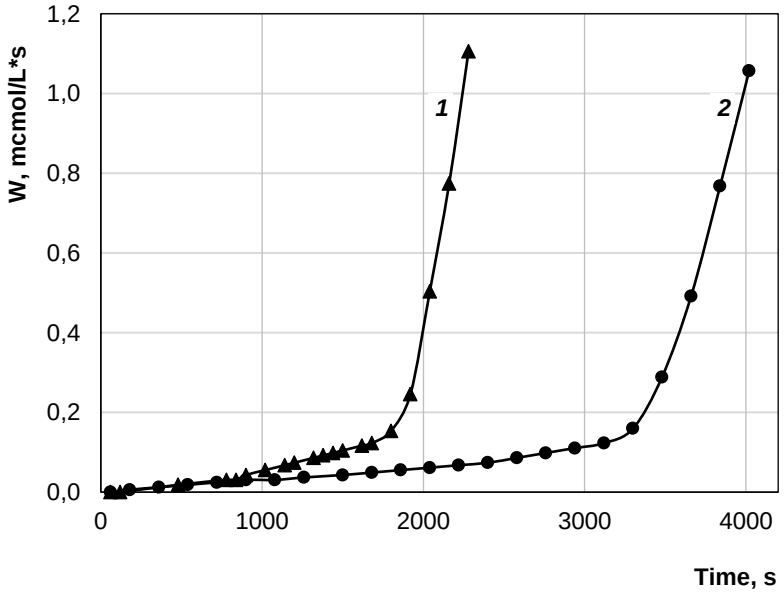

Figure 1. Absorption of oxygen by sunflower oil under initiated oxidation without (1) and with (2) addition of 2%oak bark extract

Effect of pH on the extraction of oak bark antioxidants

It was reported (Corbin et al., 2015) that using of alcoholic solution of sodium hydroxide had increased in the concentration of flavonoids in extracts compared to the "non-alkali" alcoholic solution.

Our results have shown that alkali solvent under extraction did not increase the extractive substances yield, their amount remained approximately at 2,3% (Table 2). Extracts, obtained in the alkaline solvents, did exhibit the antioxidant activity, mainly addition of 2% of the extract resulted in 52 minutes induction period of sunflower oil oxidation (for a sample with 5% of sodium hydroxide in extraction solvent). However, the increase in the pH of the medium did not enhance the efficiency of extraction of antioxidants from oak bark and inhibition of sunflower oil oxidation by the obtained extract.

Table 2

Influence of alkali addition on the extractive substances yield

Amount of NaOH (%) relatively to the mass of the oak bark	Yield of extractives (%) relatively to the oak bark sample weight
1	2,30±0.023
2	2,30±0.018
5	2,32±0.017
10	2,44±0.026

The effect of acid medium on the oak bark flavonoids extraction was studied also with using ascorbic, citric, lactic acids. Addition of food acids to a water-ethanol mixture had shown a positive effect on the process of oak bark extraction. The effect of the acid addition on the yield of the extractive substances and the induction period of the oil oxidation in the presence of the obtained antioxidants are shown in Table 3. It was demonstrated that increase of acid concentration had resulted in the higher extract concentration. Addition of 1% ascorbic acid (relatively to the oak bark) increased the yield of extractive substances from 2.3 to 3.1%, addition of 1% citric and lactic acid increased the yield up to 4,3% and 3%, respectively (Table 3). Further increase of food acids content also slightly increased the yield of extractive substances, but the maximum growth of extraction rate was observed when the content of food acids is close to 1%, so this content can be recommended for practice use. Citric acid (at all introduced acid concentrations) had the most prominent effect on the process of extract obtaining from oak bark. Addition of 1% citric acid had showed a highest yield of extractive substances (increased from 2.3 to 4.3%).

Table 3

Influence of acid addition on the oak bar extractive substances yield

Food acid	Yield of extractive substances (%) of oak bark			
	Amount of acid (%) relatively to the mass of oak bark			
	0,5	1	2	3
Ascorbic acid	2.6±0.034	3.1±0.033	3.2±0.024	3.35±0.022
Citric acid	3.1±0.029	4.3±0.040	4.5±0.044	4.8±0.036
Lactic acid	2.7±0.019	3.0±0.025	3.1±0.010	3.4±0.030

Our results are in accordance with the data obtained in (Halee et al., 2018) on the maximum yield of the flavonoids from colored rice under adding of citric acid to the solvent at concentration 0.1 mol/dm^3.

In addition, in the presence of antioxidant extracts, obtained in 1% acid water: ethanol solution, the induction period of sunflower oil oxidation was prolonged (Table 4). However, the highest effect on the prolongation of the induction period of sunflower oil oxidation had the extract, obtained with ascorbic acid. The induction period of sunflower oil oxidtion with addition of the extracts, obtained in acid medium, changed as follows: lactic acid − from 52 (extract without acid, extractives content 2,3%) to 60 min (extract containing lactic acid, extractive content 3%), citric acid − from 52 to 70 min, ascorbic acid – from 52 to 80 min. This effect is probably due to synergic effect of ascorbic acid that itself is an antioxidant and is able to inhibit the oxidation of fats (Wang et al., 2019).

Table 4

Influence of acid oak bar extracts sunflower oil oxidation

Food acid	**Sunflower oil induction period of oxidation in the presence of 2% of extract (1% of food acid), min.**	**Antioxidation activity of the extracts**
Ascorbic acid	78±3,1	2,5
Citric acid	70±1,3	2,2
Lactic acid	60±2,5	1,9

In general, all studied food acids significantly increase the yield of extractive substances and extend the shelf life of fat. Increasing induction period of sunflower oil oxidation by extracts of antioxidants containing ascorbic acid by 2.5 times (Table 4) or citric acid – by 2.2 times indicates the suitability of extraction of antioxidants in the presence of these substances (23). This approach to antioxidant extraction is the most promising due to its simplicity, low cost and safety.

Influence of the microwave treatment on the extractive substances yield from oak bark

The well-known method of extraction acceleration is a microwave extraction. It was shown (Li et al., 2012) that microwave treatment is an effective method to destroy the cell walls without damage of the plant substances. The main advantage of microwave extraction is significant reduce of the extraction time: from seconds to 15–20 minutes compared to 1.5–6 hours for traditional extraction. It was shown that the properties of biologically active substances are preserved and their content in the extract is higher comparing to traditional extraction (Li et al., 2012).

Study of the influence of microwave extraction time on the extractive substances yield had shown that yield maximum had reached after about 15 min extraction. There was not substantial increase of extractive substances yield from 15 to 100 min (Figure 2). Thus, there is no need to carry out extraction under microwave irradiation longer than 15 minutes. The microwave extraction does increase the extraction rate by eight times (2 hours for a traditional method vs. up to 15 min. Solids-to-solvent ratio and solvent composition remained the same). Microwave extraction also increased the yield of extractive substances by 1.5 times (from 2.3 to 3.5% of dry substances in the extract). Therefore, the microwave treatment has positively influence on the extraction rate and the extraction yield.

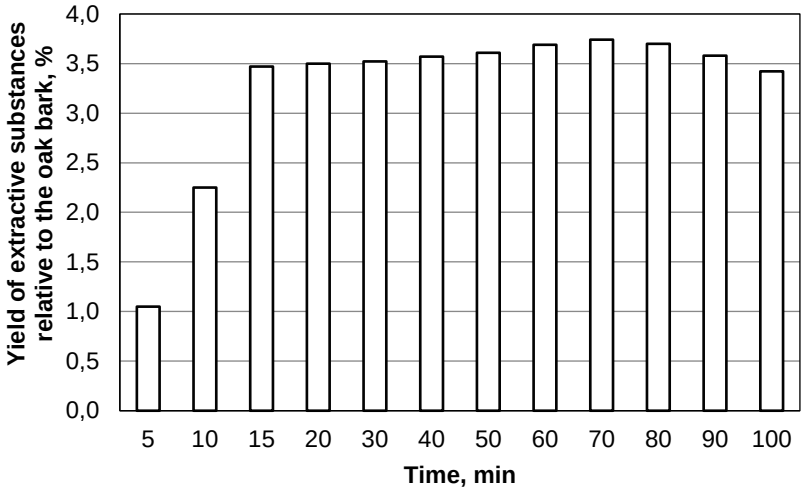

Figure 2. Influence of microwave extraction time on the extractives substances yield

Development of the method for stabilization of hydrophilic extracts in oil and fat

The extracts obtained are hydrophilic systems and are insoluble in fats. It is impossible to form a stable emulsion of water-alcohol extracts in oil and fat (Castro et al., 2020) and, consequently, the extracted antioxidants are not able to protect fats and oils from oxidative deterioration during storage.

Therefore, we had forwarded the working hypothesis of the possibility to obtain a stable system by dispersion of aqueous-alcoholic antioxidant solution in the oil phase with a particle size up to 100 nm. Such systems with an extremely high degree of dispersion are known as ultradisperse systems (the particle size in such systems is in the range from 1 to 100 nm) (Castro et al., 2020). Recently numerous studies have shown that under transition from micro- to nanoparticles the qualitative changes of number of physical and chemical properties of systems, including their solubility, occur (Castro et al., 2020; Summ et al., 2001). This happens due to the fact that for particles which dimension at least one of the directions are comparable or smaller than the correlation radius of any physical or chemical property (for example, the size of the new phase nucleation), the dimensional effects occur. These specific properties are the basis for considering the ultradisperse state as the fifth state of the matter.

To obtain the ultradispersed system the water-alcohol solution of flavonoids was added to the oil or fat together with the emulsifier, the system was dispersed to particle sizes not more than 2-3 μm. The water and alcohol had been distillated under reduced pressure. The antioxidant molecules remain in the volume of the fat. They are dispersed so tightly that they cannot be removed from the hydrophobic system. As a result, after evaporation of solvent molecules, the diameter of the hydrophilic phase particles were decreased to nano-sizes.

It was shown that the size of the particles of the emulsion (water-alcohol solution of antioxidant − oil) was 2–3 μm. The size of the antioxidant particles after water and alcohol evaporation was determined mathematically: the content of antioxidant (flavonoids) in the aqueous alcohol solution was approximately 3%, so, after evaporation of the solvents, the

particles diameter of the dispersed phase decreased by more than 2 orders and was equal to the value less than 100 nm.

It is known that when the size of the disperse system's particles is reduced, two opposite processes compete. They are dispersion and aggregation of the particles. It was noted already that ultradispersed systems are characterized by a highly developed interphase surface and, respectively, a significant excess of the Helmholtz energy ΔF_s, i.e. they tend to aggregation (Castro et al., 2020; Summ et al., 2001). The aggregation of particles will also occur during storage of the disperse system. Emulsifiers are commonly used to maintain the stability of the obtained disperse system during storage (Cassiday et al., 2016).

In this study it was proposed to use the emulsifier E 471 (mono- and diglycerides of fatty acids) in order to reduce energy consumption on dispersion and to increase the dispersion ability of water-soluble antioxidants.

We had not observed the gravitation segregation of the resulting systems during the whole period of their storage (2 months). The rate of hydroperoxide accumulation in sunflower oil under addition of oak bark antioxidant nanoparticles had decreased dramastically. The oxidation kinetics for refined deodorized sunflower oil during storage without and with addition of flavonoids (2% of aqueous-alcoholic solution relatively to oil) are shown in Figure 3. The induction period of sunflower oil oxidation was 21 days (without antioxidant) and 37 days (with antioxidant) that is, the oil's shelf life increased by 1.8 times, that correlates with another method of oxidation kinetics (data given in Figure 1). Similarly, it was reported a 2.5-fold increase in the period of biodiesel (that is methyl esters of fatty acids) oxidation induction with the addition of green tea leaf extract (one of the most flavonoid-rich plant materials) (Bharti et al., 2020).

In addition, fats usually contain natural oxidation inhibitors − tocopherols (Jung et al., 1990). It was previously shown a synergic effect of tocopherols and flavonoid extracts (Demidova et al., 2016), that also indicates that injection of flavonoid extracts is reasonable to inhibit the oxidation of oil and fat products.

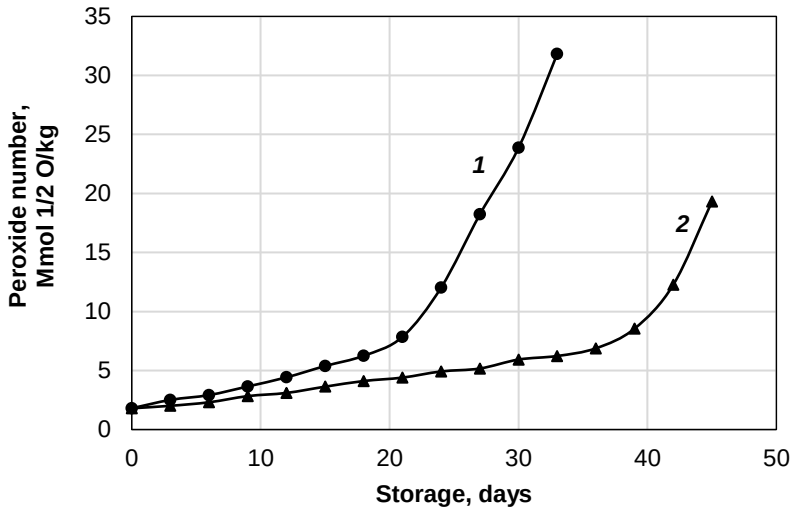

Figure 3. Oxidation rates of refined deodorized sunflower oil without (1) and with (2) addition of the developed antioxidant extracts according to the peroxide values

Conclusions

Suitable conditions for polyphenols extraction from oak bark were established in this study in order to use them as inhibitors of vegetable oil oxidation. It was shown that polyphenols of oak bark were effective as natural inhibitors of oil oxidation that makes these compounds promising to use in various fat-containing foods. The mixture of extractives of oak bark and ascorbic acid has the most significant effect on prolongation of sunflower oil shelf life.

The use of food acids in combination with a water-alcohol solvent and microwave field were effective for the enhancing of the extraction process of oak bark. The method of creation of the stable fat and hydrophilic antioxidants containing system was developed. Its efficiency was proved by the significant prolongation of induction period of sunflower oil oxidation during storage.

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Modification of potato starch with adipic acid and research of modification product as raw materials for food biodegradable packaging

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Abstract

Introduction. The aim of our work was to study the modification of potato starch with adipic acid chloride in order to further physicochemical study of the obtained product and use, for example, as an effective film former of biodegradable films/coatings.

Materials and methods. Potato starch, adipic acid (E 355), thionyl chloride. Solvents – DMSO, DMF, methanol, ethanol. IR spectroscopy was performed on a Nexus-475 Nicolet device. NMR spectra were recorded by NMR spectrometer Mercury, VARIAN. X-ray diffraction analysis was carried out by the DRON-3M device in CuKα emission with Ni filter; U = 35 kV, I = 20mA; counterdisplacement angle $\Delta 2\Theta$ is 0,04°; time of intensity reckoning is 3 s. Thermogravimetric study was performed on the device Q-1500B.

Results and discussion. There are no signals of the acyl chloride group (1785–1815 cm^{-1}) and signals of hydrolysis products of adipic acid chloride, namely the fluctuation band of carboxylic acid group $\nu C = O$ (1750-1770 cm^{-1} and their salts 1640 cm^{-1}) in the IR spectrum This gives reason to believe that the crosslinking of glucopyranose rings occurred due to the reaction of both acid chloride groups. The elemental analysis results of acylated potato starch with adipic acid chloride are as follows: found Carbon 42.33%, Hydrogen – 6.65%; $C_{54}H_{86}O_{42}$. Calculated 41.96% for Carbon and 6.58% for Hydrogen. The thermographimetric analysis results indicate that the modification of potato starch with adipic acid chloride causes a change in the shape and amount of potato starch water. NMR research did not determine the degree of starch glucopyranose chains crosslinking and the position of the substituent. The native starch X-ray showed that the degree of native starch crystallinity is 12%. Modification of potato starch with adipic acid reduces the degree of crystallinity to 5%. In addition, the destruction of the primary (crystalline) structure of the starch grains was also confirmed by optical microscopy.

Conclusions. The modified product has a number of different characteristics from the original product. The use of starch as a natural substance that is capable of biodegradation makes it possible to is recommend the resulting product as a raw material for environmentally friendly packaging materials.

Introduction

Modification of starch allows to change its physical and chemical properties and to create new materials with the set properties (Kryazhev V. N. et al., 2010). Starch, modified chloride, phosphate, sulfuric acid, acetic anhydride, acetic and succinic acid, potassium hydroxide, ammonium persulfate propionic acid chloride (Hui Chi et al. 2008) acetyl chloride malic acid (Shulga O.S. et al., 2018) etc. are produced industrially (Shulga O. et al. 2018).

A significant amount of work concerns the interaction of starch different types (Bhosale R. et al., 2006) with fatty acid chlorides (Fathi F. et al., 2014; Junistia L. et al. 2008). In addition, materials based on native and modified starch are biodegradable and are therefore used as environmentally friendly packaging materials (Suvorova A. I. et al., 2000). It is important to expand the range of raw materials for environmentally friendly packaging materials (Chorna A. I. et al., 2016).

The modification reaction occurs, as a rule, due to the interaction of starch with anhydrides and acid chlorides in organic solvents – pyridine, toluene, dimethylformamide, N, N-dimethylacetamide (Fan, Y. et al., 2020). However, organic solvents are toxic and difficult to remove from the final product, which prevents its use in the food and pharmaceutical industries. Some authors (Shulga O. S. et al., 2017) propose a method for the synthesis of esters of fatty acids of starch without solvents as the most effective, but this method has its drawbacks (incomplete interaction, reaction by-products remain in the reaction mixture, poor crystallization of reaction products, etc.).

According to the literature data (Kryazhev V. N. et al., 2010), most of the proposed schemes for the polysaccharides modification are multi-stage, complex, require expensive and toxic reagents, which is unacceptable for the food industry. All of the above complicates the implementation of new modified starch derivatives. Thus, the starch modification potential is far from exhausted.

A convenient method for modifying starch with adipic acid chloride using dimethyl sulfoxide as a solvent is proposed (DMSO). This effective solvent, which is widely used in organic synthesis, has a number of advantages, the main of which is its complete solubility in water, which allows you to completely remove its residues after the reaction, in addition, DMSO has low toxicity, is used as a drug in pharmaceuticals, for example, as part of the anti-inflammatory drug “Dimexid”.

Therefore, the work in the field of starch modification is relevant and continues in order to obtain starch with the specified technological properties. The issue of improving the modification method, which should not be complicated and cost-effective, is also relevant.

The modification of natural polymers has a positive effect on the properties of the polymer as a raw material for biodegradable materials based on previous research (Aburto J. et al., 1999).

The *aim* of our work was to study the modification of potato starch with adipic acid chloride in order to further physicochemical study of the obtained product and use, for example, as an effective film former of biodegradable films/coatings.

Materials and methods

Materials

The research subject is modificaced potato starch. In order to make modifications were used potato starch, adipic acid (E 355), thionyl chloride, solvents – DMSO, DMF, methanol, ethanol.

The starch was prepared as follows: 100.02 g of potato starch was placed in a 500 cm^3 flask, connected to a water jet pump and heated on a boiling water bath for 8 hours. Product yield: 88.00 g (loss due to drying 12%).

Preparation of DMSO

300 cm^3 of DMSO was kept over calcined at the temperature of 400 °C CaO for 2 days. Next, DMSO was transferred to a 500 cm^3 flask and 30 cm^3 of dry benzene was added. Residual water was distilled off at atmospheric pressure as an azeotrope with benzene, then DMSO was distilled in a vacuum water jet pump.

Synthesis of adipic acid chloride

29.2 g (0.2 M) of adipic acid were placed in a 100 cm^3 reactor equipped with a heated magnetic stirrer, a dropping funnel, a thermometer and a reflux condenser with a gas meter and a gas discharge tube. 53.5 g (0.45 M) of thionyl chloride were added in small portions with stirring through a dropping funnel. Calm gas evolution and slight heating of the reaction mass were observed. After 1 h, three drops of DMF were added, and the temperature was maintained at 50–60 °C for another 5 h. Excess thionyl chloride was distilled off in a vacuum water jet pump. Obtained 34.6 g (94.5%) of product, which was used without further purification.

Esterification of starch with adipic acid chloride

9 g (0.05 M based on glucose) of dried starch and 300 cm^3 of dried DMSO were placed in a 500 cm^3 three-necked reactor equipped with a heated magnetic stirrer, a dropping funnel, a thermometer and a reflux condenser with a gas meter and a gas discharge tube. Stirred for 3 h at 70 °C to form a clear colorless gem. Next, heat was removed and 0.5 g of K_2CO_3 (approximately 2% by weight of starch) was added. 27.5 g (0.15 M) of adipic acid chloride were added dropwise with stirring. The reaction mass thickened, after 1 h the reaction mass was a very thick gem of light yellow color. The mixture was heated with stirring for another 2 h for temperatures 50–60 °C. The reaction mixture was left overnight at room temperature. Then 200 cm^3 of methanol was added and stirred vigorously for 1 h. The precipitated white precipitate was filtered off under vacuum with a water jet pump and washed with distilled water (2x50 cm^3) and ethanol (2x50 cm^3). The resulting product was dried in air at room temperature.

Research methods

FT-IR. Infrared studies conducted on the device Nexus – 475 firm Nicolet, KBr tablet (Chung, C. et al., 2004).

XRD. X-ray diffraction analysis was carried out by the DRON-3M device in CuKα emission with Ni filter; U = 35 kV, I = 20mA; counterdisplacement angle Δ2Θ is 0,04°; time of intensity reckoning is 3 s (Namazi H. et al., 2010).

TGA. TGA (Thermogravimetric Analysis) research was carried out by the Q-1500B device, at a heating rate of 20 °C/min (Prime R. B. et al., 2009).

NMR. NMR spectra were recorded by the Mercury NMR spectrometer, Varian, 400MHz in DMSO-d6 (Namazi H. et al., 2010).

Results and discussion

IR research

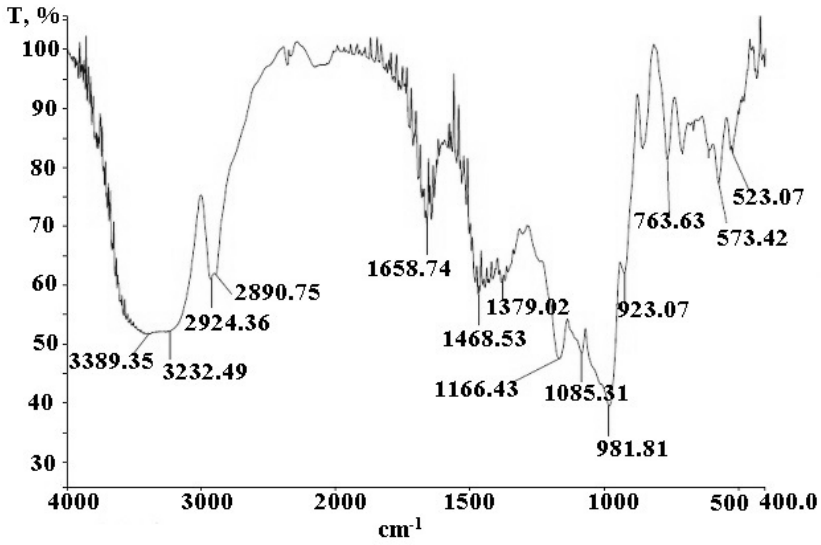
The changes that occurred as a result of the acylation reaction of potato starch with adipic acid chloride are confirmed by a number of factors. Thus, in the IR spectrum of modified starch (Figure 1 b) appeared a maximum at 1710.82 cm^{-1}, which is characteristic of C = O in the ester group, which appeared in the modified starch as a result of the acylation reaction.

In the spectrum of native starch (Figure 1) and there is a fairly wide intense band, which is at 3389.35 cm^{-1}, and in the spectrum of the modified sample (see Figure 1 b) the band lies in the region of stronger fluctuations at 3411.08 cm^{-1}, which is due with the degree of participation of the OH group in the formation of hydrogen bonds. The more hydroxyl groups involved in the formation of hydrogen bonds (native starch), the greater the range of their oscillations is shifted to a weak field. It is known (Silversteyn R. et al., 2011), that the position and nature of the band depends on the participation degree of the hydroxyl group in hydrogen bonds. In addition, when comparing the IR spectra of native and modified starches (Figure 1 a, b) it was found that the spectra have different fluctuations in the uncharacteristic region.

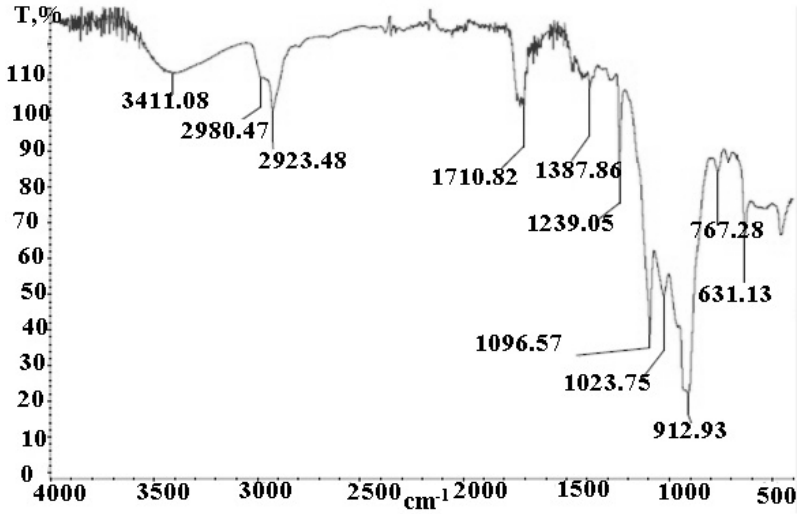
The IR spectrum of native starch (Figure 1 a) contains a number of oscillations in the uncharacteristic region, in particular 981.1 cm^{-1}, 763.63 cm^{-1}, 573.42 cm^{-1}.

When comparing these data, it is obvious that in the spectrum of native starch the oscillation frequency at 981.81 cm^{-1} increased and in the spectrum of modified starch lies at 1023.75 cm^{-1}, the band with an oscillation frequency of 763.63 cm^{-1} shifted to 912.93 cm^{-1}, and the band with an oscillation frequency of 573.42 cm^{-1} shifted to 631.13 cm^{-1}, indicating a change in the uncharacteristic region of the spectrum of the sample of native starch after acylation. There are no signals of the acyl chloride group (1785-1815 cm^{-1}) and signals of hydrolysis products of adipic acid chloride, namely the fluctuation band of carboxylic acid group νC = O (1750-1770 cm^{-1} and their salts 1640 cm^{-1}) in the IR spectrum. This gives reason to believe that there was a crosslinking of glucopyranose rings, due to the reaction of both acid chloride groups.

Therefore, according to the IR analysis results, the sample of native and modified starches have different chemical composition as a result of potato starch chemical modification with adipic acid chloride.



a



b

Figure 1. IR spectra of native (a) and modified (b) potato starch

Elemental analysis

The results of acylated potato starch elemental analysis with adipic acid chloride are as follows: found Carbon 42.33%, Hydrogen – 6.65%; $C_{54}H8_6O_{42}$. Calculated Carbon 41.96%, Hydrogen – 6.58%.

The results of IR spectra (see Figure 1) and elemental analysis show that the acylation of starch with adipic acid chloride occurred due to crosslinking of starch glucopyranose chains with adipic acid chloride at the ratio of adipic acid chloride one molecule.

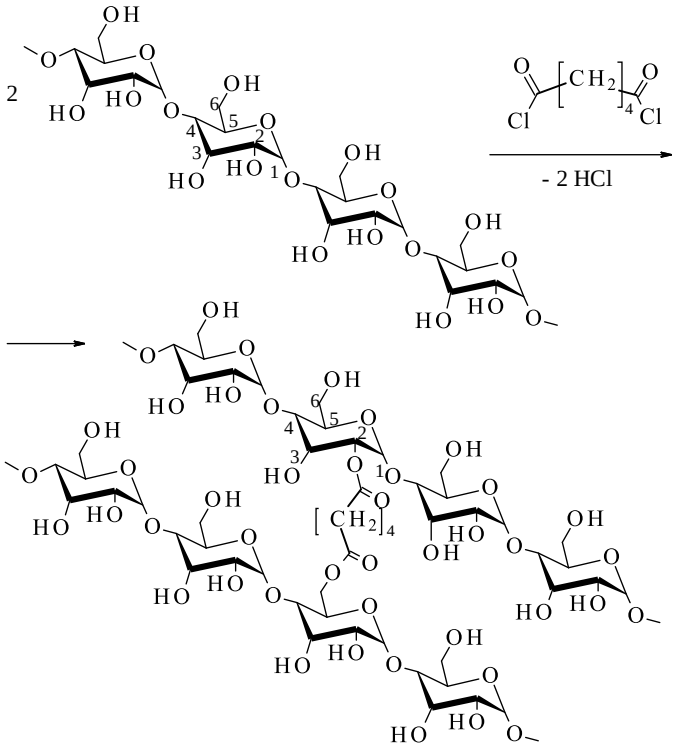


Figure 2. Structural formula of the potato starch modification product with adipic acid chloride

According to Patent by Vesa Myllymaki, Reijo Aksela the most easily acylated primary alcohol group and hydroxyl alcohol group at C_2. The authors (Junistia L. et al., 2008) prefer the hydroxyl group at C_2.

Thermogravimetric reaserch

If the crystalline to amorphous structure changes, the properties of the substance to bind and retain water can be expected to change. Using thermal analysis results obtained TGA are shown in Table 1.

Table 1

Zones of thermolysis of native and modified starches

Kind of starch	Thermolysis zones									
	I Adsorbed water		II Crystallization water		III				IV **Burning char**	
					Thermolysis of anhydrous products		Charring			
	t_s-t_f, °C	Δm, %	t_s-t_f, °C	Δm, %	t_s-t_f, °C	Δm, %	t_s-t_f, °C	Δm, %	t_s-t_f, °C	Δm, %
Native	**65-100**	**3.8**	**100-140**	**3.8**	**300-380**	**57.5**	**380-500**	**15.0**	**500-600**	**17.5**
Modified	60-100	4.9	100-240	13.3	240-370	46.5	370-500	13.8	500-670	21.5

t_s-t_f – temperature range of thermolysis zones

According to the results of Table 1 modified starch contains more adsorbed water by 1.1% due to the modification in an aqueous medium and it is due to this that the amount of crystallization water in the modified starch is 9.5% higher. During the modification, the starch is gelatinized, and if it is crosslinked, water is retained. It is due to the presence of crystallization water in the modified starch that the anhydrous products amount is less and, as a consequence, in the third thermolysis zone the anhydrous products amount is 10% less than for native starch. In addition, the thermolysis of anhydrous products begins at slightly lower temperatures of 240 °C, against 300 °C for native starch. Therefore, the potato starch modification with adipic acid chloride causes a change in the shape and amount of potato starch water.

NMR reaserch

There are 2 peaks, which are at 1498 ppm and 2,203 ppm (Figure 3) which indicates the glucopyranose chains crosslinking. The first peak at 1.498 ppm due to the methylene group appearance, which is in the α-position relative to the carbonyl group and as a result of the screen shifts to a stronger field. The second peak at 2.203 ppm is caused by other methylene groups, which also confirms the potato starch modification with adipic acid chloride. It was impossible to determine the glucopyranose crosslinking degree chains of starch, as well as to determine the substituent position by NMR spectra.

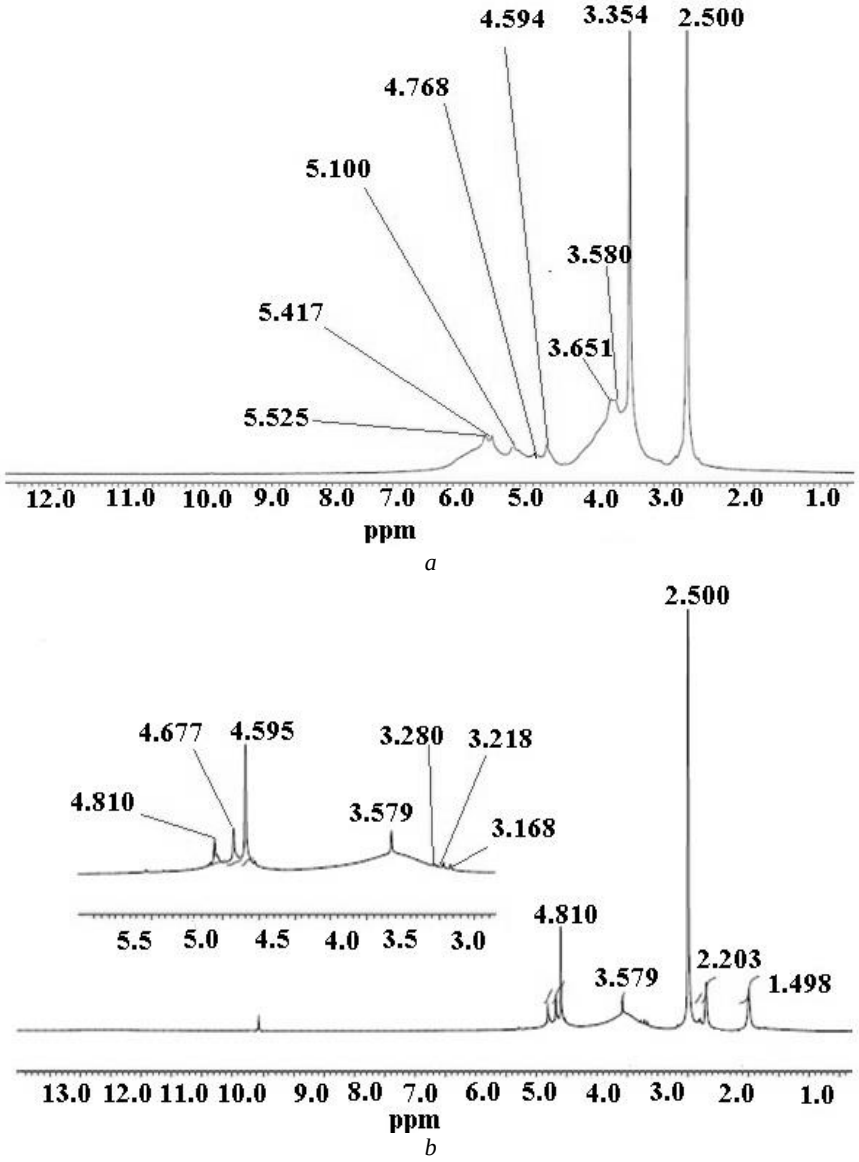


Figure 3. NMR spectra of native potato starch (a) and modified with adipic acid chloride (b) (*n=3, p≤0,05*)

X-Ray structural study

In order to determine the effect of the adipic acid chloride esterification process on the crystallinity of the potato starch structure. The obtained diffraction patterns are shown in Figure 4.

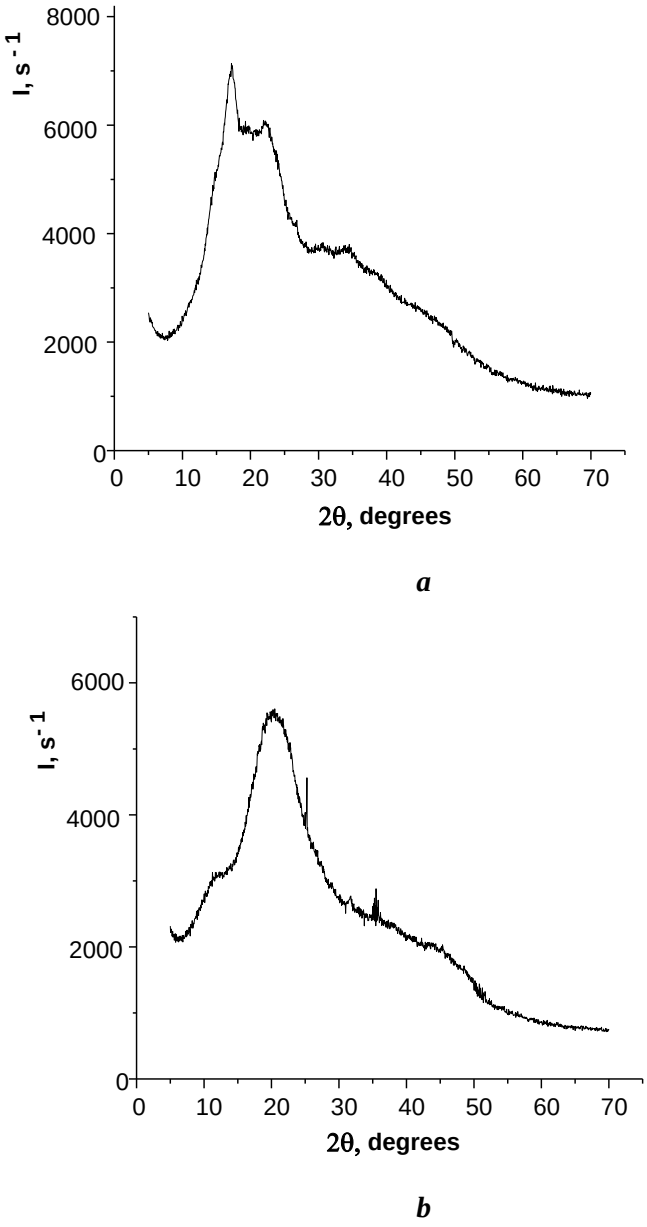
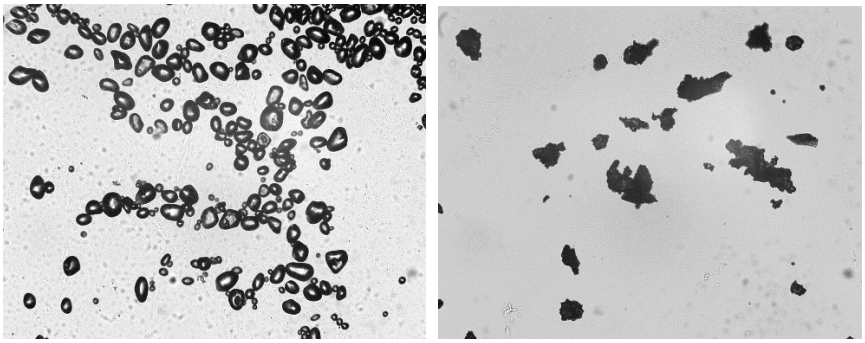


Figure 4. X-ray native starch (*a*) and adipic acid-modified chloride (*b*) (*n=3, p≤0,05*)

The native starch X-ray indicates its amorphous-crystalline structure with a crystallinity degree of 12%. The modified starch has an amorphous structure with the crystallinity of only 5%. In addition, the starch grains destruction primary structure was also confirmed by optical microscopy, as follows. Therefore, chemical modification with adipic acid chloride causes a decrease in the original starch crystal structure.

Microscope research

Native starch is in the grains form that can be seen under a microscope. For each species, depending on the origin, it has a different shape and size. Since in the process of modification there was a crosslinking of glucopyranose chains, so we can expect a change in the shape of the grains. The study results are shown in Figure 5.



a ***b***

Figure 5. The type of grains of native (a) potato starch and modified with adipic acid chloride (b) (×400)

Appearance (Figure 5) shows that the potato starch chemical modification by crosslinking, leads to the grains destruction of the initial shape. Therefore, the change in the external starch grains shape also confirms the chemical potato starch modification with adipic acid chloride.

Application of the obtained modification product

Starch as a natural polymer suitable for biodegradation (Lu D. R. et al., 2009; Versino F. 2016) is most widely used to create environmentally friendly packaging materials, using starch of different types: potato (Torabi Z. et al., 2013), corn, sago, cassava (Bangyekan C. et al., 2006; Bergo P. V. A. et al., 2009), oat (Galdeano M. C., 2013), pea (Han J. H., 2006), rice (Laohakunjit N. et al., 2004) or rice starch nanocrystals (Piyada K. et al., 2013), sweet potatoes (Mali S., 2002), bananas, mango (Romero-Bastida C. A., 2005), etc.

The proposed modification is due to the need to expand the range of raw materials (Shulga O.S. et al., 2017) for environmentally friendly packaging materials.

We have already carried out a number of natural polymers modifications (Shulga O. S. et al., 2016), which have given positive results in obtaining environmentally friendly materials from them (Fan, Y. et al., 2020). From the obtained product modifications will receive films that can be used for food packaging.

Conclusions

1. The potato starch modification with adipic acid chloride was performed, which was confirmed by elemental analysis.
2. There were changes in the frequency bands of native starch in the uncharacteristic region in the IR spectra: the frequency of oscillations at 981.81 cm^{-1} increased and in the modified starch lies spectrum at 1023.75 cm^{-1}, the band with a frequency of 763.63 cm^{-1} shifted to 912.93 cm^{-1}, and the band with an oscillation frequency of 573.42 cm^{-1} shifted to 631.13 cm^{-1}, indicating a change in the uncharacteristic region of the native starch after acylation spectrum.
3. NMR confirmed the crosslinking of glucopyranose chains as well as elemental analysis.
4. The obtained modified product properties were studied and it was found that the modification changed the moisture bonds shape with starch, the grains appearance, the crystallinity degree decreased from 12 to 5%.
5. The obtained modified product will be used as raw material for the production of environmentally friendly packaging materials.

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Effect of bioactivated amaranth grain on the quality and amino acid composition of bread

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Abstract

Introduction. This work is aimed at studying of baking properties and amino acid composition of bioactivated disintegrated amaranth grain as an ingredient for wheat and spelt bread making.

Materials and methods. Amaranth grain of Kharkivskyi-1 variety, soaked at the hydro module of 1:1 for 12–48 hours, was used to obtain bioactivated disintegrated amaranth grain. Consumer, sensory, physicochemical characteristics of wheat and spelt bread, and its protein biological value were analyzed at 15–25% of the semi-finished product substitution of wheat or spelt flour. Amino acid composition was determined by the ion exchange liquid chromatography.

Results and discussion. Incorporation of the bioactivated disintegrated amaranth grain (BDAG) into wheat and spelt bread formulations at 15–20% of the flour substitution led to increase in the specific volume of the loaves by 7–21% due to increased enzymatic activity and improved bioavailability of essential minerals during dough fermentation. The amaranth grain soaking should be 36 hours to achieve the improved sensory properties of the product. The complex quality of the wheat and spelt bread significantly depended on the flour substitution by the BDAG (56%, p= 0.007) and duration of the amaranth grain soaking (62%, p=0.038), respectively. The duration of the amaranth grain soaking had the dominant effect on the quality of the wheat bread and spelt bread with BDAG. Owing to biochemical processes, bioactivated disintegrated amaranth grain showed the improved amino acid score by 1.5–2.7 times for all essential amino acids, and 1.8–2.1 times increased content of essential and nonessential amino acids. Incorporation of 20% of the BDAG into the wheat bread formulation led to 2.4-fold increase of lysine content in the products, and the scores of essential amino acids reached up 133–213%. Bread with added BDAG contains 1.6–1.7 times more essential and nonessential amino acids. Utility of the protein followed the ascending order: wheat flour → amaranth grain → wheat bread → bioactivated disintegrated amaranth grain → wheat bread with bioactivated disintegrated amaranth grain. Protein biological value of the bread with BDAG increased up to 76%.

Conclusions. The duration of the amaranth grain soaking was the dominant factor influencing the bread quality, and providing a significant improvement in the protein biological value.

Introduction

Wheat flour and bread made of it have the imperfect amino acid composition, poor in lysine, and contain a small amount of essential macro- and micro-nutrients (Escarnot et al., 2012). Compared to the wheat flour, the flour made of spelt is characterized by the nutritional benefits (Mykolenko et al., 2016), but lower technological properties (Bojnanska, 2002). Bioactivation of grain is a controlled process of grain saturation with moisture and beginning of its sprouting, during which the macromolecular substances are converted into easily digestible ones and biologically active compounds are accumulated (Platel et al., 2016). In particular, bioactivation leads to the increase in the number of amino acids and improves the product amino acid composition. Bread with partial or complete substitution of flour with bioactivated grain previously disintegrated requires searching for raw materials of high protein biological value (Ruzhylo, 2015) and provides formation of high consumer properties of the product.

Amaranth grain differs from other cereals and legumes by the full value of its amino acid composition (Juan et al., 2007). It is considered that in terms of amino acid composition the amaranth protein the amount of which depending on the genotypic characteristics of the crop and variety varies from 12 to 24% (Bojórquez-Velázquez et al., 2018) is close to the composition of an ideal protein. Amaranth grain is characterized by high content of lysine, which is the limiting amino acid for vegetable proteins.

It is known that amino acid composition of the product is changing significantly under the influence of the processing technology factors such as temperature, enzymes, and pH (Martinez-Lopez et al., 2020). The most of studies on the introduction of amaranth grain processing products into bakery goods relate to flour (Mykolenko et al., 2020). Taking into account significant increase in the biological value and bioavailability of grain substances due to sprouting (Platel et al., 2016), studies of the effect of the bioactivated disintegrated amaranth grain (BDAG) on the quality of bread and changes of its amino acid composition are very promising.

Therefore, purpose of the research was the study of the baking properties and amino acid composition of BDAG as an ingredient of wheat and spelt bread making. The research objectives included the following:

- Determination of the effect of soaking time and BDAG dosing on physico-chemical and sensory properties of wheat and spelt bread;
- Cluster analysis of products by consumer quality indices;
- Study of amino acid composition and protein biological value of grain, flour, bioactivated disintegrated grain and bread with high consumer properties enriched with BDAG.

Materials and methods

Materials

The amaranth grain of Kharkivskyi-1 variety of the Ukrainian breeding (*Amaranthus hypochondriacus*) was used in the study. This variety is positioned by the plant breeders as a medicinal one (Ghopcij et al., 2018). High-grade wheat flour and whole-grain spelt flour were used for the dough preparation. For the wheat flour, the moisture content was 14.5%, amount of gluten – 26%, gluten deformation index (GDI) – 63 units; for the spelt bread, the

moisture content was 11.6%, amount of gluten – 38%, GDI – 88 units. For the baking trial at the laboratory, pressed baker's yeast and table salt were also used.

The amaranth grain cleaned of impurities by optical and vibro-sorting was washed with main water and then soaked at the hydro module of 1:1 (Pivovarov et al., 2018). during 12, 24, 36 and 48 hours. After bioactivation, the grain was disintegrated to form the paste-like disintegrated grain mass, further introduced into the bread formulation in doses, %: wheat bread – 0 (W), 15 (W15), 20 (W20), 25 (W25); spelt bread – (S), 15 (S15), 20 (S20), 25 (S25). At different durations of soaking of the amaranth grain introduced in the bread as BDAG, hours, the following samples of bread were received: 0 (W and S), 12 (W15-12, W20-12, W25-12; S15-12, S20-12, S25-12); 24 (W15-24, W20-24, W25-24; S15-24, S20-24, S25-24); 36 (W15-36, W20-36, W25-36; S15-36, S20-36, S25-36); 48 (W15-48, W20-48, W25-48; S15-48, S20-48, S25-48).

Baking trial

For the dough preparation, we used the composite mixes including wheat flour-BDAG and spelt flour-BDAG in the ratios of 85:15, 80:20, 75:25. The dough was prepared of 200 g of flour (control sample) or composite mix (test samples), 5 g of pressed yeast, 3 g of salt and water as per calculation. The estimated moisture content of the dough for wheat-amaranth mix was equal to 44.5%, and for spelt-amaranth mix – 49%. The dough was fermented in the thermostat at the temperature of 31 ± 1 °C for 170 minutes for bread based on wheat flour and at the temperature of 28 ± 1 °C for 210 minutes for samples based on spelt flour. The bread was baked in the laboratory oven at the temperature of 220–230 °C for 30 minutes for wheat bread and 200–210 °C for 55 minutes for spelt bread.

Determination of physico-chemical characteristics of the bread quality

Specific volume of the finished products was determined by the method of AACC 10-05.01. The complex quality of bread was determined 2-4 hours after baking, taking into account sensory characteristics by scoring on five-point scale with the use of weigh coefficients of the indicators such as volume (3), shape (1), crust color (1), surface condition (1), crumb color (2), crumb porosity structure (1.5), crumb rheological properties (2.5), aroma (2.5), taste (2.5), and chewability of bread crumb (1). Moisture content of the bread crumb was determined by thermographic method according to AACC 44-15.02. Porosity of the finished products was determined using the Zhuravlev device. From the middle of the product a piece of bread of about 7-8 cm thick was cut. From this piece, the central parts of crumb were taken by the cylinder of the device at a distance of minimum 1 cm from the crust in the place most typical for its porosity. The cylinder filled with crumb was placed onto the tray so that its rim fitted tightly into the slot of the tray. After that, the column of bread crumb was pushed out of the metal cylinder with a wooden sleeve to the distance of about 1 cm and cut at the edge of the cylinder with a sharp knife. Then the crumb was pushed out of the cylinder close to the tray wall and cut off again at the edge of the cylinder. Three parts were taken for analysis of the bread. The parts of crumb taken were weighed to the nearest 0.01 g. Porosity (P, %) was determined by the formula:

$$P = \frac{V_{tot} - \frac{G}{\rho}}{V_{tot}} \times 100, \%$$

where V_{tot} – total volume of the parts taken, cm^3 (81 cm^3); G – mass of the parts taken, g; ρ – density of nonporous mass of crumb, g/cm^3 (for wheat bread – 1.31 g/cm^3, for spelt bread – 1.21 g/cm^3).

Determination of amino acid composition and biological value of proteins

Amino acid composition was determined by the method of ion exchange liquid column chromatography (Csapó et al., 2008) with the use of lithium-citrate buffers as eluents on the automatic amino acid analyzer T 339 ("Microtechna", Czech Republic). For recording of amino acids in the eluents, the method of detection with ninhydrin was used. The experiments were carried out in duplicate. Amino acid score difference factor, biological value and utility of proteins were determined by calculation using the method described in (Dubinina et al., 2015; Shvedjuk et al., 2017).

Statistical processing

Effect of factors of the amaranth grain soaking time and dosing of BDAG in the bread formulation was determined by two-way analysis of variance (ANOVA). Pearson correlation analysis was carried out at $p>0.05$, and cluster analysis followed the agglomerative hierarchical clustering procedure (Ward's method).

Results and discussion

Effect of bioactivated disintegrated amaranth grain on wheat bread and spelt bread quality indicators

Introduction of non-traditional flour raw materials into the bread formulation has a negative effect on the specific volume (Ayo, 2001; Morita et al., 1999). As can be seen from the Figure 1, addition of BDAG increased the specific volume of test samples compared to the control, except the spelt-amaranth composite mix with the use of disintegrated amaranth grain with soaking time of more than 12 hours. Samples with the duration of amaranth grain soaking of 12–36 hours (Figure 1, a-c) and introduction of disintegrated grain in the bread formulation in the amount of 15–20% (Figure 1, a-c) corresponded to the best values of the specific volume for the wheat-amaranth mix. For example, compared to the control, specific volume for test samples containing BDAG with the duration of soaking of 12–48 hours increased by 10–21%. When the amaranth grain was soaked for 48 hours, decrease in the specific volume of test samples compared to samples with soaking time of 12–36 hours was recorded. On the other hand, for spelt-amaranth composite mixes the test sample specific volume increased with the use of BDAG soaked for 12 hours (Figure 2, a) by 8–13% compared to the control after introduction of 15–20% of BDAG in the spelt bread formulation. The obtained differences between wheat bread and spelt bread with the addition of BDAG are explained by the varying biochemical composition of wheat flour and spelt flour, in particular, the state of their protein-proteinase complex. Spelt flour is characterized by the lower content of insoluble polymeric proteins and higher content of gliadins, which leads to the formation of less elastic gluten of the dough and decrease in its shape-retaining ability (Tilman, 2006). It is known that the amaranth grain introduction reduces the dough elasticity (Silva Grobelnik et al, 2018). Against the background of increased enzymatic activity in spelt dough with BDAG, in particular, with the longer duration of the grain soaking, gas-holding capacity of the dough is decreasing, which results in the products with reduced specific volume compared to the wheat bread.

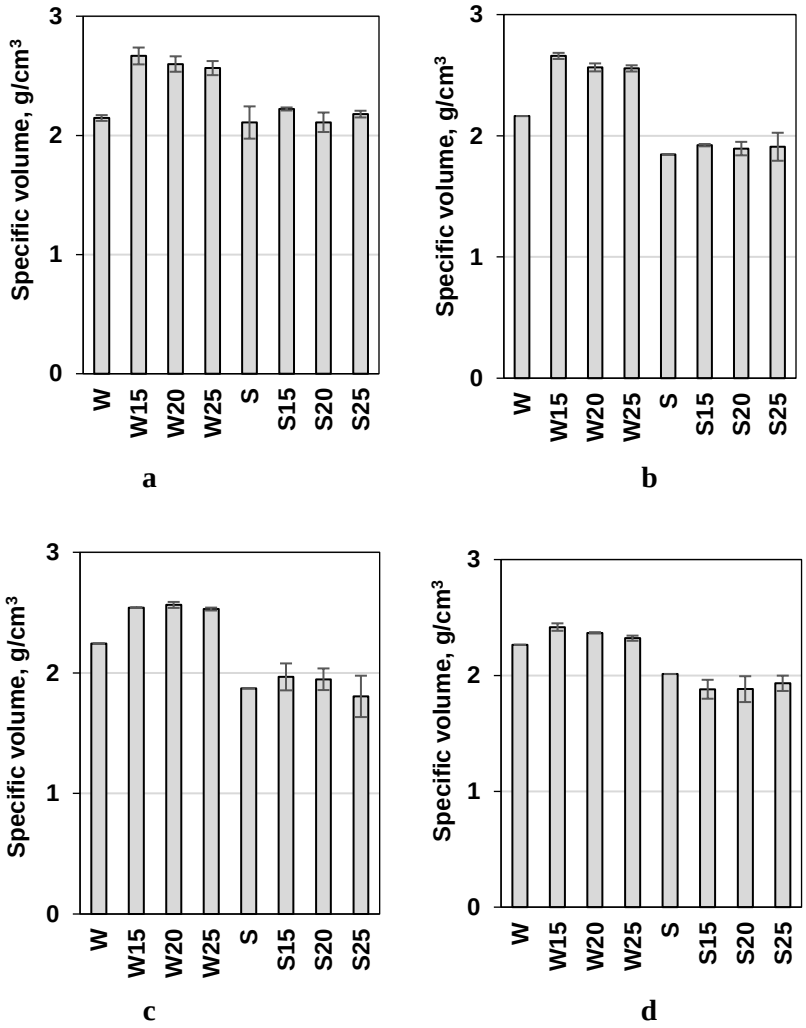
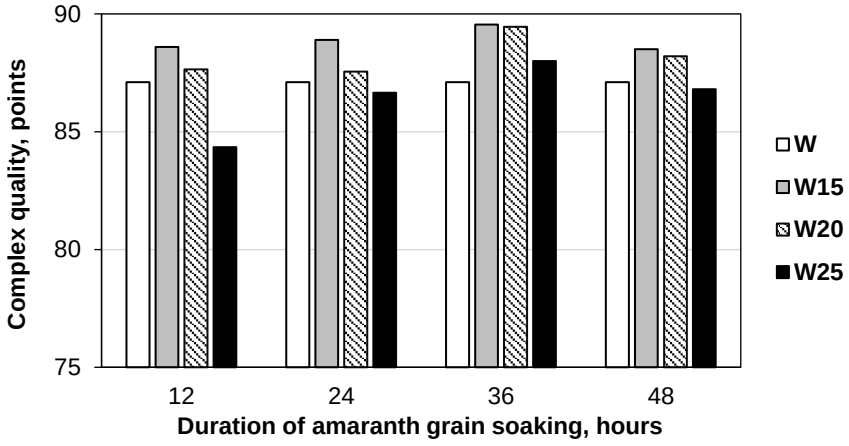


Figure 1. Specific volume of wheat bread (W) and spelt bread (S). Content of BDAG, %: W, S – 0; W15, S15 – 15; W20, S20 – 20; W25, S25 – 25. Duration of the grain soaking (hours): a – 12; b– 24; c – 36; d – 48

As can be seen from the Figure 2, in terms of complex quality, taking into account sensory characteristics, the best samples were those the formulation of which contained disintegrated amaranth grain soaked for 36 hours for both the wheat bread and spelt bread. It was found that substitution of high-grade wheat flour with BDAG in the amount of 15–25% had a positive effect on its quality compared to the control sample of the wheat flour, while for the spelt bread it was advisable to substitute max. 15%. Characteristic feature of the wheat bread and spelt bread was the gradual decrease of the finished product quality with the increase in semi-finished product substitution from 20 to 25% of the flour with BDAG. In all test samples where the amaranth semi-finished product with soaking time of 12 and 24 hours

was used, a specific taste was observed which had the negative effect on the sensory properties of bread. For the bread with the use of BDAG, soaked for 36–48 hours, no deterioration of taste was recorded. It can be explained by activation of biochemical transformations in the amaranth grain during sprouting, which result in the decrease of concentration of saponins capable of giving a specific taste to the products. For example, reduction of saponins' concentration in the flour of the sprouted amaranth grain compared to the native grain due to sprouting of grain during 48 hours was established by Kumari Beniwal et al, 2019.



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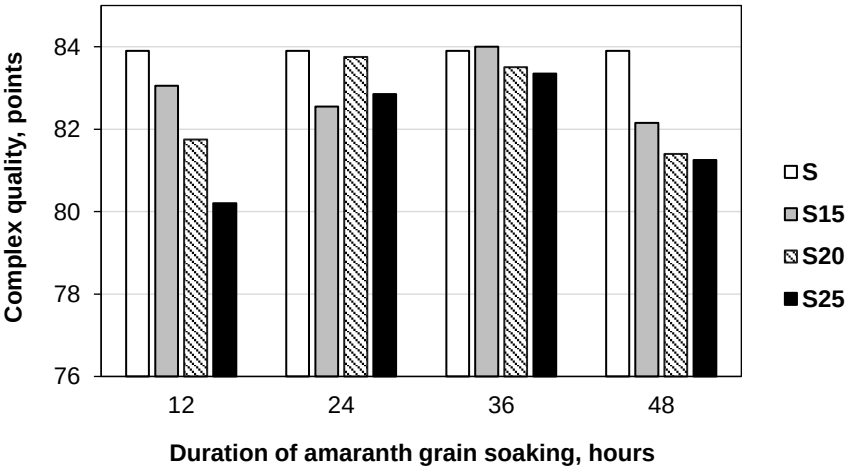


b

Figure 2. Complex quality of the wheat bread (a) and spelt bread (b). BDAG content, %: W, S – 0; W15, S15 – 15; W20, S20 – 20; W25, S25 – 25.

The obtained results differ significantly from the data of the authors (Ayo, 2001; Morita et al., 1999), stating the reduction of specific volume and sensory properties of the bread with the use of amaranth flour. Reduction of the bread specific volume in (Ayo, 2001) was apparently due to the dispersion of whole-fat flour, which was less than 0.4 mm in size, so the maximum allowable percentage of introduced amaranth flour did not exceed 15%. Shmal'ko, 2004, showed the insignificant (1–5%) growth of the specific volume compared to the obtained results (10–21%). Moreover, the use of whole-fat amaranth flour of grain of the Kharkivskyi-1 variety increased the complex quality of bread (Mykolenko et al., 2021). This is due to the fact that quality of wheat bread and spelt bread is significantly influenced by the genotypic characteristics of the grain used to obtain BDAG.

Table 1 shows that moisture content of the wheat bread and spelt bread samples under study gradually increased with the growth of BDAG content, which could be related to redistribution of free and bound moisture during soaking of the amaranth grain. Acidity of the wheat bread crumb varied in the range of 1.1–1.4 degrees, while for the spelt bread it was characterized by 2–2.5 times higher acidity. It is due to the presence of peripheral layers of kernel and, accordingly, higher enzymatic activity and acid accumulation in spelt dough during fermentation.

Table 1

Physico-chemical indicators of quality of the wheat bread and spelt bread with BDAG

Sample		Moisture content, %	Acidity, deg.	Porosity, %
BDAG content, %	Amaranth grain soaking time, hours			
Control		40.9 ± 0.14	1.20 ± 0.14	71.0 ± 0.71
15	12	43.3 ± 0.14	1.20 ± 0.01	71.0 ± 0.58
	24	43.0 ± 0.01	1.20 ± 0.01	71.0 ± 0.37
	36	43.3 ± 0.14	1.40 ± 0.01	75.1 ± 0.45
	48	43.5 ± 0.14	1.20 ± 0.01	78.0 ± 0.01
20	12	43.4 ± 0.01	1.10 ± 0.14	70.1 ± 0.01
	24	44.3 ± 0.14	1.10 ± 0.14	71.0 ± 1.17
	36	43.9 ± 0.14	1.40 ± 0.01	74.0 ± 0.47
	48	44.4 ± 0.00	1.20 ± 0,01	77.1 ± 0.01
25	12	44.1 ± 0.14	1.20 ± 0,01	73.1 ± 0.18
	24	44.0 ± 0.01	1.20 ± 0,01	74.0 ± 0.56
	36	44.6 ± 0.01	1.40 ± 0.01	77.0 ± 0,71
	48	44.6 ± 0.01	1.40 ± 0.01	77.1 ± 0.01
Control		47.2 ± 0.14	3.50 ± 0.14	64.0 ± 0.71
15	12	47.2 ± 0.01	3.10 ± 0.14	60,1 ± 0.01
	24	48.8 ± 0.01	3.10 ± 0.01	61.1 ± 2.12
	36	48.1 ± 0.14	3.20 ± 0.01	70.1 ± 0.34
	48	48.5 ± 0.14	3.10 ± 0.14	71.0 ± 1.41
20	12	48.1 ± 0.14	2.90 ± 0.14	62.1 ± 1.41
	24	49.1 ± 0.14	2.90 ± 0.14	62.1 ± 0.01
	36	49.1 ± 0.01	3.20 ± 0.01	63.0 ± 2.12
	48	48.6 ± 0.01	3.10 ± 0.14	73.1 ± 0.71
25	12	48.7 ± 0.14	2.80 ± 0.01	62.0 ± 2.12
	24	49.1 ± 0.14	2.80 ± 0.01	64.1 ± 0.71
	36	48.7 ± 0.14	3.20 ± 0.01	62.0 ± 0.71
	48	49.6 ± 0.01	3.00 ± 0.01	66.0 ± 0.71

There was the increase in the porosity of wheat bread and spelt bread for the test samples compared to the control upon the introduction of semi-finished product, soaked for 36–48 hours, by 1.1 and 1.2 times, respectively. It is consistent with the results of studies (Shmal'ko, 2004), establishing the increase in crumb porosity by 7-9% with 10 and 20% substitution of wheat flour with disintegrated amaranth grain. The resulting effect can be explained by the increase in sugars content available for fermentation by yeast. For example, Guardianelli et al., 2021, has found that due to sprouting of the amaranth grain the content of fructose and glucose increased 10 and 5 times, respectively, capable of intensifying gas formation in the dough.

Statistical analysis of results of study of the effect of bioactivated amaranth grain on the quality of wheat bread and spelt bread

Two-factor analysis of variance (Table 2) showed that BDAG dosing and duration of grain soaking had no effect at the significance level of $p>0.05$ on the specific volume of both wheat bread and spelt bread. The effect of the factor of BDAG dosing (56%) on the complex quality of the wheat bread with BDAG ($p=0,007$) has been established. On the contrary, complex quality of spelt bread was influenced by the duration of soaking of amaranth (62%) at $p=0,038$. Moisture content of the crumb depended on the factor of BDAG dosing at the level of 69% ($p=0.007$) and 37% ($p=0,038$) for the wheat bread and spelt bread, respectively.

Table 2

Effect of factors of amaranth grain soaking time (T) and dosing of bioactivated disintegrated amaranth grain (D) on the bread quality

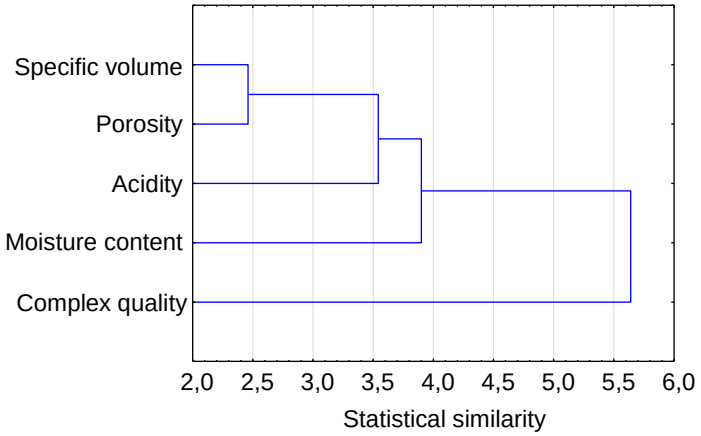
Type of bread with BDAG	Complex quality		Moisture content		Acidity		Porosity	
	T	D	T	D	T	D	T	D
Wheat bread	++	–	–	++	+++	–	+++	+
Spelt bread	–	++	–	+	++	–	–	–

Note: (–) – no effect of the factor; (+) – 10–50% effect of the factor; (++) – 50–70% effect of the factor; (+++) – 70–90% effect of the factor.

Acidity of the crumb was affected by the factor of the grain soaking duration and the level of its influence was higher: 73% ($p=0.007$) i 60% ($p=0.018$) for the wheat bread and spelt bread, respectively. Obviously, it is due to the activation of biochemical processes during grain soaking and accumulation in the amaranth grain of hydrolytic enzymes actively involved in acid accumulation in the process of preparation of the dough. In particular, increase of total and amine nitrogen by 1.3–1.4 times, proteolytic enzyme activity increase by 1.4–1.5 times, and that of lipase and lipoxygenase by 1.1–1.2 times was established in another study (Shmal'ko, 2004). Two factors simultaneously featured statistically significant effect on the porosity of the wheat bread at the level of 82% ($p=0.0008$) and 12% ($p=0.04$) for the duration of amaranth grain soaking and BDAG dosing, respectively.

The correlation analysis showed statistically significant positive Pearson correlation for the specific volume on the porosity of wheat bread with BDAG (0.67) and its acidity (0.62). For the spelt bread with BDAG, in its turn, the positive correlation (0.62) of the complex quality of bread and its acidity was statistically significant.

Formation of different clusters for the wheat bread and spelt bread with BDAG (Figure 3) was established. At the affinity level 2 for the wheat bread two clusters were formed: the first cluster included the indicators of specific volume, porosity, acidity and moisture content, and the second one – complex quality as the indicator for samples characterized by the lowest statistical affinity.



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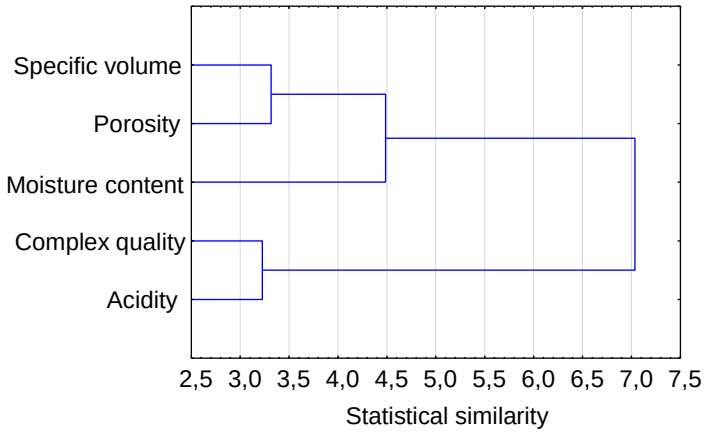

b

Figure 3. Cluster analysis of wheat bread (a) and spelt bread (b) by quality indicators

It should be noted that specific volume and porosity showed the highest statistical affinity in samples, as well as positive correlation between themselves. This subcluster formed a separate group with acidity, but statistically significant correlation was found only for the indicator of specific volume and acidity. For samples of spelt bread with BDAG at the same level of affinity 3 clusters were formed: the first cluster included the specific volume and porosity, the second one – moisture content, and the third was formed by the complex quality and acidity of the crumb (Figure 3, b). According to the results of the correlation analysis, the last cluster also showed statistically significant positive correlation between the indicators for spelt bread in contrast to wheat bread. It indicates a significant contribution of organic acids and acid-reactive compounds in the quality of spelt bread with BDAG, which was characterized by more pronounced aroma and taste compared to wheat bread with BDAG.

Cluster analysis of dendrograms of samples evaluated by the bread quality indicators (Figure 4) showed that at the level of statistical similarity 6, four separate clusters were formed.

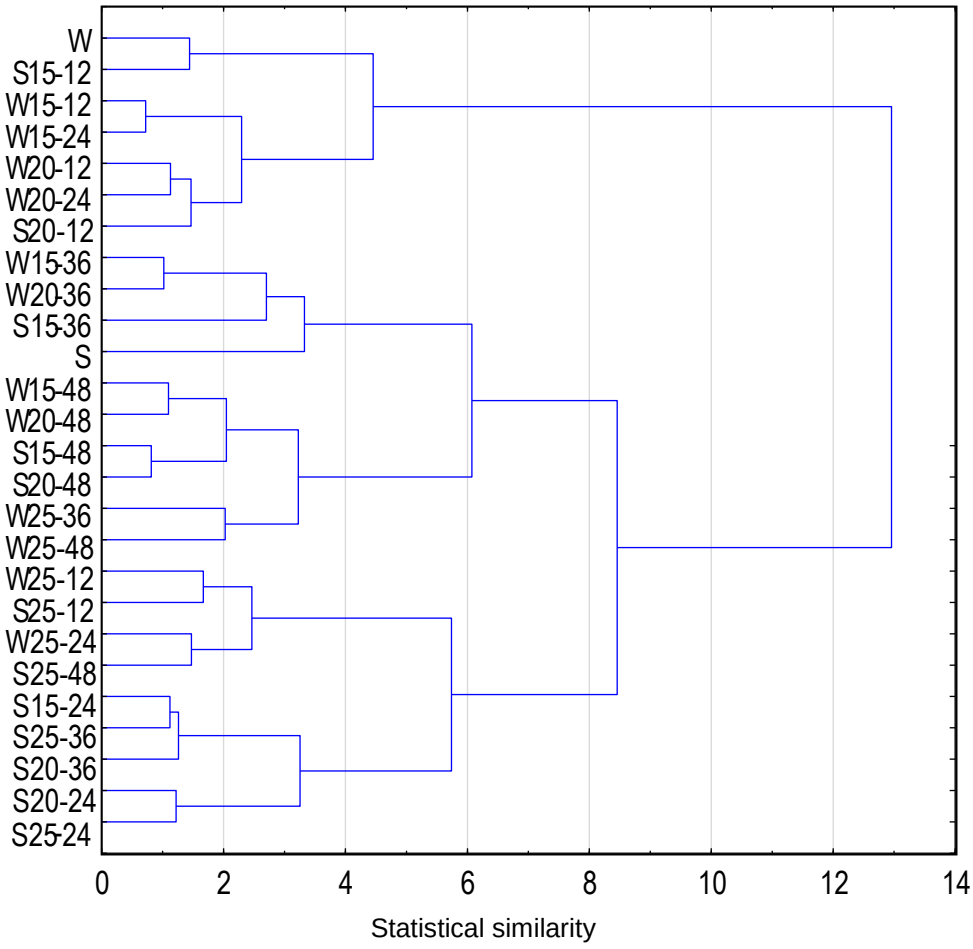

Figure 4. Cluster analysis of test samples of bread

The first one included a control sample of wheat bread and test samples of wheat bread (5) and spelt bread (2) with 15–20% of BDAG, soaked for 12-24 hours. It means that low levels of factors of the duration of amaranth grain soaking and BDAG dosing had no significant effect on the quality of bread. The second cluster included a control sample of spelt bread and test samples of wheat bread (2) and spelt bread (1) with 15–20% of BDAG, soaked for 36 hours. The third cluster combined the samples of wheat bread (4) and spelt bread (1) made on condition of 48 hours of amaranth grain soaking and all levels of BDAG introduction. The fourth cluster consisted mainly of spelt bread samples (8) and only 2 samples of wheat bread with BDAG. The latter clusters had the highest BDAG concentration and minimum 12–24 hour soaking time of the amaranth grain.

Cluster showed that the highest similarity was typical for the following bread samples in descending order: W15–12–W15–24 → S15–48–S20–48 → W15–36–W20–36 → W15–48–W20–48 → S15–24–S25–36–S20–36 → S20–24–W25–24. Along with the results of two-way analysis of variance, it indicates the dominant effect of the duration of amaranth grain soaking in the production of both wheat bread and spelt bread with BDAG.

Assessment of biological value of protein

Tables 3, 4, and Figure 5 show the results of studies of the amino acid composition of amaranth grain before and after biological activation and disintegration compared to high-grade wheat flour, as well as wheat bread and bread made with the addition of 20% of BDAG, featuring the highest consumer properties.

The amaranth grain differed significantly in amino acid composition from the wheat flour, despite the presence of the same limiting acids of the protein (isoleucine and valine), scores on which were slightly higher for the amaranth grain. In contrast to the wheat flour, valine, isoleucine, and leucine only had amino acid scores below 50%. Lysine was the dominant amino acid of the amaranth grain (with 85% amino acid score), whereas for the wheat flour its value reached up 39%. Besides, as a result of biochemical processes occurring in the amaranth grain during soaking, in particular, 1.2-1.5 times increase in the activity of proteolytic enzymes (Shmal'ko, 2004), disintegrated amaranth grain was characterized by the improved amino acid score for all essential amino acids by 1.5-2.7 times. BDAG protein featured the highest score for lysine, phenylalanine and tyrosine (137 and 165 respectively), thus bringing the protein of such raw materials closer to the composition of animal proteins.

Amino acid composition of bread was characterized by growing scores of all essential amino acids, but for the wheat bread lysine still remained the limiting amino acid (62%) in contrast to the wheat bread made with the addition of 20% of BDAG, where lysine content was 2.4 times higher compared to the control sample of bread. For the wheat bread, isoleucine remained the second limiting acid, which was not present in the wheat flour, while for test samples of bread enriched with disintegrated amaranth grain, valine and isoleucine were limiting ones. All essential amino acids of the bread under study had the scores of 133–213%, which proved a significant improvement in the amino acid composition of the product protein when using BDAG. All analyzed nonessential and conditionally essential amino acids of test samples of bread in their number exceeded the wheat bread (Figure 5): bread with the addition of BDAG contained 1.7 and 1.6 times more essential and nonessential amino acids. The process of bioactivation (grain soaking) also had a positive impact on the raw materials: the number of essential and nonessential amino acids in the amaranth grain doubled after bioactivation and disintegration.

Table 3

Amino acid content in raw materials, bioactivated disintegrated amaranth grain and bread with BDAG

Amino acid	Composition of ideal protein, g/100 g	Wheat flour		Native amaranth grain		BDAG, soaking 36 hours		Wheat bread		Bread with 20% BDAG, soaking 36 hours	
		g/100 g of protein	Score, %	g/100 g of protein	Score, %	g/100 g of protein	Score, %	g/100 g of protein	Score, %	g/100 g of protein	Score, %
Lysine	5.5	2.12	39	4.65	85	7.54	137	3.39	62	8.22	149
Histidine	–	1.07	–	1.64	–	3.51	–	2.43	–	3.79	–
Arginine	–	2.54	–	5.05	–	11.30	–	2.87	–	6.81	–
Asparagine acid	–	2.25	–	4.71	–	9.49	–	5.21	–	9.22	–
Threonine	4	1.47	37	2.36	59	5.18	130	3.44	86	5.41	135
Serine	–	3.02	–	4.31	–	7.31	–	5.30	–	8.57	–
Glutamic acid	–	23.05	–	12.93	–	18.90	–	30.70	–	46.4	–
Proline	–	7.64	–	2.43	–	1.04	–	11.78	–	19.7	–
Glycine	–	2.54	–	4.35	–	8.99	–	3.88	–	7.36	–
Alanine	–	2.11	–	3.40	–	4.48	–	3.23	–	5.73	–
Valine	5	1.68	34	1.95	39	4.58	92	4.28	86	6.81	136
Methionine+ Cystine	3.5	1.66	47	2.24	64	3.39	97	3.37	96	6.08	174
Isoleucine	4	1.20	30	1.49	37	3.90	98	3.24	81	5.32	133
Leucine	7	4.14	59	3.18	45	6.97	100	7.30	104	11.25	161
Phenylalanine +tyrosine	6	4.42	74	4.59	76	9.90	165	8.15	136	12.8	213

Table 4

Biological value of raw protein, bioactivated disintegrated amaranth grain and bread with BDAG

Indicator of protein value	Wheat flour	Native amaranth grain	BDAG, soaking 36 hours	Wheat bread	Bread with 20% BDAG, soaking 36 hours
AASDF, %	15.5	20.6	25.1	31.3	24.2
BV, %	84.5	79.4	74.9	68.7	75.8
Utility of the protein	0.63	0.64	0.77	0.65	0.83

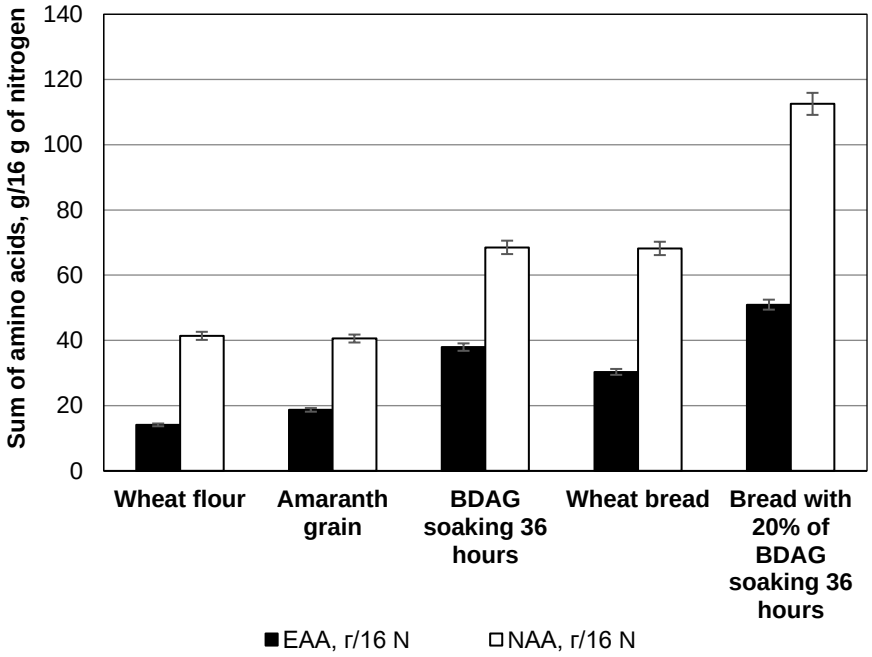

Figure 5. Content of essential and nonessential amino acids in raw materials and bread of bioactivated disintegrated amaranth grain

Amino acid score difference factor was lower for the bread with BDAG, so the protein biological value of such bread increased up to 76%, whereas for the control sample this value was equal to 69%. It should be noted that utility of protein, as its ability to be absorbed by the body for plastic needs, was arranged in ascending order: high-grade wheat flour → amaranth grain without treatment → wheat bread → bioactivated disintegrated amaranth grain → bread with bioactivated disintegrated amaranth grain. Increase in the grain protein biological value as a result of enzymatic processes agrees with (Szabóová et al., 2020; Búcaro Segura et al., 2002), where the improvement of amino acid composition of the protein and increase in the level of its digestibility were established.

Conclusions

1. Usage of BDAG in the formulation of the wheat bread and spelt bread increased the specific volume of products by 10–21% and 8–13%, when the amaranth semi-finished product was introduced in the amount of max. 20%. For the wheat-amaranth mix, according to the sensory assessment, it was expedient to substitute high-grade wheat flour with BDAG in the amount of 15–20%, and for spelt-amaranth mix this amount was max. 15% at soaking time of 36 hours. Spelt bread with BDAG compared to wheat bread was characterized by 2.4–3 times increase in the crumb acidity and 1.1–1.2 times

reduction of porosity. Complex quality of the wheat bread and spelt bread significantly depended on the flour substitution by the bioactivated disintegrated amaranth grain (56%, p=0.007) and duration of the amaranth grain soaking (62.3%, p=0.038), respectively. Acidity of the crumb was affected by the factor of the duration of the grain soaking at the level of 73%, p=0.007, and 60%, p=0.018, for the wheat bread and spelt bread, respectively. It was found that porosity of bread at the statistically significant level depends more on the duration of the amaranth grain soaking (82%, p=0.0008) than on the amount of BDAG introduced into the formulation (12%, p=0.04). Positive correlation between the specific volume of wheat bread and its porosity (0.67) and acidity (0.62) was found. For spelt bread with BDAG, positive correlation between the complex quality of bread (0.62) and its acidity was statistically significant.

2. According to the results of cluster analysis of dendrograms of samples assessed by the indicators of bread quality, it was found that low levels of factors of duration of amaranth grain soaking (12–24 hours) and BDAG dosing (15–20%) had no significant effect on the quality of bread. The highest statistical similarity was typical for the following bread samples in descending order: W15–12–W15–24 → S15–48–S20–48 → W15–36–W20–36 → W15–48–W20–48 → S15–24–S25–36–S20–36 → S20–24–W25–24. Along with the results of two-way analysis of variance, it indicated the dominant effect of the duration of amaranth grain soaking in the production of both wheat and spelt bread with the use of BDAG.
3. For the amaranth grain, valine, isoleucine and leucine had amino acid scores below 50%, whereas for wheat flour – lysine, threonine, valine, methionine, cystine and isoleucine. In terms of lysine content, the amaranth grain exceeded the wheat flour twofold. As a result of biochemical processes occurring in the kernel during soaking, disintegrated amaranth grain was characterized by the improved amino acid score for all essential amino acids by 1.5-2.7 times. BDAG protein featured the highest scores for lysine, phenylalanine and tyrosine (137 and 165 respectively), thus bringing the protein of such raw materials closer to the composition of animal proteins. For the wheat bread made with the addition of 20% of BDAG, lysine content was 2.4 times higher compared to the control sample of bread. All essential amino acids of the bread under study had scores of 133–213%, which proved the significant improvement of amino acid composition of protein in the product with the use of BDAG. As a result of bioactivation, the amaranth grain had 1.8–2.1 times higher content of essential and nonessential amino acids, the amount of which in the bread with BDAG increased by 1.6–1.7 times.
4. Protein biological value for the bread with BDAG increased up to 76%, whereas for the control sample this value was equal to 69%. Utility of the protein followed the ascending order: high-grade wheat flour → amaranth grain without treatment → wheat bread → bioactivated disintegrated amaranth grain → bread with bioactivated disintegrated amaranth grain.

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Effects of the sugar and fat substitution on the rheological properties of the pie dough

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Abstract

Introduction. The aim of this research was to determine the impact on the rheological properties of the pie dough in the case of substitution of a percentage of sugar and fat with apple puree.

Materials and methods. The evaluation of the empirical rheological characteristics of the pie dough was performed using the Alveograph tool. For the evolution of the loss and storage behaviour of the dough during processing were performed two dynamic methods: frequency and creep test.

Results and discussion. The rheological properties of the doughs showed significant changes in the case of samples obtained by substituting a lower percentage of sugar and fat with apple puree. Lower values of modulus of elasticity and viscosity were obtained with the frequency of the samples obtained by substituting with puree a lower percentage of sugar and fat compared to the control sample. The sample obtained by substituting 40% of the amount of sugar and fat had the values of the viscosity module with the frequency closest to those of the control sample.

In the case of samples with a substitution of 20 and 50% of the amount of sugar and fat, the maximum gelatinization temperature had higher values than the control sample, and the samples with a substitution of 10 and 30% of the amount of sugar and fat had showed a lower maximum gelatinization temperature than the control sample. In contrast, the sample with a substitution of 30% of the amount of sugar and fat had a maximum gelatinization temperature as the control sample.

The behaviour of the dough at creep and recovery most similar to the control sample was in the case of the sample obtained by substituting 40% of the amount of sugar and fat, followed by the one with 50% substitution.

This is because the sugar and fat in apple puree have fulfilled the functions of sugar and fat added in the control sample.

Conclusions. A reduction of sugar between 10 and 50% was achieved in 5 samples of pie dough. The use of apple puree as an ingredient in substituting a percentage of the amount of sugar and fat produced a dough with rheological properties approximately as the control sample depending on the percentage of sugar and fat substituted.

Introduction

The pie dough displays a viscoelastic behaviour, therefore the rheological properties and its creep behaviour are followed. In addition, by substituting a quantity of sugar and fat, the dough can change its rheological properties and viscoelastic behaviour, which is the study of our research. Substituting an ingredient in the pie making process requires evaluating the behavior of the dough. The present study aimed to perform dynamic rheological measurements to evaluate the viscoelastic properties of the dough.

Dough rheology plays an essential role in baking products quality prediction and may give information about mechanical behavior (Song et al., 2007). Dynamic oscillatory measurement is a fundamental approach widely used for structural and fundamental characteristics of wheat flour dough evaluation (Sigman-Grant et al., 2005). In order to better understand the system behavior, rheological tests like creep-recovery are also required (Dobraszczyk et al, 2003) to highlight the importance of mixing energy in dough structure development. These tests can also give information about bread volume potential (Thompson et al., 2008), and dough behavior during processing (Berland et al., 1995). Dough and bread characteristics suffer modifications when dairy ingredients are added in the technological process. Therefore, it is necessary to consider certain aspects, such as the percentage added, the type of dairy ingredient and the changes that occur during the production process. The influence of dairy ingredients on dough rheology depends on the composition, and particularly on the dairy protein–gluten interaction (Mann et al., 2013; Mironeasa et al., 2018).

As regards the effect of sugar and fat on the typical rheology of dough and pie dough, sucrose is hydroscopic and therefore binds to the water found in the cake batter. This results in an increase in the viscosity of the batter which is important as this helps to retain gas bubbles, increasing the final volume of the cake. As sugar binds with water, this prevents the full hydration of the gluten proteins (found in the flour), preventing theformation of a gluten network (Perego et al., 2007). Sucrose increases the temperature of starch gelatinisation and egg protein denaturation, allowing gas bubbles to expand before the formation of the gel (Christ et al., 2005; Psimouli et al., 2012)

The objective of the present study was to investigate the effects of reducing sucrose and fat in a cake formulation and include natural sugar alternatives.

The aim of this research was to determine the impact on the rheological properties of the pie dough in the case of substitution of a percentage of sugar and fat with apple puree.

Materials and methods

Sample preparation

According to the recipe (Huțu et al., 2021), the flour: sugar ratio was 12.5:1 (g:g), and after substitution the ratio varied from 13.8:1 (in the case of a substitution of 10% of the amount of sugar with apple puree) up to 25:1 (in case of a substitution of 50% of the amount of sugar with apple puree).

In the case of fat, the ratio for the control sample was 6:1 (flour: fat, and after substitution the ratio varied from 7:1 (in the case of a substitution of 10% of the amount of fat with apple puree) to 12:1 (in case of a substitution of 50% of the amount of sugar with apple puree) (Huțu et al., 2021).

Sample preparation:

M – sample obtained without substitution of sugar and fat,

P1 – sample obtained by substituting 10% of the amount of sugar and fat,

P2 – sample obtained by substituting 20% of the amount of sugar and fat,

P3 – sample obtained by substituting 30% of the sugar and fat content,

P4 – sample obtained by substituting 40% of the amount of sugar and fat,

P5 – sample obtained by substituting 50% of the amount of sugar and fat (Huțu et al., 2021),

Rheological characteristics

Characteristics were determined with the help of the alveographic method (Bordei et al., 2007). Each Alveograph chart was analyzed for the following parameters: *P*, the maximum pressure needed to blow the dough bubble, expresses dough resistance; *L*, length of the curve, expresses dough extensibility; *P/L*, configuration ratio of the Alveograph curve; *G*, index of swelling; *W*, baking strength (surface area of the curve).

The alveographic curve allows to determine a series of rheological characteristics of the dough:

- The maximum pressure, P (mm), expresses the resistance to deformation of the dough;
- Length, L (mm), expresses the extensibility of the dough. It shows the ability of the dough to form bubbles, until it breaks;
- The swelling index, G, is the average of the swelling indices of the five samples. Similar to L, G is the expression of the extensibility of the dough;
- Conformation ratio (shape) of the curve, P / L, expresses the ratio between the elastic properties and the viscous properties of the dough;
- The deformation energy, W, expresses the energy required to inflate the dough bubble before it breaks (Bordei et al., 2007).

Dough Rheological Properties

The fundamental rheological characteristics of dough were assessed by the dynamic oscillatory method in the linear viscoelastic region. For this purpose, a HAAKE MARS 40 stress controlled rheometer (Thermo-HAAKE, Karlsruhe, Germany) with smooth parallel plates measuring system and a gap width of 2 mm were used. The dough samples were formulated without yeast, at the optimum water absorption capacity previously determined. Dough formulations rested for 5 min to allow relaxation and temperature stabilization, then were placed between the plates and kept for 120 s prior testing. The measuring temperatures were controlled by using an external thermostatic bath during the tests. The excess of dough was removed, and a Vaseline layer was applied to the exposed edge of dough, to prevent the loss of moisture during testing. Before analysis, dough samples were tested for the limits of the linear viscoelastic region (LVR) based on the strain sweep determination, in which the strain was increased from 0.01 to 1%, at a constant oscillation frequency of 1 Hz (Iuga et al., 2020). The oscillatory measurements were performedat a maximum strain of 0.15%, which was found to be in the LVR where dough samples have a linear relationship between stress and strain.

Frequency Sweep Test

The frequency sweep test was performed at a frequency variation from 1 to 20 Hz, with a constant stress previously established, in the LVR, at a temperature of 20 °C. The changes of the storage modulus (G'), loss modulus (G") and complex modulus (G*) were registered. The experimental data were fitted to the Power law model using the Equations (1)–(3) (Mironeasa et al., 2019). The loss tangent (tan δ) was calculated as the ratio between G" and and G'.

$$G'(\omega) = K' \cdot \omega^{n'} \tag{1}$$

$$G''(\omega) = K'' \cdot \omega^{n''} \tag{2}$$

$$G^*(\omega) = K^* \cdot \omega^{n^*} \tag{3}$$

where G' is the storage modulus (Pa), G" loss modulus (Pa), G* complex modulus (Pa), ω angular frequency (rad/s), K', K", K* (Pa·s$^{n'}$) are consistency indices, n', n" and n* are flow behaviour indices (Bordei et al., 2007).

Creep and Recovery Test

Creep-recovery tests were performed by applying a constant shear stress of 50 Pa over a creep time of 60 s and allowing strain recovery during 180 s after stress removal, at 20 °C temperature. The compliancerheological parameter (Steffe, 1996; Lupi et al., 2020) was recorded (Equation (4)):

$$J(t) = \frac{\gamma(t)}{\sigma} \tag{4}$$

where J (Pa^{-1}) is the compliance, γ – the strain and σ – the constant stress applied (Pa^{-1}) (Iuga et al., 2020).

The experimental data of creep-recovery test were fitted to the Burgers model, made up of four components, which comprises the association in series of the Maxwell model and the Kelvin–Voigt model [23,24].

The percentage recovery, which highlighted the relative elastic part of the maximum creep compliance, was also determined as the ratio between J_{max} and J_r (Barnes, 2000).

Temperature Sweep Test

The temperature sweep test was performed at a constant strain of 0.15% and a frequency of 1 Hz, the dough samples being heated from 20 to 100 °C at a rate of 4.0±0.1 °C per min. The storage (G') and loss modulus (G'') were recorded as a function of temperature, allowing the determination of maximumgelatinization temperatures (T_{max}) (Iuga et al., 2020).

Results and discussion

Rheological characteristics

The table includes the numerical values of the rheological characteristics of the pie dough obtained by substituting in different percentages the sugar and fat with apple puree.

Table 1

Rheological characteristics of pie dough

Characteristics	M	P1	P2	P3	P4	P5
P (mm)	80	58	62	73	106	76
L (mm)	110	112	125	78	74	91
G	23,3	23,6	24,9	19,7	19,1	21,2
W (10^{-4}j)	234	155	187	168	253	190
P/L	0,73	0,52	0,50	0,94	1,43	0,84

Where:
M-sample obtained according to the manufacturing recipe without substitution of sugar and fat,
P1 – sample obtained by substituting 10% of the amount of sugar and fat;
P2 – sample obtained by substituting 20% of the amount of sugar and fat;
P3 – sample obtained by substituting 30% of the amount of sugar and fat;
P4 – sample obtained by substituting 40% of the amount of sugar and fat;
P5 – sample obtained by substituting 50% of the amount of sugar and fat;
P – maximum pressure;
L – dough extensibility;
W – baking strength;
G –index of swelling;
P / L – configuration ratio of the Alveograph curve.

This is because sucrose is hydroscopic and therefore binds to the water found in the cake batter. This results in an increase in the viscosity of the batter which is important as this helps to retain gas bubbles, increasing the final volume of the cake. As sugar binds with water, this prevents the full hydration of the gluten proteins (found in the flour), preventing theformation of a gluten network (Perego et al., 2007). Sucrose increases the temperature of starch gelatinisation and egg protein denaturation, allowing gas bubbles to expand before the formation of the gel (Christ et al., 2005; Psimouli et al., 2012).

Regarding the maximum pressure (Figure 2c) exerted on the pie dough, which expresses its deformation resistance, it was found, following the determinations, that they will be obtained by substituting 10 and 20% of the amount of sugar and fat have a lower deformation resistance. compared to the control sample, the samples obtained by substituting 30% and 50% of the amount of sugar and fat have a deformation resistance similar to the control sample, whereas the sample obtained by substituting 40% of the amount of sugar and fat has a significantly higher deformation resistance than the control sample.

The capacity of the dough to form bubbles until breaking is expressed by its extensibility (Figure 2a), which in the case of the sample in which the amount of sugar was replaced by 10% is closest to the value of the control. In the case of the sample in which the replacement was 20%, the extensibility showed higher values, and in the case of samples with a replacement of 30, 40 and 50% the extensibility is significantly lower.

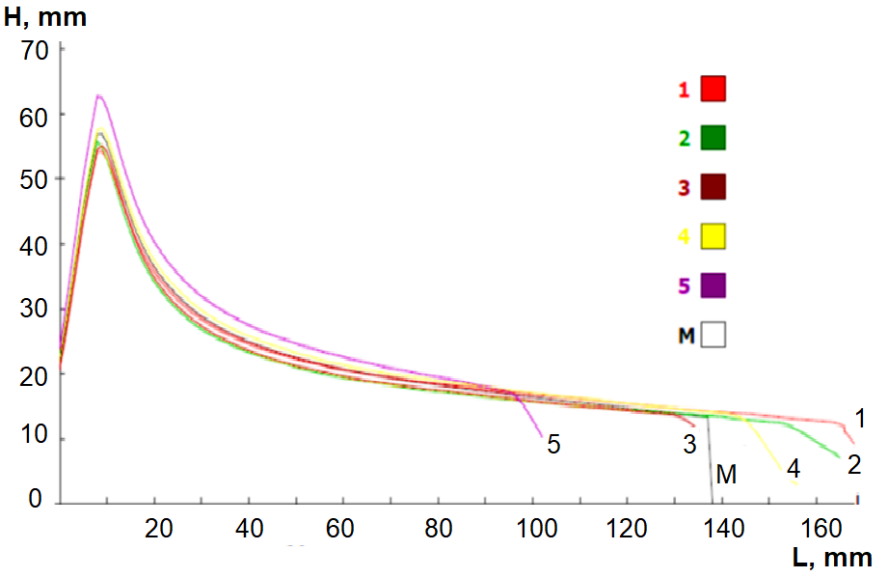
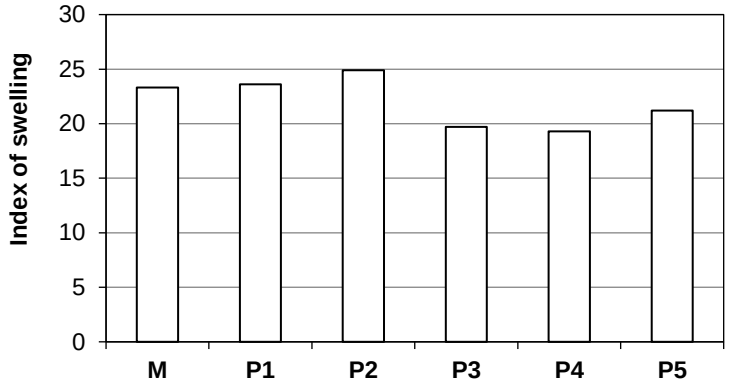
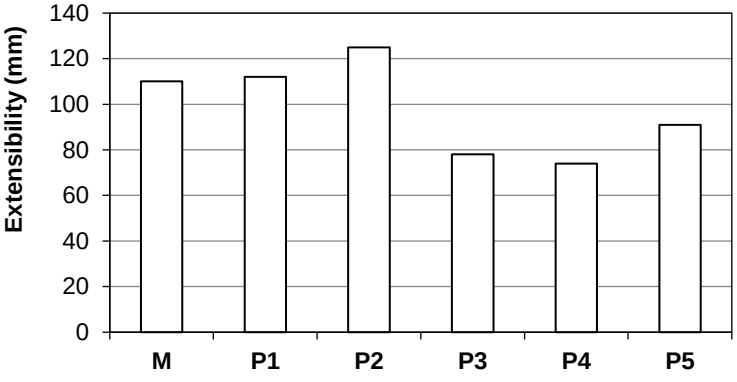


Figure 1. Alveographic curve for pie dough:
M – sample obtained according to the manufacturing recipe without substitution of sugar and fat;
P1 – sample obtained by substituting 10% of the amount of sugar and fat;
P2 – sample obtained by substituting 20% of the amount of sugar and fat;
P3 – sample obtained by substituting 30% of the amount of sugar and fat;
P4 – sample obtained by substituting 40% of the amount of sugar and fat,
P5 – sample obtained by substituting 50% of the amount of sugar and fat.

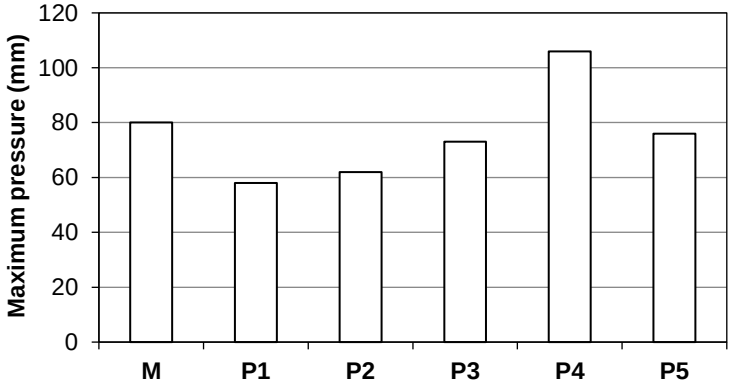
As in the case of extensibility, the swelling index, as an expression of extensibility, showed a similar rheological behavir as in the case of extensibility. In this case, the values of the samples obtained by substituting in different proportions the sugar with apple puree, respectively 10, 20, 30, 40 and 50%, the swelling index (Figure 2b) does not show significant variations compared to the control sample obtained according to the manufacturing recipe of pie dough.



a



b



c

Figure 2. Graphical representation of the swelling index (a), extensibility (b) and maximum pressure (c).

Alveographic curve for pie dough:

M – sample obtained according to the manufacturing recipe without substitution of sugar and fat;
P1 – sample obtained by substituting 10% of the amount of sugar and fat;
P2 – sample obtained by substituting 20% of the amount of sugar and fat;
P3 – sample obtained by substituting 30% of the amount of sugar and fat;
P4 – sample obtained by substituting 40% of the amount of sugar and fat,
P5 – sample obtained by substituting 50% of the amount of sugar and fat.

Frequency Sweep Test

The frequency sweep tests data obtained showed that, in the range of the considered frequencies, the mechanical spectra of the storage modulus (G') (Figure 3) was greater than of the loss modulus (G") for the control, the samples samples obtained by substituting sugar and fat with apple puree, indicating that the dough have a viscoelastic behavior, as was expected (Figure 4).

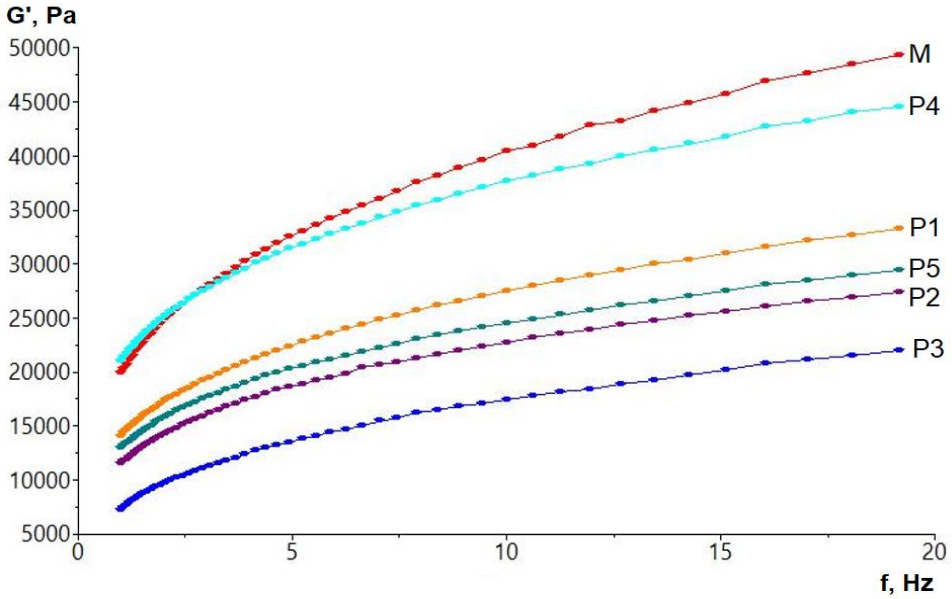


Figure 3. Storage modulus (G') variations with frequency:
M – sample obtained according to the manufacturing recipe without substitution of sugar and fat;
P1 – sample obtained by substituting 10% of the amount of sugar and fat;
P2 – sample obtained by substituting 20% of the amount of sugar and fat;
P3 – sample obtained by substituting 30% of the amount of sugar and fat;
P4 – sample obtained by substituting 40% of the amount of sugar and fat,
P5 – sample obtained by substituting 50% of the amount of sugar and fat.

The tested samples exhibited a predominant elastic solid-like behavior (G' > G") over the entire experimental frequency range. The G' and G" moduli increased with the frequency increase, which means that dough recovery after a stress application was slow, due to the fact that the network is not completely elastic.

The replacement of sugar and fat with apple puree to a significant ($p < 0.001$) increase of storage and loss moduli compared to the control, except for sample obtained by replacing 40% of the amount of sugar and fat with apple puree.

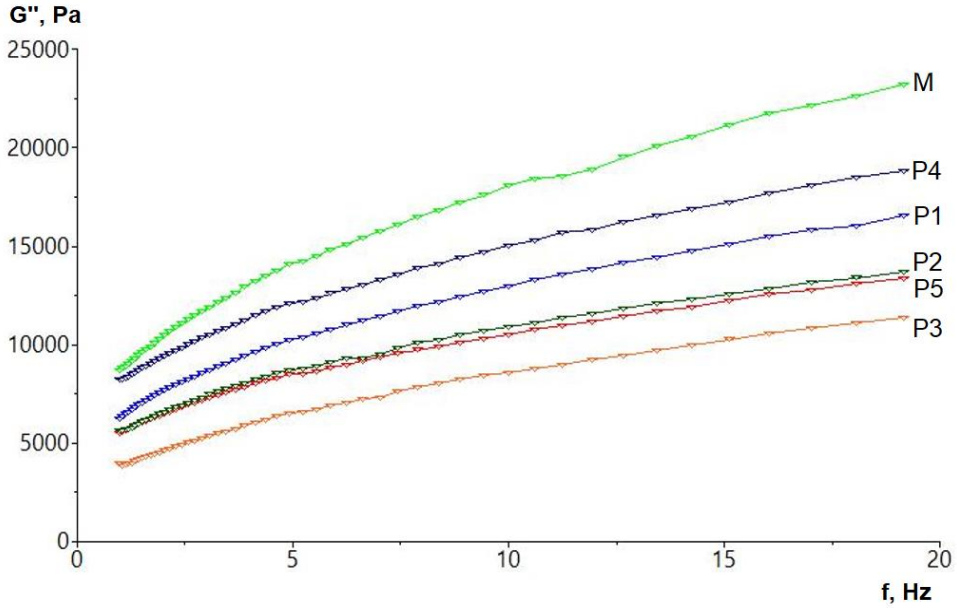


Figure 4. Loss modulus (G") variations with frequency:
M – sample obtained according to the manufacturing recipe without substitution of sugar and fat;
P1 – sample obtained by substituting 10% of the amount of sugar and fat;
P2 – sample obtained by substituting 20% of the amount of sugar and fat;
P3 – sample obtained by substituting 30% of the amount of sugar and fat;
P4 – sample obtained by substituting 40% of the amount of sugar and fat,
P5 – sample obtained by substituting 50% of the amount of sugar and fat.

Temperature Sweep Test

The storage modulus (G') variation with temperature are shown in Figure 5. At first, the storage modulus (G') appears at a minimum level up to a certain temperature, then it suddenly increases until the maximum gelatinization temperature is reached, then decreases again. Samples obtained by substituting 20 and 50% of the amount of sugar and fat showed higher gelatinization temperatures compared to the control, samples obtained by substituting 10%, 30% of the amount of sugar and fat showed higher gelatinization temperatures low compared to the control, and the sample obtained by substituting 40% of the amount of sugar and fat showed the same value of the gelatinization temperature as the control (Figure 4). The peak values of the storage modulus G′ were significantly higher for the samples obtained by substituting 20 and 50% of the amount of sugar and fat.

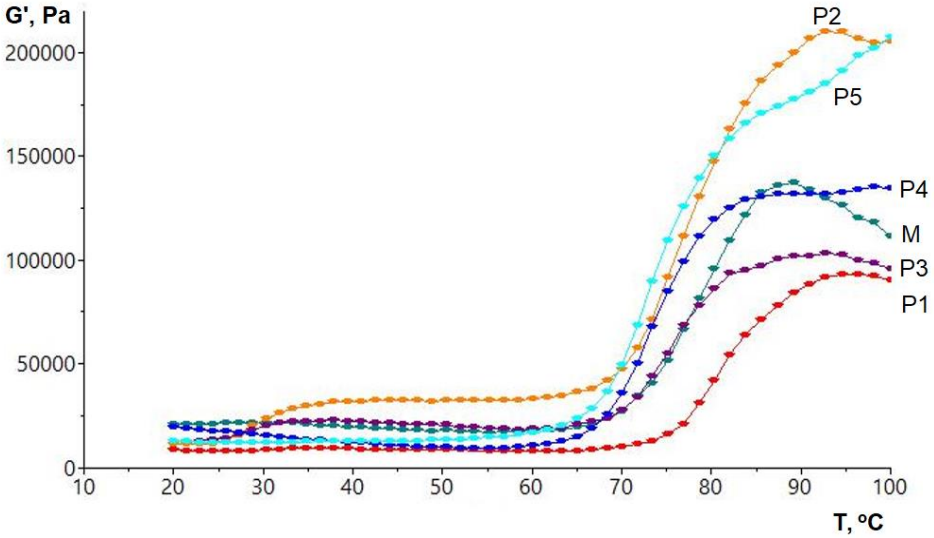


Figure 5. Storage modulus (G') variations with temperature

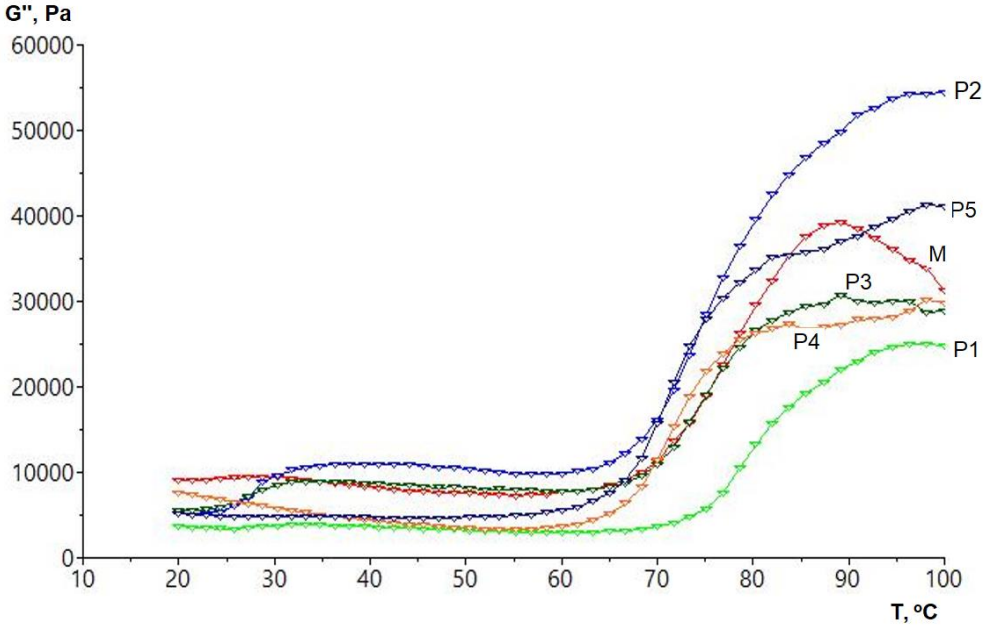


Figure 6. Loss modulus (G'') variations with temperature

For figures 5 and 6:

M – sample obtained according to the manufacturing recipe without substitution of sugar and fat;
P1 – sample obtained by substituting 10% of the amount of sugar and fat;
P2 – sample obtained by substituting 20% of the amount of sugar and fat;
P3 – sample obtained by substituting 30% of the amount of sugar and fat;
P4 – sample obtained by substituting 40% of the amount of sugar and fat,
P5 – sample obtained by substituting 50% of the amount of sugar and fat.

The loss modulus (G") variation with temperature are shown in Figure 6.

At first, the loss modulus (G") appears at a minimum level up to a certain temperature, then it increases sharply until the maximum gelatinization temperature is reached, then decreases again. Samples obtained by substituting 20 and 50% of the amount of sugar and fat showed higher gelatinization temperatures compared to the control, samples obtained by substituting 10, 30 and 40% of the amount of sugar and fat showed temperatures lower gelatinization compared to the control (Figure 4). The peak values of the loss modulus G′ were significantly lower for the samples obtained by substituting 20, 30 and 40% of the amount of sugar and fat

Creep and Recovery Test

According to the results shown in Figure 7, the replacement of sugar and fat with apple puree clearly influenced the storage and loss properties of the doughs.

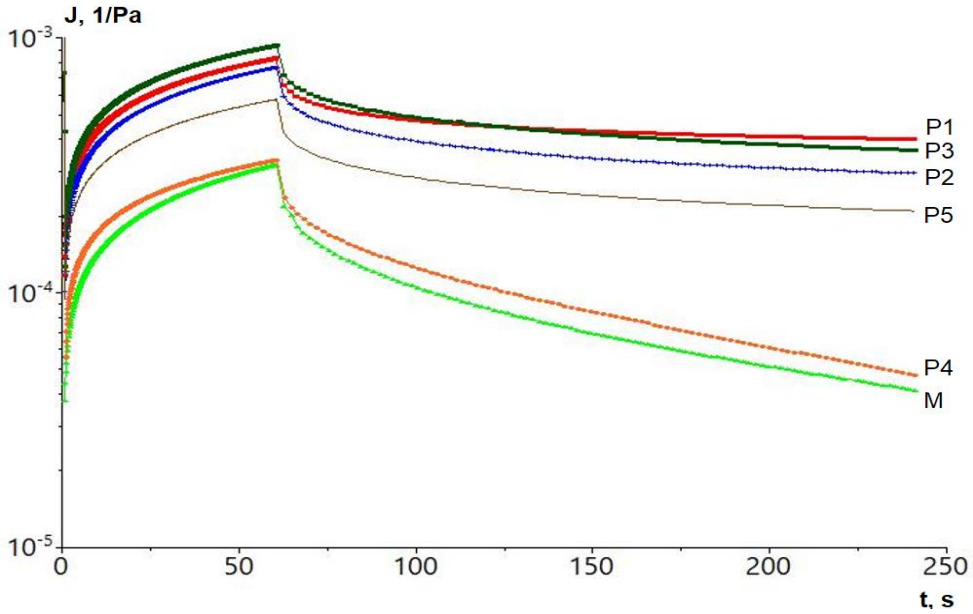


Figure 7. Creep and recovery curves of pie dough:
M – sample obtained according to the manufacturing recipe without substitution of sugar and fat;
P1 – sample obtained by substituting 10% of the amount of sugar and fat;
P2 – sample obtained by substituting 20% of the amount of sugar and fat;
P3 – sample obtained by substituting 30% of the amount of sugar and fat;
P4 – sample obtained by substituting 40% of the amount of sugar and fat,
P5 – sample obtained by substituting 50% of the amount of sugar and fat.

The doughs obtained by replacing 10, 20, 30 and 50% of the amount of sugar and fat (P1, P2, P3) showed significantly higher conformity values during the creep test (Figure 7). The sample obtained by replacing sugar and fat in a proportion of 40% showed a creep and return behavior almost identical to that of the control sample. Compared to the control

sample, the instantaneous (J_{Co}, J_{Ro})and retarded (J_{Co}, J_{Ro}) compliances for both the creep and the recovery phase were similar to the control are those of the sample obtained by substituting 40% of the the amount of sugar and fat, higher for the sample obtained by substituting 50% of the amount of sugar and fat and significantly higher than the samples obtained by substituting 10%, 20%, 30% of the amount of sugar and fat, which indicated a moreinstantaneous and retarded deformation increased.

Conclusion

Replacing some quantity of sugar and fat with apple puree has led to an increase in the nutritional, functional and technological value of pastries. The nutritional value has increased due to the content of nutrients, especially fiber. A reduction of sugar and fat between 10 and 50% was achieved in 5 samples of pie dough. The use of apple puree as an ingredient in substituting a percentage of the amount of sugar and fat produced a dough rheological properties approximately as the control sample depending on the percentage of sugar and fat substituted.

The rheological properties of the dough showed significant changes even in the case of a lower puree substitution percentage of sugar and fat. Lower values of modulus of elasticity and viscosity were obtained with the frequency of the samples obtained by substituting with puree a lower percentage of sugar and fat. Compared to the control sample, the sample obtained by substituting 40% of the amount of sugar and fat had the values of the modulus of viscosity and elasticity with frequency, respectively the values of the modulus of viscosity and elasticity with the temperature closest to those of the control sample.

In the case of samples with a substitution of 20 and 50% of the amount of sugar and fat, the maximum gelatinization temperature had higher values than the control sample, and the samples with a substitution of 10 and 30% of the amount of sugar and fat had showed a lower maximum gelatinization temperature than the control sample. In contrast, the sample with a substitution of 30% of the amount of sugar and fat had a maximum gelatinization temperature as the control sample.

The behaviour of the dough at creep and recovery most similar to the control sample was in the case of the sample obtained by substituting 40% of the amount of sugar and fat, followed by the one with 50% substitution.

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Intensification of the inverted sugar syrup production using the rotor-pulsation processing

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Abstract

The aim of the work is to intensify the process of inverted sugar syrup production using the treatment of water-sugar solution in a rotor-pulsation apparatus.

Materials and methods. The aqueous solutions of chemically pure sucrose were used. The studies were conducted using a rotor-pulsation apparatus in the range of flow shear rates from 20×10^3 $s^{-1}$to 50×10^3 s^{-1}. Determination of carbohydrate content was carried out by high-performance liquid chromatography (HPLC).

Results and discussion. The increase in temperature, processing time, and decrease in pH value at a constant flow shear rate led to an increase of the amount of inverted sugar formed. Complete inversion of sucrose occured at a treatment of sugar solution at temperature of 80 °C, pH 3.0, and a flow shear rate of 20×10^3 s^{-1} for 30 minutes. At a flow rate of 50×10^3 s^{-1} almost all sucrose was hydrolyzed at pH 3.5, and the duration of the process was just 5 minutes under conditions of five-time processing in the rotor-pulsation apparatus in the circulating mode.

In the syrup prepared by the proposed technology at a temperature of 70 °C and the duration of treatment 5 minutes, all sucrose in the solution was inverted, and no traces of hydroxymethylfurfural were detected.

It is assumed that critical stresses occur at the site of the sucrose chain, and the chemical covalent bonds are broken. The break of these bonds during the process of mechano-chemical destruction occurs on the weakest in terms of energy bonds. As a result of mechano-chemical influence on the section of the sucrose chain (C – O – C), there are critical stresses and the connection is broken. This leads to the formation of free radicals. One radical attaches to the OH^- ion and another to the H^+ ion forming glucose and fructose.

Conclusions. The use of treatment of water-sugar solution in a rotor-pulsation apparatus by the proposed technology allows to intensify process of sugar inversion, namely, reduces the duration of the inversion from 120 to 5 minutes, and ensures almost complete inversion of sucrose excluding the formation of hydroxymethylfurfural.

Introduction

Inverted sugar syrups, product of hydrolyzed sucrose, which contains instead of sucrose the mixture of glucose and fructose in ratio 1: 1, become a major sweetener and additive used extensively in the production of a wide variety of foods and beverages. Inverted sugar syrups have many advantages compared to sucrose that makes them attractive to food manufacturers. These include its sweetness, solubility, acidity, and its relative cheapness (Parker et al., 2010). Starch produced from corn, wheat, or barley due to enzymatic hydrolysis is converted into glucose, and then due to isomerization into inverted sugar syrup (Ermolaeva et al, 2012).

There are several ways to obtain inverted sugar syrup from sucrose, namely enzymatic hydrolysis (Mouelhi et al., 2014); hydrolysis by ion exchange (Khan et al., 1996), but the simplest and most common is the method of inverting sucrose by the treatment with citric acid. This method, however, has a number of disadvantages, namely the high process temperature (80–100 ºC), long process duration (up to 2 hours), and most importantly, that this method allows to invert no more than 55% of sucrose due to increased accumulation of hydroxymethylfurfural (Vasilishina et al., 1986).

The task is to increase the yield of inverted sugar by using additional methods of the inversion process.

In the food industry, rotor-pulsation apparatus (high-shear mixers) are used to create emulsions with both high and low viscosity such as salad dressings, sauces, cottage cheese, fruit, vegetable and sour milk desserts, purees, and cream. Rotor-pulsation apparatus are also used for dispersing artificial sweeteners, and cloud agents in carbonated soft drinks, for blending miscible liquids of very different viscosities, to deagglomerate and uniformly disperse nanoparticles in liquids, and also to suspend fine air bubbles (e.g., ice cream) (Rodgers et al., 2016).

The physical effects of rotor-pulsation apparatus have been studied by many authors. For example, in (Avdeeva et al., 2011) the influence of hydrodynamic cavitation on the production of phospholipid nanostructures was studied.

The influence of alternating impulses of pressure occurring in the rotor-pulsation apparatus on liquid binary systems such as water systems and water-ethanol mixtures was studied in (Dubovkina, 2017).

Under the influence of alternating impulses of pressure, the quantity of the dissolved oxygen in the water and water-ethanol mixtures in comparison with the initial maintenance decreased practically by 50–55%.

Studies (Dubovkina, 2017a) of hydrodynamic oscillations generated to activate the hydrated lime suspension for beet juice treatment have shown an increase in the hydrogen potential of the hydrated lime suspension by 15%.

Intensification of the process of inverted sugar syrup production can be achieved by including of the rotor-pulsation apparatus in the appropriate technological scheme (Myronchuk et al., 2019).

Therefore, there is a need to study the influence of physicochemical effects that occur during the processing of glucose-fructose syrups in a rotor-pulsation apparatus on the intensity of sucrose inversion.

The main technological parameters of the sucrose inversion process are temperature, pH of the solution, and the process duration. Given the fact that the proposed technological scheme includes the processing of syrup in a rotor-pulsation apparatus, it is advisable to enter another parameter influenced on the intensity of the hydrolysis process. This parameter is the

shear flow rate γ, s^{-1}, in the gap between the rotor and the stator of the rotor-pulsation apparatus (Dolinskij et al., 1998; 1999; 1999a).

The aim of this work was to intensify the technology of inverted sugar syrup production from sucrose using the treatment of water-sugar solution in a rotor-pulsation apparatus.

Materials and methods

The object of study was the process of inverted sugar syrup production. The subject of research was determination of the effect of flow rate in the rotary pulsation apparatus on the inversion of sucrose in aqueous solutions of sucrose.

Experimental instalation

Experimental studies of this process were conducted on a specially created pilot plant, the scheme of which is presented in Figure 1. The volume of the sugar syrup vessel was 100 liters. The productivity of the rotor-pulsation apparatus (**RPA**) was 6 m^3/h.

The rotor-pulsation apparatus consists of two coaxial cylinders namely a fixed stator and a movable rotor with radial channels in the side walls of different cross- sections (Fig. 2) (Zhang et al., 2012). The substance to be treated is fed into the rotor cavity, through the channels passes into the inter-cylinder gap, and subsequently through the stator channels.

The shear rate of the flow was regulated by changing the speed of the rotor shaft using a frequency converter.

The range of flow shear rates was from 20×10^3 s^{-1} to 50×10^3 s^{-1}.

During the studies, the dependence of sucrose inversion on temperature, pH and process duration was determined at a constant flow shear rate, which was 20×10^3 s^{-1}. The amount of inverted sugar formation was determined depending on the flow shear rate, pH and process duration at a temperature of 70 °C.

Technological operations were performed in the following order. In the sugar syrup vessel 3, the sugar was mixed with water to a dry matter concentration of 65%. In citric acid solution preparation vessel 1, a 25% solution of citric acid was prepared and added to the sugar syrup vessel 3 in an amount of 0.75 kg of citric acid per 100 kg of sugar.

This solution was treated in a rotor-pulsation apparatus in a recirculation mode until complete hydrolysis of sucrose was occurred, and then sent for cooling and storage. The dry matter content in inverted glucose-fructose syrup was about 65%.

Determination of carbohydrates

Determination of carbohydrates content was carried out by high-performance liquid chromatography (HPLC) (Costa et al., 2015).

An Agilent 1100 chromatograph with a diode array detector was used in this study. UV detection was performed at 195 nm at a column temperature of 30 °C and a column with the aminopropyl stationary phase (Zorbax Carbohydrate 250x4.6 mm, 5 μm, manufactured by Agilent). The ratio of acetonitrile used and deionized water was from 82 to 18% vol.

Determination of pH value

To determine the degree of activity of hydrogen ions in the liquid used a pH meter Ezodo 5011.

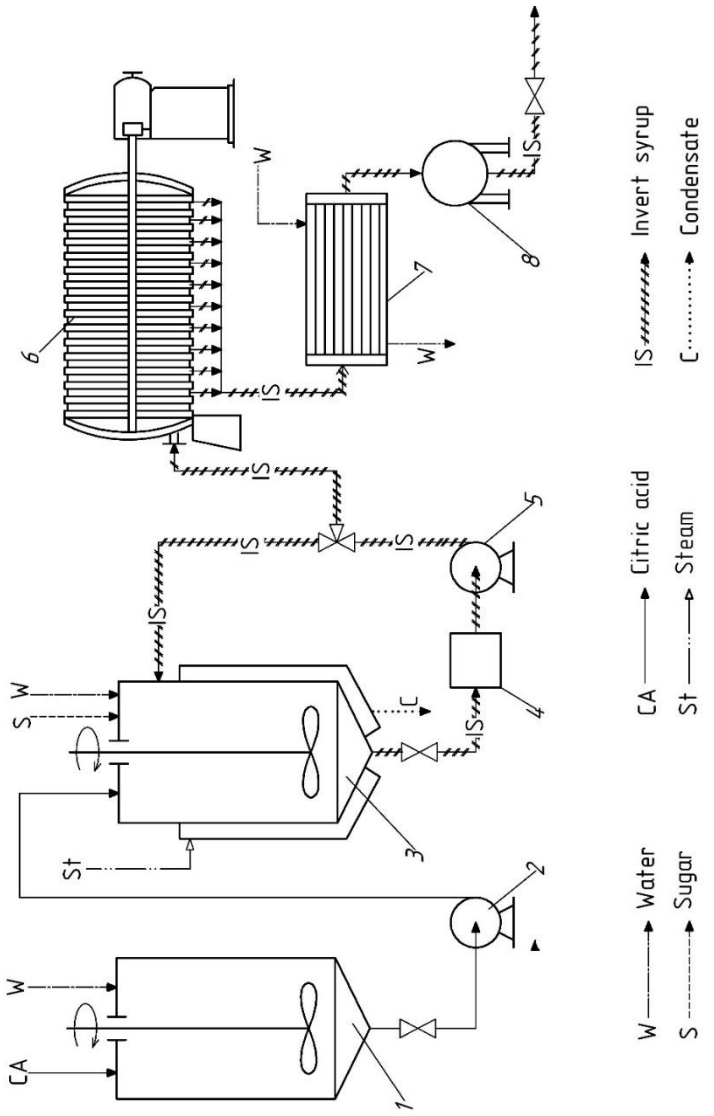

Figure 1. Technological scheme of the inverted sugar syrups preparation using a rotor-pulsation apparatus:
1 – citric acid solution preparation vessel: 2 – pump;
3 – sugar syrup vessel; 4 – filter; 5 – rotor-pulsation apparatus;
6 – filter; 7 – heat exchanger; 8 – storage vessel

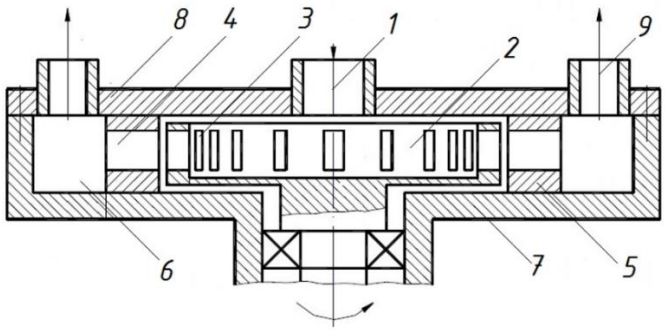

Figure 2. The design of rotor-pulsation apparatus:
1 – outlet; 2 – rotor; 3 – channels in the rotor; 4 – channels in the stator;
5 – stator; 6 – sound camera; 7 – housing; 8 – cover; 9 – inlet pipe.

Results and discussion

Dependence of the formation of inverted sugar on the temperature, pH, and duration of the process at a shear rate of 20×10^3 s^{-1}

The dependence of the inverted sugar formation on the temperature, pH, and the process duration at a flow shear rate of 20×10^3 s^{-1} is shown in Table 1. It was determined that with the process duration increasing from 30 to 120 minutes at a flow shear rate of 20×10^3 s^{-1}, the amount of the formed inverted sugar increased from 43 to 46% at pH = 4.0; from 51 to 57% at pH = 3.5; from 61 to 66% at pH = 3.0; from 69 to 71% at pH = 2.5 at a temperature of 50 °C. Increasing the temperature to 80 °C led to the fact that with increasing process duration from 30 to 120 minutes at a flow shear rate of 20×10^3 s^{-1} the amount of the formed inverted sugar increased from 53 to 58% at pH = 4.0; from 72 to 75% at pH = 3.5; from 98 to 100% at pH = 3.0. Increasing the temperature to 90 °C led to the fact that with increasing process duration from 30 to 120 minutes at a flow shear rate of 20×10^3 s^{-1} the amount of the formed inverted sugar increased from 98 to 100% at pH 4.0.

Dependence of the inverted sugar formation on the flow shear rate, pH, and the process duration at a constant temperature of 70 °C

The dependence of the inverted sugar formation on the flow shear rate, pH, and the process duration at a constant temperature of 70 °C is given in Table 2.

It was determined that with increasing of the process duration from 5 to 20 minutes at the flow shear rate of 30×10^3 s^{-1} the amount of inverted sugar increased from 65 to 73%; at a shear rate of the flow of 40×10^3 s^{-1} from 75 to 85%; at a flow shear rate of 50×10^3 s^{-1} from 87 to 93%. It was determined that with increasing the process duration from 5 to 20 minutes at the flow shear rate of 30×10^3 s^{-1} the amount of inverted sugar increased from 65 to 73%; at a flow shear rate of 40×10^3 s^{-1} from 75 to 85%; at a flow shear rate of 50×10^3 s^{-1} from 87 to 93%. Reducing the pH to 3.5, depending on the process duration from 5 to 20 minutes at a flow shear rate of 30×10^3 s^{-1}, amount of the inverted sugar increased from 74 to 81%; at a flow shear rate of 40×10^3 s^{-1} from 89 to 96%; at a flow shear rate of 50×10^3 s^{-1} almost all sucrose was hydrolyzed in 5 minutes of processing (Patent UA 9399. A method of preparing inverted sugar syrup).

Table 1

Formation of inverted sugar depending on the temperature, pH and the process duration at a flow shear rate of 20×10^3 s^{-1}

t, ºC	pH	Process duration, min	Amount of inverted sugar, %
50	4.0	30	43
		60	45
		120	46
	3.5	30	51
		60	55
		120	57
	3.0	30	61
		60	65
		120	66
	2.5	30	69
		60	71
		120	71
80	4.0	30	53
		60	57
		120	58
	3.5	30	72
		60	74
		120	75
	3.0	30	98
		60	99
		120	100
90	4.0	30	98
		60	100
		120	100

Table 2

Formation of inverted sugar depending on the flow rate, pH and the process duration at a temperature of 70 °C

pH	Process duration, min	γ, $\times10^3$ s^{-1}	Amount of inverted sugar, %
40	5	30	65
	10		71
	20		73
	5	40	75
	10		83
	20		85
	.	50	97
			91
			92
3.5	5	30	74
	10		79
	20		81
	5	40	89
	10		94
	20		96
	5	50	100
	10		100
	20		100

The traditional technology of the acid inversion of sucrose is accompanied by the formation of a toxic by-product of the deep decomposition of sucrose hydroxymitylfurfural. This is due to the long duration and the high temperature of treatment. The use of rotor-pulsation apparatus in the process of inverted sugar syrup production eliminates the formation of hydroxymethylfurfural due to reducing the inversion process duration to 5 minutes.

Determination of the composition of inverted sugar syrup depending on the processing method

The characteristics of inverted sugar syrup prepared according to the existing and proposed technology are presented in Table 3.

Table 3

Characteristics of inverted sugar syrup obtained in the traditional way and using a rotor-pulsation apparatus

Method of treatment	Duration, min	Amount of inverted sugar, %	Content of hydroxymethylfurfural, mg/1000 g of syrup
90 ºC, without treatment in a potor-puslation apparatus	120	55	0.09
70 ºC, with treatment in a potor-puslation apparatus	5	100	-

The results indicate that application of proposed technology for production of inverted sugar syrup reduces the process duration from 120 min to 5 min, ensures almost complete hydrolysis of sucrose, and increases the amount of inverted sugar in solution from 55 to 100% without the formation of hydroxymethylfurfural.

Effect of the inversion duration on the composition of sugars at a temperature of 70ºC, pH 3.5 and the flow rate of 50×10^3 s^{-1}

Figure 3 shows the results of studies of the effect of the inversion duration on the composition of sugars at a temperature of 70 ºC, pH 3.5 and a shear flow rate of 50×10^3 s^{-1}.

Effect of the number of processing cycles in the rotary pulsation apparatus on the degree of sucrose inversion.

Results shown in Figure 4 indicate that complete inversion of sucrose was achieved by five times treatment in a rotor-pulsation apparatus in a circulating mode for 5 minutes at a temperature of 70 °C, pH 3.5 and shear flow rate of 50×10^3 s^{-1}.

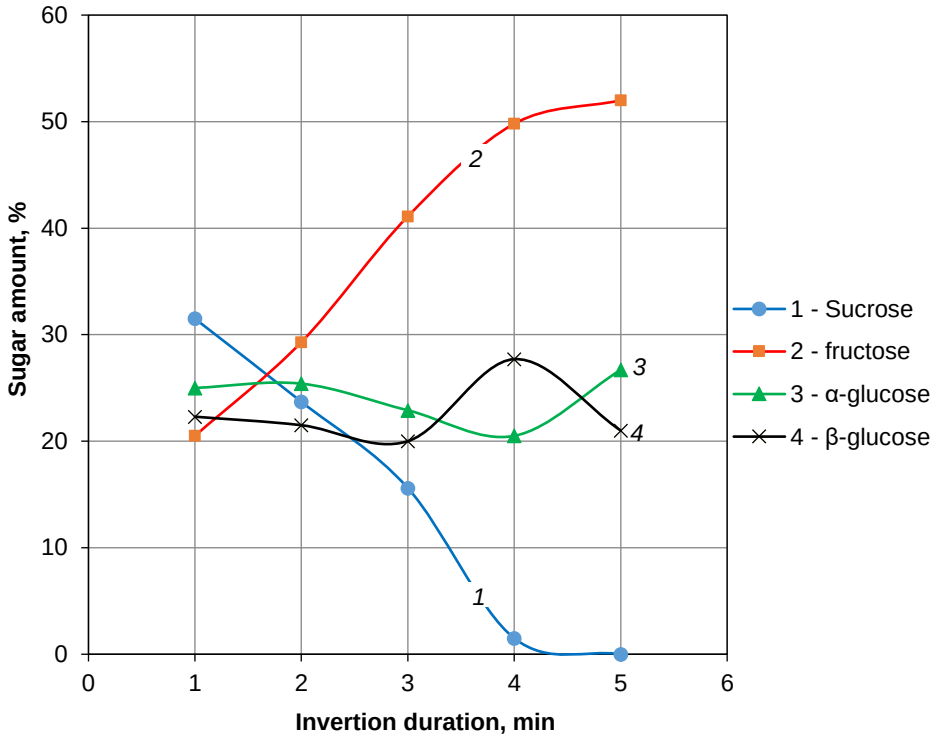

Figure 3. Production of sugars depending on the inversion duration at a temperature of 70 °C, pH 3.5 and a shear flow rate of 50×10^3 s^{-1}

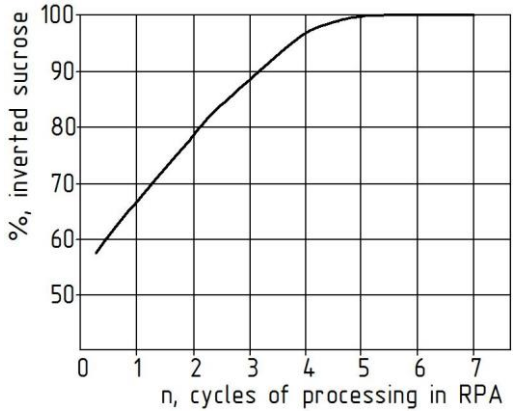

Figure 4. Amount of inverted sucrose depending on the number of cycles of glucose-fruit syrup processing in a rotor-pulsation apparatus

Therefore, the intensive inversion of sucrose using of rotor-pulsation treatment causes destruction of an acetal bond (an acetal oxygen bridge) (C – O – C) which joins glucose and fructose units in molecule of sucrose due to the simultaneous action of chemical and physical influences. The breaking energy of this bond is 1076 kJ/mol.

Critical stresses occur at the site of the sucrose chain and chemical covalent bonds are broken. The break of these bonds in the process of mechano-chemical influence occurs on the weakest in terms of energy bonds. In the case of mechanically activated hydrolysis, the destruction of the chains is localized at heterogeneous bonds, and in this respect, such processes do not differ from the corresponding purely chemical ones.

The breakdown of the sucrose chain usually follows the acetal bonds, but under certain conditions of the process, there is a breaking of carbon (C–C) bonds (Stick, 2001).

Action of citric acid weakens the (C–O) bond in sucrose and allows implementing a mechanically activated chemical process. Because of mechano-chemical influence on the section of the sucrose chain (C–O–C) there are critical stresses and the connection is broken. This leads to the formation of free radicals. One radical attaches the OH^- ion and another to H^+ ion As a result, the process of production of inverted sugar is intensified, i.e., the formation of a mixture of monosaccharides of glucose and fructose.

It is important that the proposed technology has significant advantages in terms of energy and resource saving characteristics (Dolinskiy et al., 2012).

Conclusion

The use of circulating five-time rotor-pulsation treatment of water-sugar solution in the circulating mode in the technology of inverted sugar syrups production at a temperature of 70 °C, pH 3.5 and a flow rate of 50×10^3 s^{-1} allows intensifying this process, namely:

– Reduce the duration of inversion to 5 minutes;
– Increase the amount of inverted sucrose from 55% to almost complete inversion;
– Exclude the formation of hydroxymethylfurfural.

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Biosynthesis and characterictics of silver nanoparticles obtained using *Saccharomyces cerevisiae* M437

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Abstract

Introduction. Due to the wide antimicrobial spectrum, silver nanoparticles (AgNPs) have great potential for use in the food industry to control foodborne pathogens.

Materials and methods. The culture supernatant and cell-free aqueous extract from biomass *Saccharomyces cerevisiae* M437 were used for the synthesis of AgNPs. The fact of the synthesis of biogenic AgNPs was confirmed by analysing the absorption spectra of the samples in the range of 200-700 nm. The size and zeta potential of AgNPs were determined using Zetasizer Nano ZS. The morphology of nanoparticles was examined using electron microscopy.

Results and discussion. Using spectral analysis in the UV-visible region, it was confirmed the formation of AgNPs in the investigated solutions. A pronounced absorption peak of AgNPs obtained using a cell-free aqueous extract from *S. cerevisiae* M437 was recorded in the wavelength range from 300 to 540 nm with a peak at 425 nm. For nanoparticles obtained using the supernatant, a widening spectral range of absorption was observed, which may be associated with the aggregation of AgNPs.

AgNPs synthesized using the supernatant *S. cerevisiae* M437 had a spherical shape with a diameter of about 15 nm. The polydispersity index (PdI) of AgNPs solutions was 0.3, and the zeta potential was 13.6 mV. After storage for 45 days at 4 °C, the PdI value increased 1.6 times, and the zeta potential increased by 11.7%. This may indicates a possible change in the shape of AgNPs, the formation of an agglomerate, or other processes that takes place in a colloidal solution during storage.

AgNPs that were obtained using a cell-free aqueous extract from biomass of *S. cerevisiae* M437 had an oval shape with a size of 21.3×14.2 nm. The PdI and zeta potential values were similar to the nanoparticles obtained using the supernatant. However, after storage, these values differed significantly: the value of PdI increased 1.3 times, and the zeta potential decreased by 29%. So, the solution of silver nanoparticles obtained in this way is more stable after storage under the specified conditions.

Conclusions. The possibility of extracellular synthesis of silver nanoparticles using the yeast *Saccharomyces cerevisiae* M437 has been shown. The shape, size, and zeta potential of biogenic AgNPs are described and their stability after storage is proved.

Introduction

Silver nanoparticles (AgNPs) have great potential to be used in the food industry due to a wide range of antimicrobial activity against foodborne pathogens such as *Listeria monocytogenes (*Du et al., 2019; Amer et al., 2021*), Campylobacter* (Silvan et al., 2018), *Vibrio parahaemolyticus*, *Escherichia coli*, *Salmonella typhimurium* (Du et al., 2019; Chandhru et al., 2019), *Staphylococcus aureus (*Yahya et al., 2021; Reddy et al., 2021*), Klebsiella pneumoniae (*Huang et al., 2020*),* and fungi of the genus *Aspergillus (Bocate* et al., 2019*).*

AgNPs can be used for the manufacturing of packaging materials for food products – nuts *(*Tavakoli et al., 2017*),* apricots *(*Shahat et al., 2020*),* strawberries (Oliveira et al., 2021*),* bell peppers (Kandasamy, 2020), shrimps (Paidari *et al., 2021*), poultry meat (Zhao et al., 2021*), and pork (Kuuliala* et al., 2015). Such packaging prolongs the shelf life of food and prevents the development of pathogenic bacteria (Sachdev et al., 2021).

The use of AgNPs in nanobiosensors allow increasing the sensitivity of detection in food and drinking water of bacterial pathogens such as *Escherichia coli* (Zhou et al., 2015; Qiao et al., 2021), *Staphylococcus aureus* (Gasparyan and Bazukyan, 2013; Hovhannisyan et al., 2017), and *Salmonella typhimurium* (Ma et al., 2021).

One of the potential uses of AgNPs is winemaking. Traditionally, sulfur dioxide is used as a preservative in winemaking, which poses certain risks for some groups of consumers. AgNPs, due to their antimicrobial action, reduce the use of sulfur dioxide in winemaking (Gil-Sanchez et al., 2019; Loira et al., 2020).

Another area of application of AgNPs in the food industry is their use as nanocatalysts, in particular, to accelerate the decomposition of starch due to the immobilization of α-amylase on the surface of AgNPs (Ernest et al., 2012; Krishnakumar et al., 2018).

Given the wide range of applications for AgNPs in the food industry, the demand for AgNPs is also growing. At the same time, the problems of economic and environmentally safe synthesis of AgNPs remain unresolved. There are various methods for the synthesis of AgNPs: chemical and physical methods, as well as biogenic synthesis. Due to a number of disadvantages inherent in the chemical and physical synthesis of nanoparticles – the use of aggressive, toxic and expensive reagents, high synthesis temperature or pressure, negative impact on the environment (Lekha et al., 2021; Halder et al., 2021), based on the above, the biological synthesis of nanoparticles provides promising alternative (Gaurav et al., 2019).

Plants, filamentous fungi, bacteria and yeast cells can be used for biogenic synthesis of nanoparticles (Kumar et al., 2021). The choice of each of these objects for the biosynthesis of nanoparticles has its advantages and disadvantages. Among the disadvantages of using plants are the use of large areas for their cultivation, the duration of growth, the cost of collecting plants and the extraction of biomolecules involved in the synthesis of nanoparticles. (Castillo-Henriquez et al., 2020). The main disadvantage in usage of filamentous fungi is in the long cultivation time (Bahrulolum et al., 2021). The use of bacteria compared to plants and fungi has a number of advantages. But it should be noted that bacteria are prokaryotes, so the products of their metabolism will be less biocompatible compared to eukaryotic models. Bacteria lack a system of capping and polyadenylation that protect biomolecules and nanoparticles from degradation and formation of toxic compounds in the body (Liu et al., 2021). Among the advantages of using yeast compared to bacteria is that yeast, unlike most bacteria, is simpler and safer to work with, since it does not require specific biosafety measures (Grasso, 2020).

Depending on the choice of biological producer, it`s cultivation conditions, as well as the parameters of biogenic synthesis, AgNPs of different sizes and shapes can be formed.

That will affect their further biological properties and applicability in the food industry. That is why the purpose of our work was to study the synthesis of silver nanoparticles using the culture fluid supernatant and a cell-free aqueous extract of the yeast *Saccharomyces cerevisiae* M437 and to check the stability after storage.

Materials and methods

Culture media and chemicals

Medium 1. Saburo medium (dextrose – 40.0 g/l, bacteriological agar – 15.0 g/l, a mixture (1:1) of fermented animal tissue and pancreatic casein hydrolysate – 10.0 g/l, pH = 5.6). This medium was used to store and passivate the culture of *S. cerevisiae* M437 (in test tubes on agar slant).

Medium 2. YPD medium (glucose – 20.0 g/l, peptone – 20.0 g/l, yeast extract – 10.0 g/l). This medium was used for the cultivation of *S. cerevisiae* M437 for further biosynthesis of silver nanoparticles.

Silver nitrate salt ($AgNO_3$, 99.99%) Sigma-Aldrich (Steinheim, Germany).

Cultivation of *Saccharomyces cerevisiae* M437

The culture of *S. cerevisiae* M437 from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technologies was maintained in a test tubes on agar slant at 4 °C. To prepare the inoculum, the culture was washed from the surface of the agar slant (the amount of inoculum with a titer of 10^4 to 10^5 cells/ml was 5% of the volume of the medium), and added to 150 ml of sterile YPD medium. Cultivation was carried out in 750 ml flasks with 150 ml of medium at 30 °C, 200 rpm for 24 hours. Cultivated until reaching OD_{600} = 2.

Biosynthesis of silver nanoparticles

Obtaining of supernatant. After 24 hours of the yeast culturing, the following procedure was performed: the culture fluid was centrifuged at 5.000 rpm for 30 min to separate the cells. The supernatant was separated from the cells by pouring into a sterile flask. Obtained supernatant was filtered through a sterile 0.22 μm syringe filter.

Obtaining of a cell-free aqueous extract. The culture fluid was centrifuged at 5000 rpm for 30 minutes. The supernatant was drained, and the cell pellet was washed three times from residual nutrient medium with sterile double-distilled water. The washed cells were resuspended in 150 ml of sterile double-distilled water and incubated at 30 °C, 320 rpm for 72 hours. After incubation, centrifugation was performed at 5000 rpm for 30 minutes. The precipitate was separated and the supernatant was used for further studies as a cell-free extract. The obtained cell-free extract was filtered through a sterile syringe filter with a pore diameter of 0.22 μm.

Biosynthesis conditions. A solution of silver nitrate was added to the supernatant or water extract to a final concentration of 1 mM. The samples were kept at 45 °C under static conditions for 72 hours. A 1 mM aqueous solution of silver nitrate, a YPD nutrient medium supplemented with 1 mM silver nitrate, an aqueous cell-free extract without the addition of silver nitrate solution, and a supernatant without the addition of silver nitrate solution were used as control samples. The control samples were incubated under the same conditions as the experimental ones.

Isolation of silver nanoparticles was performed as follows: centrifugation at 14.000 rpm for 20 min, the supernatant was drained, and the precipitate of nanoparticles was washed with deionized water. These steps were repeated three times (Jalal et al., 2018).

UV-vis spectroscopy of synthesized silver nanoparticles

The absorption spectra of the samples were measured using a UV-Vis (Thermo Spectronic UV300, Spectronic Unicam, England) spectrophotometer in the wavelength range of 200-700 nm (Hashim et al., 2020). The measurements were carried out in quartz cuvettes. Absorbance was measured with a resolution of 2 nm. Measurements were carried out 24, 48, and 72 h after the addition of silver nitrate to the test samples (cell-free aqueous extract, supernatant). Double distilled water was used as a blank experiment.

Analysis of the silver nanoparticles size and zeta potential

Analysis of nanoparticle's size were performed by determining the hydrodynamic diameter (HD) (Foujdar et al., 2021). The HD was monitored by dynamic light scattering (DLS) using a two-angle particle and molecular size analyzer Zetasizer Nano ZS (Malvern, UK). We performed all measurements at a constant temperature (25 °C) in a neutral medium (pH = 7.0) three times. For statistical calculations, we used Stat Plus Pro 5.9.8 software. Software and STATISTICA, version 8.0 (StatSoft, Inc. 2007).

Electron microscopy

Nanoparticle sizes and their general morphology were determined by electron microscopy (Kthiri et al., 2021). To achieve this, we prepared alcohol suspensions of nanoparticles, dried them at room temperature, and applied to copper grids with a carbon coating. Subsequently, we analyzed the samples using a transmission electron microscopy (JEM-1400 Jeol, Japan) at an accelerating voltage of 80 kV and an instrumental magnification of 50000 – 100000x.

Statistical analysis

All results are presented as the median of the values with interquartile range – Me [LQ – UQ], where Me = median (50% percentile), LQ = 25% percentile and UQ = 75% percentile. We tested the null hypothesis using the nonparametric Mann-Whitney test and the Wilcoxon matched pairs test (WMP-test). The difference between the compared groups was considered statistically significant at a value of $p < 0.05$ (Fay and Malinovsky, 2017). Calculations of median values with interquartile range with Microsoft Office Excel, 2019, and all statistical calculations – with Stat Plus Pro 5.9.8 software. Software and STATISTICA, version 8.0 were provided (StatSoft, Inc. 2007).

Results and discussion

Synthesis of Silver Nanoparticles and UV-Vis Spectral Analysis

After the addition of silver nitrate to the cell-free aqueous extract of *S. cerevisiae* M437, the color of the reaction mixture began to change from transparent to light brown and turned dark brown until the end of biosynthesis (72 h at 45 °C under static conditions) (Figure 1, A). When silver nitrate was added to the supernatant of *S. cerevisiae* M437 culture liquid, the

color of the reaction mixture at the end of biosynthesis changed from light brown to almost black (Figure 1, B). The color change is the first evidence of a successful biogenic synthesis of silver nanoparticles (Kthiri et al., 2021). The indicated color change of the reaction mixture (cell-free aqueous extract, supernatant) upon addition of silver nitrate is explained by the excitation of surface plasmon resonance (SPR), which indicates a decrease in silver ions (Ag^+) and their bioreduction to AgNPs (Rosman et al., 2020).

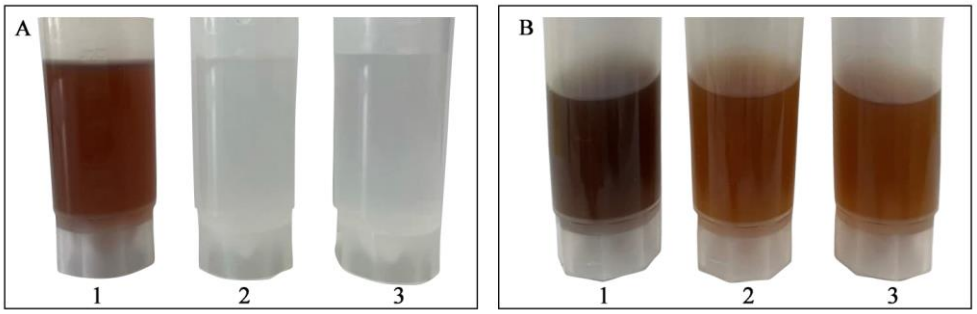

Figure 1. AgNPs biosynthesis. A: cell-free aqueous extract of *S. cerevisiae* M437 1 –with $AgNO_3$, 2 – without $AgNO_3$; 3 – solution of $AgNO_3$.
B: culture fluid supernatant of *S. cerevisiae* M437 1 – with $AgNO_3$, 2 – without $AgNO_3$; 3 – YPD medium with $AgNO_3$

The formation of silver NPs in the solution was confirmed by UV-Vis spectral analysis (Figure 2). As shown in the spectra, the absorption intensity increased with time without shifting the wavelength at which the maximum absorption was observed. This indicates a continuous decrease of the concentration of silver ions and an increase of the concentration of AgNPs, as well as a uniform distribution of nanoparticles in size (Xue et al., 2016; Win et al., 2020).

When a cell-free aqueous yeast extract was used for the biosynthesis of AgNPs (Figure 2, A), a pronounced absorption peak was observed in the wavelength range from 300 to 540 nm with an average wavelength at which a peak took place at about 425 nm. A similar surface plasmon resonance peak is described for AgNPs smaller than 50 nm (Win et al., 2020). When the supernatant of *S. cerevisiae* M437 culture fluid was used for the biosynthesis of AgNPs, an expansion of the absorption spectra was observed (Figure 2, B). This may be due to the aggregation of AgNPs or an increase in their size (Kumari et al., 2020).

Other authors have also shown the possibility of biosynthesis of AgNPs using yeast. When using psychrotrophic yeast *Yarrowia lipolytica* NCYC 789, the maximum absorption peak was 410 nm and the average nanoparticle size was 15 nm (Apte et al., 2013). The absorption peak of AgNPs during their synthesis using extremophilic yeast (genus and species were not indicated by the authors) was 420 nm, and the size was 4-15 nm (Mourato et al., 2011). While using *Saccharomyces cerevisiae* for the biosynthesis of AgNPs, obtained nanoparticles with a similar absorption peak, and sizes of 60-110 nm were obtained (Badhusha and Mohideen, 2016). It is considered that the shift of the light absorption peak towards the red spectral range indicates an increase in the size of AgNPs (Win et al., 2020), however, the following experimental data do not always confirm this. Thus, at a peak of 430 nm, the size of AgNPs synthesized using *Saccharomyces cerevisiae* was 2-20 nm (Korbekandi et al., 2016) and 60-80 nm (Saravanan et al., 2013), at the peak of 440 nm – 10-

60 nm (Sowbarnika et al., 2018), at the peak of 450 nm – 10 nm (Roy et al., 2015). Using the yeast *Candida* sp. VITDKGB, synthesized AgNPs had a size of 87 nm, and the absorption peak was at 430 nm (Kumar et al., 2011). When using the yeast *Rhodotorula* sp. ATL72 for the biosynthesis of AgNPs the nanoparticles with a size of 8-21 nm were received, and the light absorption peak was at 450 nm (Soliman et al., 2018).

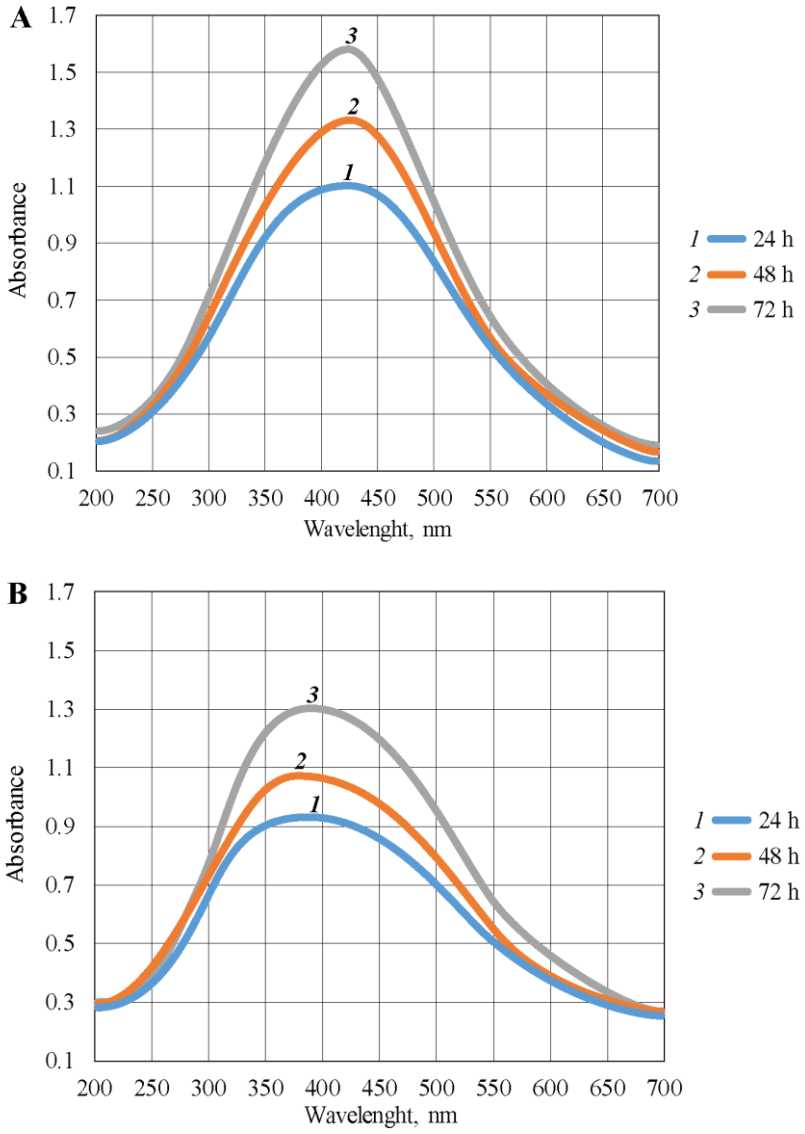

Figure 2. UV–vis spectra of biosynthesized AgNPs using a cell-free yeast extract (A) and culture fluid supernatant (B)

Size, zeta potential and stability of biosynthesized silver nanoparticles

The primary results obtained with the DLS are the particle intensity distribution values. The value of the intensity of the distribution of particles in solution is proportional to the value of the square of the molecular weight. This distribution is used to determine the increase in particle size in the sample. To analyze the reliability of the formation of nanocomplexes, we compared the PdI (polydispersity index) and Z-Average (average semi-variant) indices. Z-average is the main and most stable parameter of this method. It defines the average particle size as "the average value of the harmonic average particle diameter". The polydispersity index can determine the distribution of particles by size or mass. PdI is defined as the ratio of weight average molecular weight to number average molecular weight. This is a dimensionless value of the width of the particle size distribution. The calculations used for the determination of size and PDI parameters are defined in the ISO standard documents 13321:1996 E and ISO 22412:2008 (Worldwide, 2011). PDI value which is higher than 0,7 indicates that the sample has a very wide particle size distribution and is probably not suitable to be analysed by the dynamic light scattering (DLS) technique (Danaei et al., 2018). The higher the PdI value, the less monodisperse nanoparticles are in solution. Based on this, the lower the PdI value, the less capable of aggregation of nanoparticles in the sample and their size is in a narrow range of values (Skora et al., 2021).

The isolated nanoparticles were dispersed in double-distilled water at neutral pH and studied using Zetasizer Nano ZS. The size of AgNPs, which was synthesized using the supernatant of the culture medium of *S. cerevisiae* M437, was determined. The major part of nanoparticles has a size of 152.2 nm, which is close to the value of Z-average (Table 1). When testing the correctness of the null hypothesis using the Wilcoxon test, it was found that the values of the size distribution in Peak 1 and Z-average differ significantly ($p < 0.05$). The value of PdI is 0.3, which corresponds to a rather narrow range of particle size distribution.

Table 1

Characteristics of synthesized AgNPs using supernatant of culture medium *S. cerevisiae* M437

Index	Measurements were performed immediately after biosynthesis		
	Peak 1	**Peak 2**	**Peak 3**
Size, nm	152.2 [146.1; 154.2]	4848.0 [4519.0; 4997.0]	0.0 [0.0; 13.1]
Volume, %	94.4 [91.2; 96.2]	5.1 [3.9; 7.2]	0.0 [0.0; 1.9]
Z-average, nm	129.6 [127.5; 132.0]		
PdI	0.3 [0.3; 0.4]		
	Measurements were performed after 45 days of storage at 4 °C		
	Peak 1	**Peak 2**	**Peak 3**
Size, nm	204.4 [176.5; 229.1]	3779.0 [2448.5; 4504.0]	0.0 [0.0; 2148.0]
Volume, %	91.2 [80.7; 91.9]	8.8 [8.2; 15.6]	0.0 [0.0; 3.8]
Z-average, nm	156.1 [153.3; 163.8]		
PdI	0.5 [0.5; 0.6]		

In addition, the stability of dispersed in double-distilled water nanoparticles after 45 days storage at 4 °C was evaluated (Table 1). After storage, some changes in AgNPs size were observed. When testing the correctness of the null hypothesis using the Wilcoxon test,

it was found that the size distribution in Peak 1 and Z-average value was not differing significantly ($p > 0.05$). This indicates that after storage at these conditions, AgNPs have a spherical shape. The PdI value was 0.5, which corresponds to the average range of the nanoparticle size distribution range. Therefore, AgNPs after storage were likely to be degraded, or there were continuous transformation processes in the suspension, which led to an increase in the range of hydrodynamic diameter values. It should be noted that after saving the volume distribution changed a little (Table 1) and these changes were insignificant ($p > 0.05$). However, there was a significant ($p < 0.01$) increase in PdI by 40%, which directly indicated the increase in the range of hydrodynamic diameter values. This in turn confirms the presence of disintegration reactions and aggregation of the formed nanoparticles.

After storage, the hydrodynamic diameter of the synthesized AgNPs (Peak 1, Figure 3) increases by 25.5% ($p < 0.05$) and the Z-Average value increases by 17% ($p < 0.01$).

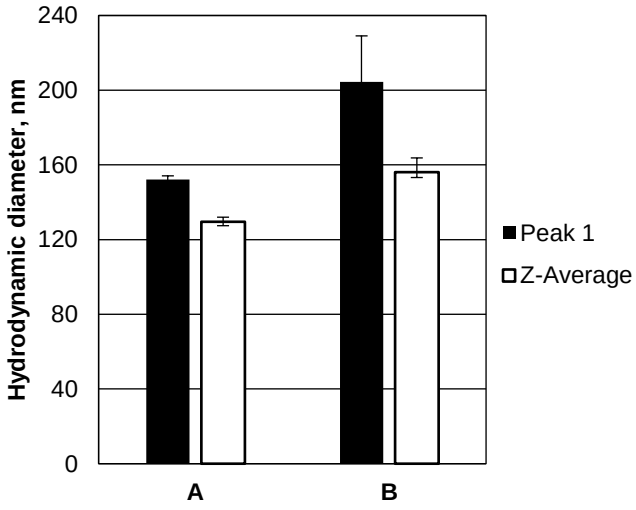

Figure 3. Sizes of only synthesized (A) AgNPs using *S. cerevisiae* M437 supernatant and after storage for 45 days (B)

Characteristics of AgNPs that were obtained using cell-free aqueous extract of *S. cerevisiae* M437 are given in table 2. The value of the distribution of nanoparticles in size in Peak 1 and Z-Average do not differ significantly ($p > 0.05$). The PdI value corresponds to a narrow range of particle size distribution. After storage of AgNPs for 45 days, there were some changes in the size and distribution of nanoparticles (Table 2). The value of PdI after storage increases by 25%, but this increase is not significant ($p > 0.05$). This suggests that AgNPs remain stable in colloidal solution.

When comparing the sizes of AgNPs obtained using a cell-free aqueous extract of *S. cerevisiae* M437 before and after storage (Peak 1, Figure 4), we see that the change in their hydrodynamic diameter is statistically insignificant ($p > 0.05$). The Z-Average decreases by 7% ($p > 0.05$), which is also unreliable. That is, there are no changes in the shape of the nanoparticles.

Table 2

Size distribution of AgNPs obtained using cell-free aqueous extract of *S. cerevisiae* M437

Index	Measurements were performed immediately after biosynthesis		
	Peak 1	**Peak 2**	**Peak 3**
Size, nm	163.4 [144.2; 186.1]	0.0 [0.0; 4444.5]	0.0 [0.0; 0.0]
Volume, %	100.0 [97.8; 100.0]	0.0 [0.0; 2.2]	0.0 [0.0; 0.0]
Z-Average, nm	143.6 [138.4; 164.1]		
PdI	0.3 [0.3; 0.4]		
	Measurements were performed after 45 days of storage at 4 °C		
	Peak 1	**Peak 2**	**Peak 3**
Size, nm	175.7 [173.7; 183.9]	4561.0 [343.0; 4714,0]	0.0 [0.0; 2485.5]
Volume, %	95.2 [90.8; 96.4]	4.8 [3.6; 6.1]	0.0 [0.0; 1.6]
Z-Average, нм	133.3 [132.0; 133.9]		
PdI	0.4 [0.4; 0.4]		

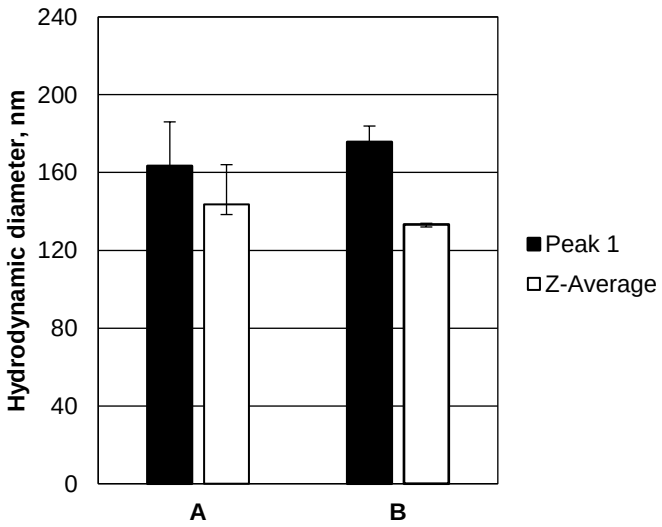

Figure 4. Sizes of synthesized (A) AgNPs using a cell-free aqueous yeast extract and after storage for 45 days (B)

Comparing the sizes of biogenic AgNPs obtained using the above two methods, it was evident that the value of their hydrodynamic diameter did not differ statistically (Figure 5). The difference between the Z-average values was significant ($p < 0.01$). This suggests that the shape of AgNPs obtained by different methods was different, which was confirmed by the TEM data.

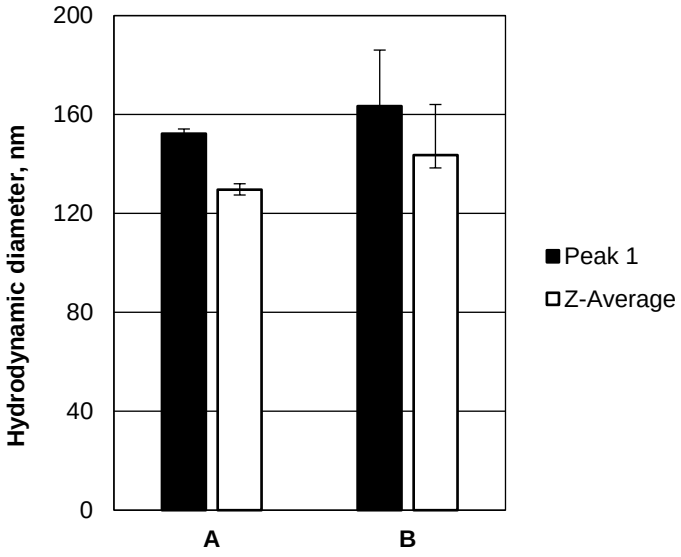

Figure 5. Hydrodynamic diameter of AgNPs obtained using supernatant (A) and cell-free aqueous extract (B) *S. cerevisiae* M437

We also compared the characteristics of AgNPs obtained using cell-free aqueous extract or supernatant of *S. cerevisiae* M437 after storage for 45 days at 4 °C (Figure 6). The hydrodynamic diameter of nanoparticles and the Z-average value were not statistically different ($p > 0.05$).

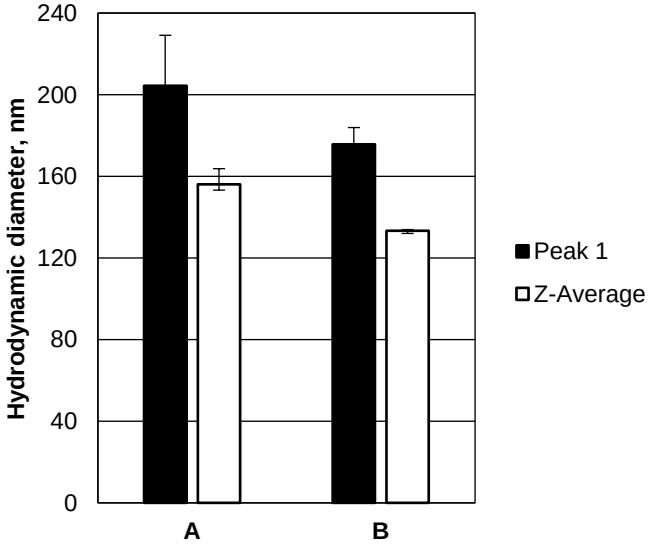

Figure 6. Hydrodynamic diameter of AgNPs obtained using supernatant (A) and cell-free aqueous extract (B) from *S. cerevisiae* M437 after storage for 45 days

Comparing the polydispersity of the studied AgNPs shows that the nanoparticles obtained using a cell-free aqueous extract from *S. cerevisiae* M437 are 27.2% ($p < 0.01$) less polydisperse than the nanoparticles obtained using the supernatant of the *S. cerevisiae* M437 culture liquid (Tables 1 and 2). This indicates a more uniform distribution of nanoparticles in size after storage under the specified conditions.

Zeta potential is an important indicator of the surface charge of nanoparticles (Kthiri et al., 2021). The surface charge of AgNPs is related to their dispersity and stability (Leo et al., 2013). The high value of the negative zeta potential indicates long-term stability and dispersion, lack of flocculation and the tendency to form agglomerates of biogenic AgNPs due to the repulsion of negatively charged particles (Skoglund et al., 2017; Win et al., 2020; Foujdar et al., 2021).

The value of the zeta potential for AgNPs obtained using the supernatant of the culture liquid of *S. cerevisiae* M437 was -13.6 [-13.8; -13.1] mV. After 45 days of storage at 4 °C, the zeta potential increased by 11.7% and was -12.0 [-12.6; -11.0] mV. For AgNPs obtained using the cell-free aqueous extract from *S. cerevisiae* M437, the value of the zeta potential was -13.7 [-14.5; -13.5] mV, and after storage it was -19.3 [-20.1; -18.6] mV. That is a 29% decrease in the zeta potential.

Obtained results indicate that negatively charged ions are present on the surface of AgNPs. Due to the repulsion between them, biogenic AgNPs were stable in solution for 45 days of storage. At the same time, nanoparticles obtained using a cell-free aqueous extract from *S. cerevisiae* M437 were more stable due to the more negative charge of their surface.

Electron microscopy

The morphology and size of biogenic AgNPs were examined by transmission electron microscopy (TEM). Silver nanoparticles were relatively homogeneous and close to a spherical shape (Figure 7) with dimensions of less than 30 nm and tended to form groups or aggregates.

The median size of nanoparticles and their aggregates was calculated according to the internal scale mark (bar) indicated on the microphotographs (Table 3).

Table 3

Size of biogenic silver nanoparticles

Origin of the nanoparticles	Aggregate		Separate nanoparticle	
	Width, nm	Length, nm	Width, nm	Length, nm
Obtained using a cell-free aqueous extract from *S. cerevisiae* M437	39.1 [23.1; 69.2]	32.0 [23.1; 47.9]	21.3 [14.2; 24.9]	14.2 [14.2; 21.3]
Obtained using the supernatant of the culture liquid of *S. cerevisiae* M437	237.6 [132.0; 257.4]	109.2 [60.6; 195.0]	15.6 [10.8; 32.4]	15.6 [10.8; 27.0]

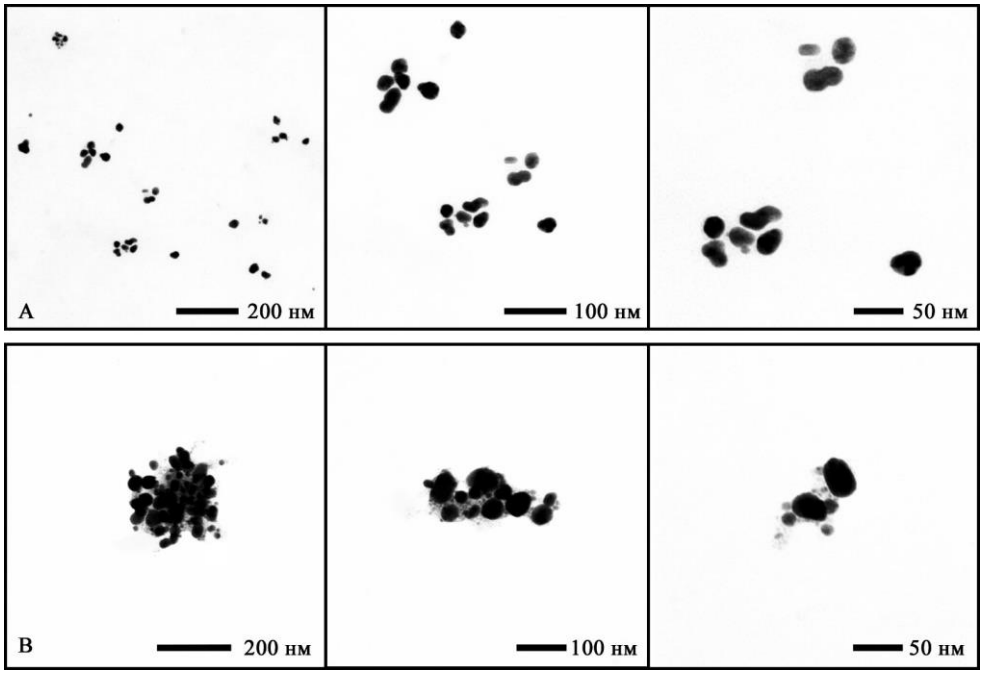

Figure 7. Electronic micrographs of AgNPs obtained using cell-free aqueous extract (A) and supernatant of culture liquid *S. cerevisiae* M437 (B)

There are no significant differences in size between AgNPs synthesized using the supernatant and using a cell-free aqueous extract of the studied yeast (Table 3). During the synthesis of AgNPs using a cell-free extract, there is a tendency to the formation of nanoparticles close to oval, and during the synthesis of AgNPs using the supernatant, they are close to spherical. It is also noticeable the feature of AgNPs synthesized using the supernatant *S. cerevisiae* M437 to form relatively large aggregates of nanoparticles.

It was previously reported that when using the supernatant of the culture liquid *Candida utilis* NCIM 3469, no biosynthesis of AgNPs was observed when $AgNO_3$ was added to a final concentration of 2 mM at a temperature of 30 °C (Waghmare et al., 2015). At the same time, using the supernatant of *Candida glabrata* at room temperature of biosynthesis, it was possible to obtain AgNPs of spherical and oval shapes with sizes of 2-15 nm (Jalal et al., 2018). An interesting study is where *S. cerevisiae* BY4741 was grown under the influence of a moderate static magnetic field, after which the culture broth supernatant was used for the biosynthesis of AgNPs. The resulting nanoparticles were mostly spherical and had a size of 2-12 nm (Kthiri et al., 2021).

Using a cell-free extract of red yeast *Phaffia rhodozyma*, quasi-spherical AgNPs with a diameter of 4.1 nm were obtained. The silver nitrate was added to the reaction mixture at a final concentration of 0.1 M (Ronavari et al., 2018). Synthesis of AgNPs using cell-free aqueous extract of *S. cerevisiae* was shown by Skora with co-authors. The authors obtained spherical AgNPs with an average diameter of 20.1 nm. The temperature of biosynthesis was 60 °C (Skora et al., 2021).

Thus, depending on the choice of conditions for biogenic synthesis of nanoparticles, it is possible to obtain different sizes of AgNPs. The sizes of AgNPs, which were determined using TEM, were smaller than sizes determined using the Zetasizer Nano ZS. This can be explained by the presence of surface proteins, carbohydrates, and other cellular compounds that can participate in the stabilization of biogenic nanoparticles, which were measured by Zetasizer. However, these compounds are not fixed when using the TEM method, since they cannot be retained on the surface of nanoparticles in vacuum under an electron beam (Xue et al., 2016). Also, it should be said that the Zetasizer Nano device cannot differentiate individual nanoparticles from their aggregates, therefore it perceives a separate aggregate as a separate particle.

The obtained different values of the sizes of biogenic AgNPs can be explained by the constantly running processes of decomposition and formation of new aggregates of nanoparticles occurring simultaneously. This hypothesis can be refined in future studies using stabilizers and the study of samples using the Zetasizer Nano in dynamics for a long time.

Conclusions

The possibility of mediated synthesis of AgNPs by the *Saccharomyces cerevisiae* M437 yeast strain from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technologies have been shown. In this case, the synthesis of AgNPs was observed both when using the culture fluid supernatant and the cell-free aqueous extract from *S. cerevisiae* M437. The size and shape of the nanoparticles that were obtained using these two methods did not actually differ. But we found a difference in the specific sizes of AgNPs using different methods (Zetasizer Nano ZS and TEM). Perhaps this difference is due to organic compounds involved in the formation and stabilization of nanoparticles. However, these compounds are not fixed when using the TEM method, since they cannot be retained on the surface of nanoparticles in vacuum under an electron beam. A difference in the stability of biogenic AgNPs after storage in solution for 45 days was also observed. At the same time, the nanoparticles obtained with the use of a cell-free aqueous yeast extract turned out to be more stable. In the future, the antimicrobial activity of biogenic AgNPs with a view to their further use in the food industry will be studied.

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Intensification of microbial exopolysaccharide ethapolan synthesis on the mixture of energy-excessive substrates

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Abstract

Introduction. The cultivation conditions of *Acinetobacter* sp. IMV B-7005, providing maximum synthesis of exopolysaccharide (EPS) ethapolan on the mixture of ethanol and sunflower oil were studied, as well as the possibility of replacing refined oil in the mixture with ethanol on a waste one was demonstrated.

Materials and methods. Strain IMV B-7005 was grown in liquid mineral media, containing the mixture of ethanol and sunflower oil of various quality, as well as appropriate monosubstrates. The optimal molar ratio of the concentrations of substrates in the mixture was calculated theoretically according to Babel's concept. The EPS concentration was determined gravimetrically after precipitation with isopropanol, the EPS-synthesizing ability – as the ratio of the EPS concentration to the concentration of biomass and expressed in g EPS/g biomass.

Results and discussion. The highest rates of ethapolan synthesis were observed with the molar ratio of concentrations of ethanol and refined sunflower oil in the mixture of 1:0.056, as close as possible to the theoretically calculated (1:0.076), and the use of inoculum grown on ethanol. Further increasing of the concentrations of ethanol and oil led to a decrease in pH of the culture fluid to a suboptimal level for the EPS synthesis (4.5-4.8). To ensure the synthesis of ethapolan on a medium with high concentrations of ethanol (4%) and oil (1.2%) ammonium nitrate was replaced with an equimolar amount of nitrogen KNO_3 (0.8 g/l), which is transported into cells by the symport with proton; fractional introduction of substrates in five equal portions during cultivation was carried out and was increased the concentration of Mg^{2+} cations, which are one of the activators of acetyl-CoA synthetase in *Acinetobacter* sp. IMV B-7005 affecting the enzymatic activity of systems responsible for the catabolism of fatty acids. Under such cultivation conditions, regardless of the type of used sunflower oil (refined or mixed waste) in the mixture with ethanol, the concentration of ethapolan reached 13.5-16.0 g/l, and EPS-synthesizing ability – 3.1-3.7 g EPS/g of biomass, which were respectively 3.2-3.8 and 1.6-1.9 times higher than before optimization.

Conclusions. Based on determining the optimal molar ratio of monosubstrate concentrations in the mixture, modification of the medium composition (replacement of ammonium nitrate with potassium nitrate, increasing the content of magnesium cations, replacement of refined oil on a mixed waste one) and fractional addition of substrates the possibility of intensification of ethapolan synthesis on the mixture of energy-excessive substrates (ethanol and sunflower oil) was established.

Introduction

Production of microbial exopolysaccharides (EPS) is growing every year worldwide (Rana *et al.*, 2020). This is mainly due to the physicochemical and functional properties of these polymers (gelling and emulsifying abilities, ability to retain a large amount of water and alter rheological properties of water systems, anticancer, antioxidant, antimicrobial, antiviral, anti-inflammatory, and immunomodulatory activities, etc.), which make possible their use in various industries, from food to petroleum (Barcelos *et al.*, 2020).

Nevertheless, the same commercially successful EPS (xanthan, dextran, gelan, alginate, levan, pullulan, welan, scleroglucan, emulsan, hyaluronic acid, etc.), some of which have been known since the first half of the twentieth century, continue to be used in most industries for decades (Donot *et al.*, 2012).

At the same time, most of the new microbial polysaccharides continue to be only at the stage of fundamental research (Zayed *et al.*, 2021). Nowadays, the presence of unique properties is not enough for the industrial implementation of these polymers (Fukuda *et al.*, 2021; Rana *et al.*, 2020). In addition, they must be cheap, which in the biotechnological production of microbial EPS is mainly determined by the final concentration of the target product, the cost, and efficiency of consumption of carbon and energy sources (Barcelos *et al.*, 2020).

One approach to the intensification of microbial synthesis and increasing the efficiency of substrates transformation into biomass and secondary metabolites is the use of a mixture of growth substrates (Babel *et al.*, 1985; Pidhorskyy *et al.*, 2010). In our previous studies, this approach has been successfully used to increase the polysaccharide ethapolan synthesis (produced by *Acinetobacter* sp. IMV B-7005) (Pidhorskyy *et al.*, 2010).

The mixture usually consists of a combination of two substrates, which depending on the amount of energy generated during their catabolism to the central carbon precursor – phosphoglyceric acid (PGA), are divided into energy-excessive and energy-deficient (Babel *et al.*, 1985; Pidhorskyy *et al.*, 2010).

If the amounts of ATP and reducing equivalents produced in the transformation of the substrate to PGA are sufficient for the synthesis of cellular components, such substrate is energy-excessive (Babel *et al.*, 1985; Pidhorskyy *et al.*, 2010). The substrates which must be partially oxidized to CO_2 in order to obtain the energy necessary for constructive metabolism are energy-deficient (Babel *et al.*, 1985; Pidhorskyy *et al.*, 2010).

It should be noted that according to the classical concept of the auxiliary substrate, the use of a mixture of two energy-excessive substrates is not possible (Babel *et al.*, 1985). Meanwhile, it is known about the possibility of using mixed substrates for both energy needs and biomass synthesis (Pidhorskyy *et al.*, 2010).

We suggested that it can be possible to intensify the synthesis of ethapolan by using a mixture of two energy-excessive substrates. Ethanol and sunflower oil were selected as substrates for the studies, because during the growth on them high rates of ethapolan synthesis by strain IMV B-7005 were observed (Pidhorskyy *et al.*, 2010).

In connection with the above, the aim of this work was to establish the cultivation conditions of *Acinetobacter* sp. IMV B-7005 for the maximum indicators of the exopolysaccharide ethapolan synthesis on the mixture of ethanol and sunflower oil, as well as to study the possibility of replacing the refined oil in the mixture with a waste one.

Materials and methods

Materials

The strain *Acinetobacter* sp. 12S, deposited in the Depository of Institute of Microbiology and Virology, National Academy of Sciences of Ukraine under the number IMV B-7005 was used for microbial polysaccharide ethapolan synthesis (Pidhorskyy *et al.*, 2010).

The complex polysaccharide preparation ethapolan consists of one neutral and two acidic EPS, one of which is acylated (AP). The acylated and non-acylated (NAP) polysaccharides are identical in molar ratios of *D*-glucose, *D*-mannose, *D*-galactose, *L*-rhamnose, *D*-glucuronic acid and pyruvic acid (3: 2: 1: 1: 1: 1) and the structure of repeated unit of the carbohydrate chain. The difference between those EPS is that the acylated polysaccharide contains fatty acids (C_{12}-C_{18}) (Pidhorskyy *et al.*, 2010).

Medium composition and cultivation conditions

The IMV B-7005 strain was grown in such liquid mineral medium (g/l): *medium 1 (basic)*: KH_2PO_4 – 6.8; KOH – 0.9; NH_4NO_3 – 0.6; $MgSO_4 \times 7\ H_2O$ – 0.4 (1.6 мМ); $CaCl_2 \times 2H_2O$ – 0.1; $FeSO_4 \times 7\ H_2O$ – 0.001 (Pidhorskyy *et al.*, 2010); *medium 2* is similar to medium 1, but NH_4NO_3 was replaced with KNO_3 (1.5 g/l); *medium 3* is similar to medium 2, but the concentration of $MgSO_4 \times 7\ H_2O$ was 1.25 g/l (5.0 mM).

An additional 0.5% (v/v) of yeast autolysate was added into the medium, as well as the multivitamin complex "Complevit" at a concentration of 0.00085 (w/w by pantothenate) (Pidhorskyy *et al.*, 2010; Pirog *et al.*, 2020).

The following types of substrates were used as a carbon and energy source: *monosubstrates* – ethanol 1.66% (v/v), refined sunflower oil 1.0% (v/v); *mixed substrates* – the mixture of ethanol (1.0-4.0%) and refined oil (0.2-1.2%). In one variant refined oil was replaced with the mixed waste one (after frying meat, potatoes, onions, cheese from "RockerPub", Kyiv).

In some variants the initial concentration of ethanol in the medium was 0.66-2.0% and oil 0.2-0.6%, respectively. During the cultivation process every 24 h these substrates were fractionally applied (fed-batch) in portions (total 1-4 portions) of 0.66-2.0% (ethanol) and 0.2-0.6% (oil).

The culture in exponential growth phase, grown in a medium with ethanol (0.5%), oil (0.5%) or the mixture of ethanol (0.25%) and refined oil (0.25%) was used as inoculum. Concentration of inoculum was 10% (Pidhorskyy *et al.*, 2010; Pirog *et al.*, 2020).

Cultivation of IMV B-7005 strain was carried out in the flasks (750 ml) with 100 ml of medium in shaker (320 rpm) at 30 °C for 120 hours (Pidhorskyy *et al.*, 2010; Pirog *et al.*, 2020).

Biomass and ethapolan estimation

Biomass concentration was determined by optical density of cell suspension with subsequent recalculation to dry biomass in accordance with the calibration curve (Pidhorskyy *et al.*, 2010).

The amount of EPS was determined gravimetrically (Pidhorskyy *et al.*, 2010). For this purpose, 1.5-2 volumes of isopropanol were added to a certain volume of the culture fluid (usually 10-15 ml). The EPS precipitate was washed with pure isopropanol and dried at room temperature for 24 hours.

EPS-synthesizing ability was calculated as the ratio of the EPS concentration to the

biomass and expressed in g EPS/g biomass (Pidhorskyy *et al.*, 2010).

Determination of the optimal molar ratio of substrates concentrations

Determination of the optimal molar ratio of substrates concentrations (ethanol and refined sunflower oil) in the mixture was based on the corresponding theoretical calculations (Pirog *et al.*, 2020). Taking into account that during the metabolism of each energy-excessive substrate to PGA a sufficient amount of ATP and reducing equivalents for the synthesis of cell components is produced, it is necessary to: 1) calculate the energy generation during the EPS synthesis on each substrate; 2) determine the energy distribution of each substrate that occurs after the biomass synthesis.

Determination of energy expenditures for the formation of ethapolan from ethanol was calculated on the basis of the activity of Krebs cycle enzymes, glyoxylate cycle and gluconeogenesis of the strain *Acinetobacter* sp. IMV B-7005, as well as on the calculations as described earlier (Pidhorskyy *et al.*, 2010). Energy generation in the catabolism of linoleic and oleic acids was calculated as described earlier (Pirog *et al.*, 2020).

Since the ethapolan contains residues of fatty acids in its composition, during calculations it was additionally assumed that part of fatty acids of the oil can be used without significant transformations for the esterification of the carbohydrate chain of the EPS. Thus, after the biomass synthesis from fatty acids of oil and ethanol is generated 25 and 75% of total amount of energy, respectively. Other assumptions were similar to those described in the paper (Pirog *et al.*, 2020).

Statistical data processing

All experiments were conducted in three repetitions; the number of parallel definitions in the experiments was from three to five. Statistical processing of experimental data was carried out as described earlier (Pidhorskyy *et al.*, 2010; Pirog *et al.*, 2020). Differences in average indicators were considered reliable at the level of significance $p < 0.05$.

Results and discussion

Theoretical calculation of the optimal molar ratio of ethanol and sunflower oil in the mixture

From the schemes shown in Figures 1 and 2, it is possible to calculate the amount of energy generated during the synthesis of the repeating unit of EPS from ethanol and fatty acids (oleic and linoleic) that prevail in the composition of sunflower oil. The corresponding summary data are represented in Table 1 and 2.

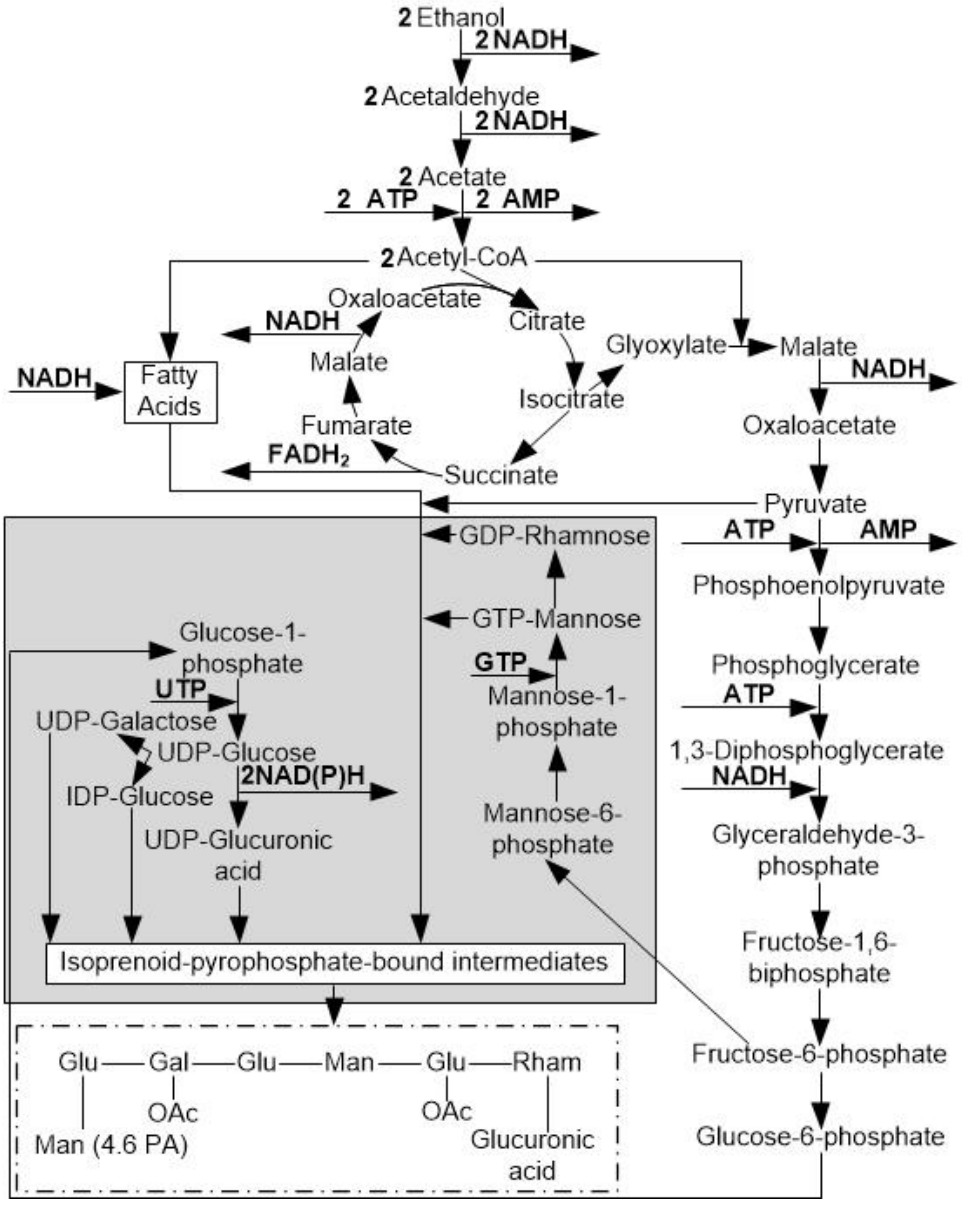

Figure 1. Synthesis scheme of repetitive units in the process of ethanol catabolism (literature data are grayed out)

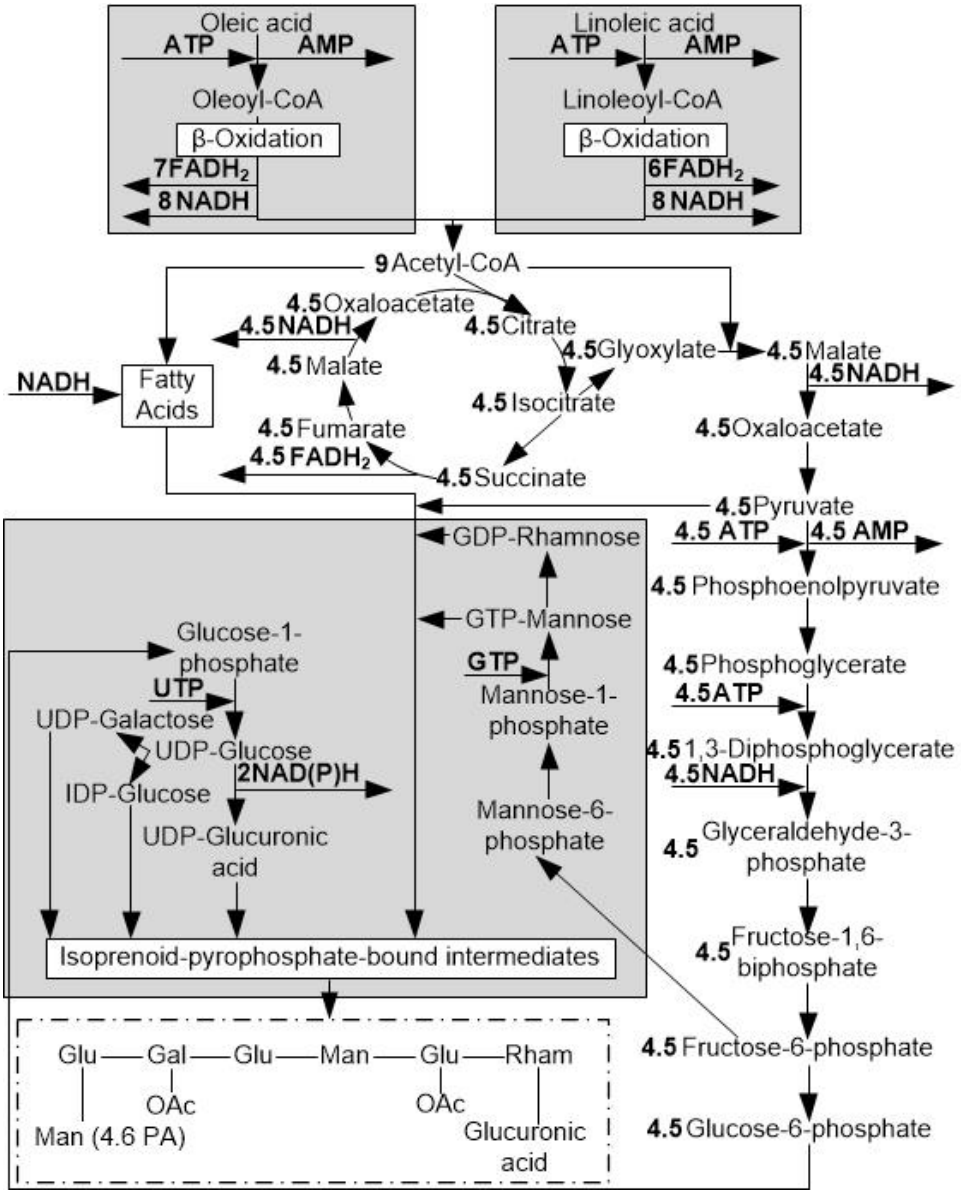

Figure 2. Synthesis scheme of repetitive units in the process of oleic and linoleic acids catabolism (literature data are grayed out)

Table 1

Energy generation during synthesis of the EPS components from ethanol and sunflower oil

Substrate for the EPS biosynthesis	Stage	Consumption of substrate for the synthesis of the EPS unit, mol	Energy generation, mol ATP
Ethanol	Synthesis of monosaccharides	16.00	51.00
	Synthesis of fatty acids	14.00	29.00
	Synthesis of pyruvate	2.00	11.00
Sunflower oil	Synthesis of monosaccharides	1.78	41.28
	Synthesis of fatty acids	2.00	12.00
	Synthesis of pyruvate	0.22	9.78

Table 2

Energy expenditures of acylated and non-acylated polysaccharides synthesis from ethanol and sunflower oil

Substrate for the EPS biosynthesis	EPS	Consumption of substrate for the synthesis of the EPS unit, mol	Energy generation, mol ATP	
			for the EPS unit synthesis	per mol of used acetate
Ethanol	AP	32	91.00	2.84
	NAP	18	62.00	3.40
	AP + NAP	50	152.00	3.04
Sunflower oil	AP	4	63.06	15.77
	NAP	2	51.06	25.53
	AP + NAP	6	114.12	19.02

Thus, the total energy expenditure for the synthesis of the repetitive unit of AP and NAP (AP + NAP) is: 3.04 + 19.02 = 22.06 mol ATP/mol of used substrate.

According to the calculations presented in (Pidhorskyy *et al.*, 2010; Pirog *et al.*, 2020), the energy generation during the conversion of ethanol and oil to PGA is 5.0 and 39.5 mol of ATP/mol of the used substrate, respectively (equations 1 and 2).

$$C_2H_5OH \rightarrow 0.5\ PGA + 5\ ATP; \quad (1)$$

$$0.5\ C_{17}H_{31}COOH + 0.5\ C_{17}H_{33}COOH \rightarrow 4.5\ PGA + 39.5\ ATP. \quad (2)$$

Synthesis of biomass from PGA (using an ammonium nitrogen source) can be represented by the equation (Pidhorskyy *et al.*, 2010):

$$4\ PGA + NH_3 + 29\ ATP + 5.5\ NAD(P)H \rightarrow (C_4H_8O_2N_1)_3, \quad (3)$$

where $(C_4H_8O_2N_1)_3$ is the formula of one biomass mole.

On the basis of the equation for biomass synthesis from PGA (Equation 3) and the equation of ethanol and oil catabolism to PGA (Equation 1 and 2), as well as the information shown in Table 2, it can be calculated that under cultivation on these substrates after synthesis of biomass and EPS remains 3.04 and 13.52 mol ATP/mol of used substrate, respectively, or 16.56 mol ATP in total.

Assuming that 75% and 25% of excess energy are generated from ethanol and oil, respectively, 4.09 mol of ethanol and 0.31 mol of oil are required to generate 16.56 mol of ATP, which corresponds to a molar ratio of 1:0.076.

For example, at an ethanol concentration of 1% (v/v, 10 ml/l or 8 g/l, 0.12 mol), the oil concentration should be 0.013 mol, or 3.66 g/l, or 3.98 ml/l, or 0.4% (v/v).

Experimental verification of the molar ratio of concentrations of monosubstrates in the mixture

It has been found that regardless of the method of inoculum preparation, the indicators of the ethapolan synthesis were 1.3-2.1 times higher than those obtained on the corresponding monosubstrates (Table 3). In further studies, the inoculum was grown on ethanol, because when used the concentration of the final product was maximum (3.18 g/l).

Table 3

Synthesis of ethapolan depending on the method of inoculum preparation

Concentration of substrates in the medium for		EPS concentration, g/l	EPS-synthesizing ability, g EPS/g biomass
biosynthesis, %	inoculum preparation, %		
Ethanol, 1.0, and oil, 0.4*	Ethanol, 0.5	3.18±0.16	1.85±0.09
	Oil, 0.5	2.14±0.11	1.39±0.07
	Ethanol, 0.25, and oil, 0.25	2.00±0.10	0.84±0.04
Ethanol, 1.66	Ethanol, 0.5	1.49±0.07	0.93±0.05
Oil, 1.00	Oil, 0.5	1.63±0.08	1.10±0.06

Notes. 1. Cultivation was carried out in the medium 1. **2.** * – molar ratio of concentrations of ethanol and sunflower oil in the mixture was 1:0.076.

Since theoretical calculations, due to several assumptions, allow only to approximately determining the molar ratio of substrates in the mixture, the synthesis of polysaccharide at different molar ratios of ethanol and oil concentrations in the mixture was further investigated.

It was shown that the highest indicators of the ethapolan synthesis (EPS concentration 4.2 g/l, EPS-synthesizing ability – 2.0 g EPS/g biomass) were observed for the 1:0.056 molar ratio of monosubstrates in the mixture, maximally approximated to the theoretically calculated (1:0.076) (Table 4). In our opinion, this deviation from the theoretical ratio is due to the uneven involvement of each substrate in energetic and constructive metabolism.

Table 4

Influence of molar ratio of ethanol and refined oil in the mixture on the ethapolan synthesis

Concentration of substrates in the mixture, %		Molar ratio of ethanol and oil	EPS concentration, g/l	EPS-synthesizing ability, g EPS/g biomass
ethanol	oil			
1.0	0.2	1:0.036	2.10±0.11	0.49±0.02
1.0	0.3	1:0.056	4.23±0.21	2.00±0.10
1.0	0.4	1:0.076	3.18±0.16	1.85±0.09
1.0	0.5	1:0.096	2.94±0.15	1.20±0.06
1.0	0.6	1:0.116	2.67±0.13	0.79±0.04

Notes. 1. Cultivation was carried out in the medium 1. **2.** Inoculum was grown on ethanol.

Intensification of ethapolan synthesis on the mixture of refined sunflower oil and ethanol

One of the important indicators of the effectiveness of technologies for obtaining practically valuable microbial metabolites that directly affects the possibility of their industrial implementation is the concentration of the target product (Fukuda *et al.*, 2021).

It should be noted that the indicators of EPS synthesis on mixed substrates depend not only on the optimal molar ratio of their concentration in the mixture, but also on the total concentration of monosubstrates in the mixture (Pidhorskyy *et al.*, 2010).

Experiments have shown that increasing the concentrations of ethanol and oil in the mixture in two-folds to 2.0% and 0.6%, respectively, was not only led to an increase in the amount of synthesized ethapolan by 1.2 times, but also was accompanied by a decrease in the pH of the culture fluid from 7.2 to 5.5 (optimum for the ethapolan synthesis 7.0-8.0). Further increase in the concentration of ethanol (3.0-4.0%) and oil (0.9-1.2%) in the cultivation media led to a drastic decrease in the indicators of EPS synthesis (EPS concentration 1.9-2.4 g/l, EPS-synthesizing ability – 1.0 g EPS/g biomass) and pH of the cultured fluid (4.5-4.8).

This decrease in pH can be due to several reasons, including the consumption of NH_4NO_3 through the antiport with proton and limitation of acetate metabolism due to low activity of acetyl-CoA synthetase, which catalyzes the assimilation of this substrate in the strain IMV B-7005. To solve this problem, the nitrogen source in the culture medium was replaced by an equimolar nitrogen concentration of KNO_3, the assimilation of which is carried out by the symport with proton and is accompanied by an increase in pH of the cultural fluid, as well as fed-batch was applied.

It was found that when replacing the nitrogen source, regardless of the mode of fractional application of substrates, the pH of the culture fluid during cultivation was maintained at an acceptable level for the synthesis of EPS (Table 5). The highest rates of ethapolan synthesis (EPS concentration 13.5 g/l, EPS-synthesizing ability – 3.5 g EPS/g biomass) were observed when reducing the initial concentration of monosubstrates in the mixture to 1/5 of their total content, followed by their fractional application during the cultivation to the final concentration of ethanol 4.0% and oil 1.2%.

Table 5

Influence of nitrogen source replacement and fractional application of substrates on ethapolan biosynthesis on the mixture of ethanol and refined oil

Nitrogen source	Concentration of monosubstrates in the mixture, %	Fractional substrate addition mode*	pH_{end}	EPS concentration, g/l	EPS-synthesizing ability, g EPS/g biomass
NH_4NO_3 (medium 1)	Ethanol, 2.0, and oil, 0.6	Without fractional application (control)	5.5	5.01±0.25	1.95±0.10
		Two portions of 1.0% ethanol and 0.3% oil	5.6	8.02±0.40	1.81±0.09
		Three portions of 0.66% ethanol and 0.2% oil	5.4	9.45±0.47	2.65±0.13
	Ethanol, 4.0, and oil, 1.2	Two portions of 2.0% ethanol and 0.6% oil	5.1	6.82±0.34	1.40±0.07
		Three portions of 1.33% ethanol and 0.4% oil	5.2	8.07±0.40	1.25±0.06
KNO_3 (medium 2)	Ethanol, 2.0, and oil, 0.6	Without fractional application (control)	7.0	5.16±0.26	1.52±0.08
		Two portions of 1.0% ethanol and 0.3% oil	6.4	9.27±0.46	2.10±0.11
		Three portions of 0.66% ethanol and 0.2% oil	6.6	8.36±0.42	2.17±0.11
	Ethanol, 4.0, and oil, 1.2	Two portions of 2.0% ethanol and 0.6% oil	5.7	7.45±0.37	1.18±0.06
		Three portions of 1.33% ethanol and 0.4% oil	5.9	11.38±0.57	1.59±0.08
		Four portions of 1.0% ethanol and 0.3% oil	5.9	11.88±0.59	3.08±0.15
		Five portions of 0.8% ethanol and 0.24% oil	6.0	13.51±0.68	3.65±0.18

Notes:

1. Inoculum was grown on ethanol.

2. * – during the cultivation process fractional addition of substrates was carried out every 24 hours

Replacement of the refined oil in the mixture with ethanol on a waste one

It is known that substrate costs can be up to 50% of the final cost of the target product (Fukuda *et al.*, 2021). To further reduce the cost of ethapolan, the refined oil in the mixture with ethanol was replaced on a mixed waste one, which is usually formed by mixing various fried oils before sending for utilization. It should be noted that these experiments were carried out with an additional increase in the cultivation medium of the concentration of Mg^{2+} cations, affecting the enzymatic activity of systems responsible for fatty acid catabolism and are one of the activators of acetyl-CoA synthetase in the producer of EPS.

It was established that with increasing content of Mg^{2+} up to 5 mM in the medium with ethanol and waste oil, regardless of the mode of fractional addition of substrates (total 4 or 5 portions), an increase in the amount of synthesized polysaccharide to 15.5-16 g/l was observed, which is higher than the synthesis indicators on the basic medium with ethanol and refined oil (10-13.5 g/l) (Table 6).

Table 6

Indicators of ethapolan synthesis on the mixture of ethanol (4.0%) and sunflower oil (1.2%) of different quality

Content of Mg^{2+} in the medium, мМ	Quality of oil in the mixture with ethanol	Fractional substrate addition mode*	pH_{end}	EPS concen-tration, g/l	EPS-synthesizing ability, g EPS/g biomass
1.6 (medium 2)	Refined oil	Four portions of 1.0% ethanol and 0.3% oil	5.9	11.88±0.59	3.08±0.15
		Five portions of 0.8% ethanol and 0.24% oil	6.1	13.51±0.68	3.65±0.18
	Mixed waste oil	Four portions of 1.0% ethanol and 0.3% oil	6.2	12.02±0.60	3.24±0.16
		Five portions of 0.8% ethanol and 0.24% oil	6.1	10.10±0.51	2.73±0.14
5.0 (medium 3)	Refined oil	Four portions of 1.0% ethanol and 0.3% oil	6.1	10.37±0.52	2.58±0.13
		Five portions of 0.8% ethanol and 0.24% oil	6.2	9.86±0.49	2.19±0.11
	Mixed waste oil	Four portions of 1.0% ethanol and 0.3% oil	6.0	15.63±0.78	3.33±0.17
		Five portions of 0.8% ethanol and 0.24% oil	5.9	15.95±0.80	3.11±0.16

Notes.

1. Inoculum was grown on ethanol.

2. * – during the cultivation process fractional addition of substrates was carried out every 24 hours.

It turned out to be unexpected, but the additional introduction of Mg^{2+} into the medium with ethanol and refined oil led to a slight decrease in the synthesis of EPS. This may be due to the different effects of magnesium cations on the enzymatic systems responsible for the catabolism of fatty acids and other components of sunflower oil. For example, in *Lactococcus lactis* ssp. *lactis* concentration of Mg^{2+}, which provides maximum enzymatic activity, differs for various enzymes of the β-oxidation system (Li *et al.*, 2014).

According to literature date, the use of mixed substrates is primarily associated with the production of various microbial metabolites (enzymes, ethanol, methane, lipids, etc.) from lignocellulosic biomass (wood, straw, pulp, etc.) (Chukwuma *et al.*, 2021). At the same time, information on the use of lignocellulose to obtain microbial EPS is limited (Liu *et al.*, 2020; Wang *et al.*, 2020; Wu *et al.*, 2021).

Wu *et al.* (Wu *et al.*, 2021) established the ability of *Sphingomonas sanxanigenens* NX02 to synthesize microbial EPS sanxan on a mixture of glucose and xylose. Regardless of the ratio of substrates in the mixture (7:3, 5:5, 3:7) their consumption rate, EPS concentration and biomass level in the first 24 h of cultivation were 1.1-2.3 times higher than those obtained on the corresponding monosubstrates. Meanwhile, at the end of cultivation the indicators were at the same level regardless of the type of used substrate (mono- or mixed). It should be noted that the replacement of refined carbohydrates with 4% (w/w by carbohydrates) of corn straw hydrolyzate (ratio of glucose and xylose 3.48:1) was accompanied by an additional increase in the amount of synthesized sanxan to 13.4 g/kg.

Other researchers (Liu *et al.*, 2020) report the ability of *Aureobasidium melanogenum* TN2-1-2 on the medium containing 11% (w/w by carbohydrates) of wheat straw hydrolyzate (glucose to xylose ratio 78%:22%) to synthesize 55.1 g/l of pullulan. During cultivation on corresponding monosubstrates (glucose and xylose) the EPS concentration was 58.3 and 50.2 g/l, respectively.

Note that the above producers due to the functioning of independent metabolic systems of glucose and xylose consumption grown in mixotrophic conditions.

Recently, new information about the EPS synthesis on mixture of waste substrates has appeared. Fathiyah *et al.* (Fathiyah *et al.*, 2021) demonstrated the ability of *Acetobacter xylinum* to accumulate 4.5 g/l of bacterial cellulose during growth on a mixture of oil palm frond juice and coconut water (optimal ratio 60:40). Under cultivation in a medium containing 8% (w/w by carbohydrates) of mixed fruit waste the strain *Bacillus* sp. SRA4 synthesized 25.1 g/l EPS for 72 h (Vaishnav *et al.*, 2020).

In the work (Gao *et al.*, 2020) it was shown that additional application of 5.09% (w/w by carbohydrates) of cane molasses powder during solid-state cultivation of *Kosakonia cowanii* TL-1 strain on the medium, which contains a mixture of cane bagasse and broadbean seed capsule (optimal 2:1 ratio), was accompanied by an increase in the EPS yield in 7.16 times (up to 0.42 g of EPS/g of substrate).

One of the features of the microbial polysaccharide ethapolan, which distinguishes it from other known EPS, is the ability to use various oil-containing (refined and waste sunflower, olive oil, etc.) and C_2-C_6-substrates (carbohydrates, ethanol, acetate, organic acids, etc.), as well as their mixtures for its production (Pidhorskyy *et al.*, 2010; Pirog *et al.*, 2020).

It should be noted that nowadays information about the use of oil-containing substrates for microbial EPS is very limited, and in available data the concentration of the target product does not exceed a few grams per liter (Pirog *et al.*, 2021; Pirog *et al.*, 2016).

At the same time, this work demonstrated the possibility of obtaining 16 g/l EPS under cultivation of strain IMV B-7005 on the mixture of ethanol and mixed waste oil.

Conclusion

Thus, during the studies the possibility of microbial EPS ethapolan synthesis on a mixture of energy-excessive substrates (ethanol and sunflower oil) was established. To intensify the EPS synthesis, a comprehensive approach was used, which included:

1. Calculation and experimental confirmation of the optimal molar ratio of ethanol and refined sunflower oil concentration in the mixture (1:0.056);
2. Stabilization of pH of the medium at an acceptable level for the EPS synthesis while using high concentrations of ethanol and refined oil in the mixture (4% and 1.2%, respectively) by replacing the nitrogen source (NH_4NO_3 with KNO_3), establishing the optimal mode of fractional addition of substrates (5 equal portions during cultivation) and increasing the concentration of Mg^{2+} cations to 5.0 mM (acetyl-CoA-synthetase activator);
3. Replacement of refined oil in the mixture with ethanol on a mixed one.

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Анотації

Харчові технології

Обґрунтування технологій зберігання і переробки насіння конопель для функціональної, дієтичної та спеціальної продукції. Огляд

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Вступ. Представлено аналітичні дослідження складу та якості насіння конопель, методів і технологій його зберігання й переробки для виробництва функціональних, дієтичних і спеціальних продуктів.

Матеріали і методи. Предметами огляду є особливості складу насіння промислових конопель, аспекти його зберігання; особливості виробництва конопляної харчової продукції (олії, ядра, борошна, білкових концентратів); аспекти використання насіння конопель і продуктів його переробки.

Результати і обговорення. Насіння конопель містить більше 30% олії і близько 25% білка, значну кількість мінеральних речовин (Ca, Mg, P, K, S, Fe, Zn тощо), харчових волокон і біологічно-активних речовин. Компонентний склад і біологічна цінність насіння конопель залежить від регіону й умов вирощування. Раціональними умовами зберігання є: вологість насіння – 11%, температура і відносна вологість повітря – 14–18 °C та 50–55% відповідно. Продуктами переробки насіння конопель є олія, ядро, борошно та білковий концентрат. Олію переважно вилучають методом механічного віджиму. Конопляна олія містить жирні кислоти, зокрема, лінолеву, ліноленову і γ-ліноленову. γ-ліноленова кислота сприяє утворенню γ-глобуліну. Токофероли конопляної олії виконують роль антиоксидантів у харчових, дієтичних і спеціальних продуктах. Ядро з насіння конопель отримують методом однократного удару з подальшим відділенням оболонок з отриманої маси. Одержаний продукт має високий вміст незамінних амінокислот. Борошно, клітковину і білковий концентрат виробляють із конопляної макухи. У публікаціях недостатньо приділено уваги взаємозв'язку факторів підготовки матеріалу, параметрів процесу виробництва конопляних продуктів, умов і тривалості їх зберігання щодо вмісту функціональних і біологічно активних компонентів. Використання насіння конопель і продуктів його переробки сприяє підвищенню біологічної, поживної цінності, функціональних сенсорних властивостей харчової продукції. Важливі подальші дослідження щодо використання препаратів для регулювання антимікробних та антиоксидантних властивостей функціональної, дієтичної та спеціальної продукції.

Висновки. Обґрунтовано необхідність використання представлених теоретичних, наукових і практичних досліджень у комплексних технологіях переробки екологічно чистого насіння промислових і медичних конопель.

Ключові слова: *коноплі, насіння, олія, ядро, борошно, функціональність, екопродукція.*

Фізико-хімічні та органолептичні властивості напоїв на основі кислого винограду та моніторинг їх якості під час зберігання

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Вступ. Метою дослідження є визначення придатності кислого виноградного концентрату в напоях і моніторинг зміни їх якості за різних умов зберігання.

Матеріали і методи. Як матеріал для отримання концентрату використовували кислий виноград Sultani Çekirdeksiz (*V. vinifera* L.). Загальний вміст фенолів визначали за методами Фоліна-Чіокальтеу. Антиоксидантні властивості зразків аналізували методами FRAP, CUPRAC, ABTS і DPPH. Вміст цукрів і окремих фенольних сполук визначали методом HPLC, а мінералів – методами атомно-абсорбційної спектроскопії.

Результати і обговорення. Загальний вміст фенолів, антиоксидантна здатність, окремі фенольні сполуки та кількість мінеральних речовин збільшувалися при зростанні частки кислого виноградного концентрату у зразках промислових газованих напоїв (CD), шербету (SH) та холодного чаю (IT). Загальний вміст фенолів коливався від 32,7 до 40,82 мг/л у CD, від 27,3 до 35,0 мг/л у SH та від 357,08 до 365,64 мг/л у зразках IT. Значення антиоксидантної здатності коливались від 66 до 4319 ммоль ТЕ/л для методу ABTS, 214 і 4893 ммоль ТЕ/л для CUPRAC і від 186 до 4319 ммоль ТЕ/л для методу FRAP. Зразки IT мали більшу антиоксидантну здатність, ніж зразки SH та CD.

Найпоширенішими мінералами в напоях були Na (77,41–692,28 мг/мл), Ca (159,03–358,25 мг/мл) і Fe (8,74–22,84 мг/мл). Загальний вміст цукру в напоях коливався від 6,29 до 10,97 г/100 мл. За вмістом цукру вони оцінені як CD>SH>IT. Галову, ванільну і кавову кислоти, (+)-катехін та (-)-епігалокатехін галат визначено у всіх напоях, тоді як р-кумарова, ферулова та синапінова кислоти, мірицетин, кверцетин, (-)-епігалокатехін та (-)-епікатехін галат не виявлено у CD і SH. Вміст фенольних сполук у IT був більшим, ніж у CD і SH для всіх досліджуваних сполук. Умови зберігання спричинили зміни pH напою, кислотності, вмісту TP та інгібування DPPH.

Висновки. Вміст мінеральних речовин, загальний вміст фенолів, антиоксидантні властивості та вміст окремих фенольних сполук у напоях збільшувалися зі збільшенням вмісту кислого виноградного концентрату. Галова кислота була найбільш поширеною фенольною кислотою в напоях, а (-)-епікатехін для зразків IT був найбільш поширеним флавонолом. PH напоїв, кислотність, загальний вміст фенолів та інгібування DPPH можуть змінюватися залежно від температури й тривалості зберігання.

Ключові слова: *кислий виноградний сік, зберігання, антиоксидант, напій, поліфенол.*

Виявлення фальсифікацій малинового меду агавовим, кленовим, рисовим, кукурудзяним та інвертованим цукровим сиропами з використанням інструментальних прийомів

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Вступ. Обґрунтовано доцільність використання «електронного язика», що складається з мідного робочого електрода, для виявленні фальсифікованого меду агавовим, кленовим, рисовим, кукурудзяним та інвертованим цукровим сиропами.

Матеріали і методи. Фальсифікацію зразків меду проводили шляхом додавання сиропу в кількості 5, 10, 20 і 50%. Аналізували фізико-хімічні параметри: колір, вміст вологи, pH, вільну кислотність, електропровідність і вміст гідроксиметилфурфуролу (ГМФ). Вимірювальна система – PGSTAT з модулем FRA32M, з'єднаним з трьома електродами: електродом порівняння (Ag/AgCl), протиелектродом (платина) і робочим електродом (мідь).

Результати і обговорення. Фальсифікація меду суттєво вплинула як на фізико-хімічні, так і на вольтаметричні параметри «язика». Зміни відбувалися залежно від агента та ступеня фальсифікації. Фальсифікований мед з інвертованим цукровим сиропом мав найвище значення параметра L* (33,14), а з рисовим сиропом – найнижче (27,10). Вологість зростала з 16,16% у справжньому меду до 19,28% у фальсифікованому, вміст сиропу в якому складав 50%. Величина pH знижувалася у фальсифікованому меді з інвертованим цукровим сиропом через наявність доданої лимонної кислоти. Вмісту ГМФ збільшувався від 58,09 мг/кг в автентичному меді до 185,07 мг/кг у фальсифікованому меді з інвертованим цукровим сиропом. Що стосується «вольтамперометричного язика», то значення сили струму для справжнього меду становило 0,192 мА. Чистий кленовий і рисовий сиропи мали найвищі значення, тоді як найнижчі значення спостерігались для інших трьох видів сиропу. Незалежно від агента фальсифікації, коливання сили струму спостерігались, починаючи від ступеня фальсифікації 5% з мідним електродом. Відповідно до аналізу основних компонентів (PCA), перший компонент (PC-1) представляв 88% дисперсії, а другий (PC-2) – 11%, що становить 99% початкової мінливості. Зразки фальсифікованого меду з вмістом 5%, 10% і 20% агавового сиропу, 5% і 10% кукурудзяного сиропу, а також 5% кленового сиропу були близькими до справжнього меду.

Висновки. Фізико-хімічні параметри (колір, pH, вологість, вільна кислотність, електропровідність, вміст ГМФ) можуть використовуватися для попереднього аналізу, але в поєднанні з «вольтамперометричним язиком» довели свою ефективність у виявленні фальсифікованих зразків меду навіть при кількості 5% різних агентів фальсифікації.

Ключові слова: *мед, фальсифікація, сироп, електронний язик.*

Вплив заквасок спонтанного бродіння з борошна круп'яних культур на показники технологічного процесу виготовлення пшеничного хліба

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Вступ. Метою досліджень є визначення вуглеводно-амілазного комплексу вівсяного борошна та борошна зеленої гречки, дослідження перебігу процесу приготування заквасок спонтанного бродіння на основі цих видів борошна, встановлення впливу отриманих заквасок спонтанного бродіння на показники технологічного процесу та якість пшеничного хліба.

Матеріали і методи. Досліджено борошно вівсяне, зеленої гречки, закваски спонтанного бродіння на основі цих видів борошна, пшеничний хліб з додаванням заквасок. Методи: прискорений та експрес-метод висушування, йодометричний, волюмометричний, автолітичної проби, титрування та pH-метрія, за відновленням забарвлення індикатора, об'єм – за об'ємом витісненого зерна.

Результати і обговорення. При проведенні досліджень контролем було борошно пшеничне першого сорту. Так, цукроутворювальна здатність вівсяного та гречаного борошна була нижчою, порівняно з контролем, на 19,4 та 56,4%; автолітична активність – на 36,9 та 43,1%; сумарне газоутворення – на 31,1 та 38,6% відповідно. Зниження ферментативної активності пояснюється відмінностями хімічного складу й технологією приготування круп, що використовуються для отримання вівсяного та гречаного борошна.

У сумішах пшеничного борошна з вівсяним або гречаним борошном цукроутворювальна здатність була вищою, порівняно з контролем, на 15,1 і 10,4%; газоутворювальна здатність – на 12,7 і 7,3% відповідно. Це пояснюється дією активної β-амілази пшеничного борошна на менші за розміром крохмальні зерна круп'яного борошна.

Встановлено, що після п'ятого поновлення закваски з вівсяного та гречаного борошна можливо використовувати для виготовлення хліба, оскільки їхня якість за фізико-хімічними показниками стабілізується (кислотність – 16,0–18,0 град, активність МКБ – 45–60 хв).

Використання досліджуваних заквасок у кількості до 12% до маси борошна в тісті дає змогу отримати вироби з органолептичними та фізико-хімічними показниками, близькими до контрольного зразка.

Висновки. Технологічні властивості вівсяного борошна та борошна зеленої гречки дають змогу використовувати їх як поживне середовище для заквасок спонтанного бродіння з метою інтенсифікації технологічних процесів і покращення харчової цінності пшеничного хліба.

Ключові слова: *технологія, борошно, заквaска, тісто, хліб.*

Вміст фітинової кислоти та засвоюваність in vitro злакових і бобових культур, оброблених різними способами

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Вступ. Це дослідження має на меті визначити взаємозв'язок між вмістом фітинової кислоти (ФК) і швидкістю засвоюваності зерна, яке широко вживається в щоденному раціоні.

Матеріали і методи. Вивчався ефект дефітинізації замочування (протягом 12 год), кип'ятіння (при 100 °C протягом 1 год), автоклавування (при 121 °C протягом 15 хвилин), ферментації (при 25-27°C) та процесів пророщування на зернові. Визначали вміст ФК, засвоюваність мінералів (MD, %) і засвоюваність білків (ЗБ, %) та порівнювали їхній вплив до та після процесів.

Результати і обговорення. За всіма застосованими методами спостерігалося зниження вмісту ФК у бобових і зернових культурах. Встановлено, що ферментація (<800 мг/100 г) борошна і пророщування інших зразків (<700 мг/100 г) дають більш ефективні результати для деградації ФК. Було виявлено зниження вмісту ФК у зразках на 20–70% шляхом ферментації та на 50–80% – за рахунок проростання. Крім того, рівень ЗМ у зернових, який був особливо низьким до пророщування, після пророщування збільшився вдвічі. Встановлено, що ЗБ, %, збільшується під впливом процесів, за винятком кип'ятіння та замочування. Особливо ефективним був процес пророщування при ЗБ. Показник збільшення ЗБ для десяти зразків бобів склав до 200%., тоді як вміст ФК у зразках борошна злаків після їх спонтанного бродіння знижувався, показники їх засвоюваності статистично підвищувалися ($p<0,05$).

Висновки. Такі процеси, як замочування, ферментація або термічне оброблення, які широко використовуються для приготування зерна, забезпечують перевагу дефітинізації з різною швидкістю. Ефективним з точки зору харчової якості є комбіноване використання таких прийомів, як замочування і відварювання, які використовуються при приготуванні для споживання різноманітних злакових і бобових продуктів.

Ключові слова: *зернові, бобові, фітинова кислота, засвоюваність, біодоступність.*

Основні напрями застосування нових натуральних інгредієнтів у виробництві харчових продуктів

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Вступ. Розглянуто застосування нових натуральних добавок у харчовому виробництві.

Матеріали і методи. Проведено аналіз наукових даних про використання натуральних добавок у харчових продуктах.

Результати і обговорення. В останні роки значна кількість досліджень була присвячена вивченню застосування нових натуральних інгредієнтів (рослин з

фармацевтичними властивостями, рослинних матеріалів з антиоксидазною активностю, ефірних рослинних олій, екстрактів з рослинних матеріалів, морських водоростей, продуктів переробки насіння, харчових волокон, борошна із зернових і псевдозернових культур, що не містять глютену, для заміни ним пшеничного борошна в безглютенових хлібобулочних виробах, їстівних покриттів рослинного походження в приготуванні різних традиційних харчових продуктів).

Основною метою цього напряму є підвищення оздоровчої цінності харчових продуктів без внесення суттєвих змін у технологічний процес і формування відповідних споживчих властивостей виробів. Ці тенденції передбачають заміну в м'ясних продуктах тваринних жирів низькокалорійними інгредієнтами з високим вмістом жирних кислот, використання рослинних матеріалів з антиоксидазними властивостями, наприклад, екстрактів з фруктів, овочів, пряностей і трав, що містять фенольні сполуки, замість синтетичних антиоксидантів при виготовленні харчових продуктів; застосування безглютенової сировини у виробництві безглютенового хліба та використання ефірних олій як природних консервантів для продовження строку зберігання харчових продуктів. У деяких дослідженнях йдеться про застосування рослинної сировини для виготовлення продуктів із вираженими оздоровчими властивостями для профілактики захворювань і забезпечення споживачів необхідною кількістю важливих для здоров'я нутрієнтів. Проаналізовано основні сучасні напрями використання нових натуральних інгредієнтів у виробництві харчових продуктів.

Висновок. Оглядова інформація може стати в нагоді дослідникам для формування пріоритетних напрямів у дослідницькій та інноваційній роботі, що сприятеме покращанню якості харчових продуктів.

Ключові слова: *харчування, добавка, оздоровчий, рослинний замінник.*

Дослідження процесу екстрагування антиоксидантів з кори дуба та їх використання для окиснювальної стійкості соняшникової олії

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Вступ. Актуальним завданням для харчової галузі є пошук антиоксидантів, отриманих із природної сировини. Додаткових досліджень також потребують технологічні параметри для підвищення ефективності вилучення антиоксидантів та їх введення в гідрофобні системи.

Матеріали і методи. Як сировину для одержання антиоксидантів використовували кору дуба. Для вилучення флавоноїдів використовували водно-спиртові розчини, додавали луги та кислоти, проводили мікрохвильову обробку. Вміст сухих речовин в екстрактах визначали гравіметрично. Кінетику окиснення соняшникової олії досліджували волюметричним методом за додавання ініціатора окиснення динітрилу азобісизобутирової кислоти та за зміною пероксидного числа олії.

Результати і обговорення. Підвищення концентрації етанолу у водно-етанольному розчиннику призводило до збільшення концентрації екстракту кори дуба. Концентрація екстракту збільшувалась на 45% при підвищенні концентрації етанолу

від 50 до 80%. Додавання лужних розчинів під час екстрагування не збільшувало вихід екстрактивних речовин, додавання 1% аскорбінової кислоти збільшувало вихід екстрактивних речовин з 2,3 до 3,1%, 1% лимонної та молочної кислоти – до 4,3 та 3%, відповідно.

Мікрохвильове екстрагування підвищувало швидкість вилучення екстрактивних речовин у 8 разів, вихід − у 1,5 раза (з 2,3% вмісту сухих речовин в екстракті до 3,5%).

Одержані водно-спиртові екстракти разом з емульгатором вводили в олію методом їх диспергування до розмірів часточок не більше, ніж 2–3 мкм. Залишки води та спирту випаровували під зниженим тиском. Після випаровування розчинників розміри гідрофільної фази зменшувались до нанорозмірів.

За зміною вмісту гідропероксидів у соняшниковій олії визначено, що одержані антиоксиданти кори дуба гальмують швидкість окиснення олії. Період індукції накопичення гідропероксидів у соняшниковій олії збільшувався від 21 доби в контролі до 37 діб для соняшникової олії з додаванням одержаного антиоксиданту. Також визанчено, що додавання 2% екстракту кори дуба до соняшникової олії збільшує тривалість індукційного періоду ініційованого окиснення олії.

Висновки. Встановлено, що використання харчових кислот у поєднанні з водно-спиртовим розчинником і мікрохвильовим полем ефективно підвищує екстрактивність антиоксидантів з кори дуба.

Ключові слова: *кора, дуб, антиоксидант, окиснення, олія.*

Модифікація картопляного крохмалю хлорангідридом адипінової кислоти та дослідження продукту модифікації як сировини екологічного пакування харчових продуктів

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Вступ. Проведено модифікацію картопляного крохмалю хлоридом адипінової кислоти з метою подальшого фізико-хімічного дослідження отриманого продукту та використання його, наприклад, як ефективного плівкоутворювача біодеградабельних плівок/покриттів.

Матеріали і методи. Картопляний крохмаль вищого сорту, адипінова кислота (Е 355), хлористий тіоніл. Розчинники – ДМСО, ДМФА, метанол, етанол. ІЧ-спектроскопію проводили на пристрої фірми Nexus-475 Nicolet. ЯМР-спектри були зареєстровані ЯМР-спектрометром Mercury, фірмою VARIAN. Рентгенофазовий аналіз проводився на приладі ДРОН-3М. Термогравіметричне дослідження проводилося на приладі Q-1500D.

Результати і обговорення. В ІЧ-спектрі відсутні сигнали ацилхлоридної групи (1785-1815 $см^{-1}$) і сигнали продуктів гідролізу хлорангідриду адипінової кислоти, зокрема смуга коливань карбонової кислоти групи νC=O (1750-1770 $см^{-1}$ та їх солей 1640 $см^{-1}$). Це дає підстави вважати, що відбулося зшивання глюкопіранозних кілець унаслідок реакції обох хлорангідридних груп. Результати елементного аналізу ацильованого картопляного крохмалю хлорангідридом адипінової кислоти: знайдено Карбону 42,33%, Гідрогену – 6,65%; $C_{54}H_{86}O_{42}$; розраховано кількість Карбону 41,96%, Гідрогену – 6,58%. Результати термографіметричного аналізу вказують на те, що модифікація картопляного крохмалю хлорангідридом адипінової кислоти зумовлює зміну форми та кількості води картопляного крохмалю. ЯМР-дослідження не дало

змоги визначити ступінь зшивання глюкопіранозних ланцюгів крохмалю та положення замісника. Рентгенограма нативного крохмалю показала, що ступінь кристалічності нативного крохмалю становить 12%. Модифікація картопляного крохмалю адипіновою кислотою зменшує ступінь кристалічності до 5%. Крім того, руйнування первинної (кристалічної) структури зерен крохмалю також підтверджено за допомогою оптичного мікроскопіюванні.

Отже, на підставі ряду досліджень визначено, що модифікований продукт має ряд відмінних характеристик від вихідного продукту. Крім того, використання крохмалю як природної речовини, яка здатна до біодеструкції, надає можливість рекомендувати отриманий продукт як сировину для екологічних пакувальних матеріалів

Ключові слова: *крохмаль, адипінова кислота, ІЧ-спектроскопія, ЯМР, екологічне пакування, біодеградабельний.*

Вплив біоактивованого зерна амаранту на якість та амінокислотний склад хліба

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Вступ. Вивчено хлібопекарські властивості та амінокислотний склад диспергованої зернової маси амаранту як інгредієнта пшеничного і спельтового хліба.

Матеріали і методи. Для отримання біоактивованого диспергованого зерна амаранту використано зерно сорту Харківський-1, яке замочували при гідромодулі 1:1 протягом 12–48 годин. У рецептуру хліба з пшеничного чи спельтового борошна вводили 15–25% амарантового напівфабрикату та досліджували його вплив на споживчі якості продукту. Амінокислотний склад визначали методом іонообмінної рідинно-колонкової хроматографії.

Результати і обговорення. Використання 15–20% біоактивованого диспергованого зерна амаранту (БДЗА) у складі пшеничного й спельтового хліба призводило до збільшення питомого об'єму виробів на 7–21% унаслідок підвищення активності ферментів, поліпшення біодоступності есенціальних мінеральних речовин під час бродіння тіста. Тривалість замочування зерна амаранту для поліпшення органолептичних якостей виробів має становити 36 годин. Комплексна якість пшеничного і спельтового хліба суттєво залежала від дозування БДЗА (56%, p=0,007) і тривалості замочування зерна амаранту (62%, p=0,038) відповідно. Переважний вплив на якість пшеничного і спельтового хліба з БДЗА чинила тривалість замочування зерна амаранту. Внаслідок біохімічних процесів диспергуване зерно амаранту має підвищений амінокислотний скор за всіма незамінними амінокислотами (у 1,5–2,7 раза), вміст незамінних і замінних амінокислот збільшився у 1,8–2,1 раза. За рахунок введення 20% БДЗА у склад пшеничного хліба вміст лізину у виробах зростав у 2,4 раза, а скори незамінних амінокислот сягають 133–213%. Хліб з додаванням БДЗА містить в 1,6–1,7 раза більше незамінних і замінних амінокислот. Утилітарність білка збільшується в такому порядку: пшеничне борошно → зерно амаранту → пшеничний хліб → диспергуване зерно амаранту → хліб з диспергованим зерном амаранту. Біологічна цінність білка хліба з БДЗА зростає до 76%.

Висновки. Тривалість біоактивації зерна амаранту суттєво впливає на якість хліба і забезпечує підвищення біологічної цінності білка продукту.

Ключові слова: *зерно, амарант, хліб, амінокислоти.*

Вплив заміни цукру та жиру на реологічні властивості тіста для пирогів

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Вступ. Визначено зміну реологічних властивості тіста для пирогів у разі заміни відсотка цукру та жиру яблучним пюре.

Матеріали і методи. Оцінку емпіричних реологічних характеристик тіста для пирогів проводили за допомогою інструменту «Альвеограф». Для оцінювання втрат і властивостей тіста час оброблення були застосовані два динамічні методи: частотний і тест на повзучість.

Результати і обговорення. Реологічні властивості тіста показали значні зміни у зразках, отриманих при заміні меншого відсотка цукру і жиру на яблучне пюре. Нижчі значення модуля пружності та в'язкості отримані для зразків із меншим відсотком цукру і жиру. Зразок, отриманий шляхом заміни 40% кількості цукру та жиру, мав значення модуля в'язкості з частотою, найближчою до контрольної проби.

У зразках із заміною 20 і 50% кількості цукру і жиру максимальна температура клейстеризації мала вищі значення, ніж у контрольного зразка, а зразок із заміною 10 та 30% кількості цукру і жиру мав нижчу максимальну температуру желатинізації, ніж контрольний зразок. Однак зразок із заміною 30% кількості цукру і жиру мав максимальну температуру желатинізації порівняно з контрольним.

Поведінка тіста при повзучості та відновленні була найбільш подібною до контрольного зразка у випадку заміни 40% кількості цукру та жиру та для зразка з 50-відсотковою заміною.

Це пояснюється тим, що з яблучним пюре у контрольний зразок вноситься доданий цукор і жиру.

Висновки. У п'яти зразках тіста для пирогів було досягнуто зниження цукру на 10–50%. Використання яблучного пюре як інгредієнта для заміни цукру і жиру дало змогу отримати тісто з реологічними властивостями, подібними до контрольного зразка залежно від відсотка замінених цукру і жиру.

Ключові слова: *тісто, пиріг, реологія, цукор, жир, яблучне пюре.*

Процеси і обладнання

Інтенсифікація процесу отримання інвертного цукрового сиропу застосуванням роторно-пульсаційного оброблення

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Вступ. Метою дослідження є інтенсифікація технології інвертного цукрового сиропу із сахарози застосуванням обробки водно-цукрового розчину в роторно-пульсаційному апараті.

Матеріали і методи. Матеріалом досліджень слугували водні розчини сахарози хімічно чистої. Дослідження проводилися на установці із застосуванням роторно-пульсаційного апарата в діапазоні швидкостей зсуву потоку $20–50\times10^3$ c^{-1}. Визначення вмісту вуглеводів здійснювали методом високоефективної рідинної хроматографії.

Результати і обговорення. Збільшення температури, тривалості обробки та зменшення значення pH при постійній швидкості зсуву потоку призводять до збільшення частки утвореного інвертного цукру. Повна інверсія сахарози відбувається за температури 80 °C, pH = 3,0 протягом 30 хв за швидкості зсуву потоку 20×10^3 c^{-1}. За швидкості зсуву потоку 50×10^3 c^{-1} практично вся сахароза гідролізується при pH = 3,5, тривалості процесу 5 хв за умов п'ятиразової обробки в роторно-пульсаційному апараті в циркуляційному режимі.

У сиропі, приготованому за запропонованою технологією за температури 70 °C і тривалості інверсії 5 хв, частка інвертованої сахарози склала 100%, слідів оксиметилфурфуролу не виявлено.

Зроблено припущення, що на ділянці ланцюга сахарози виникають критичні напруги і відбувається розрив хімічних ковалентних зв'язків у процесі механохімічної деструкції в найбільш енергетично слабких місцях. У результаті механохімічного впливу на ділянці ланцюга сахарози (С–О–С) виникають критичні напруги і відбувається розрив зв'язку. Це призводить до утворення вільних радикалів. Один радикал приєднує іон OH^-, інший – H^+. У результаті утворюється глюкоза і фруктоза.

Висновки. Застосування обробки водно-цукрового розчину в роторно-пульсаційному апараті знижує тривалість інверсії від 120 хв до 5 хв, збільшує частку інвертованої сахарози з 55% до практично повної її інверсії, виключаючи утворення оксиметилфурфуролу.

Ключові слова: *сироп, цукор, інверсія, роторно-пульсаційне оброблення.*

Біотехнологія, мікробіологія

Біосинтез і характеристика наночасток срібла, отриманих з використанням *Saccharomyces cerevisiae* M437

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Вступ. Завдяки широкому спектру антимікробної дії наночастки срібла (AgNPs) мають великий потенціал використання у харчовій галузі для боротьби із патогенами харчового походження.

Матеріали і методи. Для синтезу AgNPs використовували супернатант культуральної рідини та безклітинний водний екстракт *Saccharomyces cerevisiae* M437. Факт синтезу біогенних AgNPs підтверджували, знімаючи спектри поглинання зразків у діапазоні 200-700 нм. Розмір і дзета-потенціал AgNPs визначали за допомогою Zetasizer Nano ZS. Морфологію наночасток досліджували з використанням електронної мікроскопії.

Результати і обговорення. Використовуючи спектральний аналіз в УФ-видимій області, ми підтвердили формування AgNPs у досліджуваних розчинах. Виражений пік поглинання AgNPs, отриманих з використанням безклітинного водного екстракту *S. cerevisiae* M437, реєстрували в діапазоні довжин хвиль від 300 до 540 нм з піком при 425 нм. Для наночасток, отриманих з використанням супернатанту, спостерігали розширення спектрів поглинання, що може бути пов'язано з агрегацією AgNPs.

Синтезовані з використанням супернатанту *S. cerevisiae* M437 AgNPs, мали сферичну форму з діаметром близько 15 нм. Індекс полідисперсності (PdI) їх розчинів становив 0,3, а дзета-потенціал –13,6. Після зберігання упродовж 45 діб при 4 °C значення PdI збільшилось в 1,6 раза, а дзета-потенціал – на 11,7%. Це свідчить про можливу зміну форми AgNPs, формування агломератів або інші процеси, перебіг який відбувається в колоїдному розчині у процесі зберігання.

AgNPs, які були отримані при використанні безклітинного водного екстракту *S. cerevisiae* M437, мали овальну форму з розміром 21,3×14,2 нм. Значення PdI та дзета-потенціалу були аналогічні наночасткам, отриманим з використанням супернатанту. Проте після зберігання ці показники суттєво відрізнялись: значення PdI збільшилось в 1,3 раза, а дзета-потенціал зменшився на 29%. Тобто розчин наночасток срібла, що були отримані в такий спосіб, є більш стабільним після зберігання за вказаних умов.

Висновки. Показано можливість позаклітинного синтезу наночасток срібла з використанням дріжджів *Saccharomyces cerevisiae* M437. Описано форму, розмір і дзета-потенціал біогенних AgNPs і доведено їхню стабільність після зберігання.

Ключові слова: *наночастки, срібло, дріжджі, біосинтез, Saccharomyces cerevisiae.*

Інтенсифікація синтезу мікробного екзополісахариду етаполану на суміші енергетично надлишкових субстратів

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Вступ. Досліджено умови культивування *Acinetobacter* sp. IMB B-7005, які забезпечують максимальні показники синтезу екзополісахариду (ЕПС) етаполану на суміші етанолу та соняшникової олії, а також продемонстровано можливість заміни рафінованої олії у суміші з етанолом на відпрацьовану.

Матеріали і методи. Бактерії вирощували в рідких мінеральних середовищах на суміші етанолу та соняшниковій олії різної якості, а також відповідних моносубстратах. Оптимальне молярне співвідношення концентрацій субстратів у суміші розраховували теоретично за концепцією Бабеля. Концентрацію ЕПС визначали ваговим методом після осадження ізопропанолом, ЕПС-синтезувальну здатність як відношення концентрації ЕПС до біомаси та виражали у г ЕПС/г біомаси.

Результати і обговорення. Найвищі показники синтезу етаполану спостерігались за молярного співвідношення концентрацій етанолу та рафінованої соняшникової олії у суміші 1:0,056, максимально наближеного до теоретично розрахованого (1:0,076), та використанні інокуляту, вирощеного на етанолі. Подальше підвищення концентрацій етанолу та олії призводило до зниження pH культуральної рідини до неоптимального для синтезу ЕПС рівня (4,5–4,8). Для забезпечення можливості синтезу етаполану на середовищі з підвищеною концентрацією етанолу (4%) та олії (1,2%) заміняли нітрат амонію на еквімолярну за нітрогеном кількість KNO_3 (0,8 г/л), який транспортується у клітини симпортом з протоном; здійснювали дробне внесення субстратів п'ятьма рівними порціями впродовж культивування та підвищували концентрацію катіонів Mg^{2+}, які є одними із активаторів ацетил-КоА-синтетази у *Acinetobacter* sp. IMB B-7005 та здатні впливати на ферментативну активність систем, відповідальних за катаболізм жирних кислот. За таких умов культивування незалежно від типу використаної соняшникової олії (рафінована або змішана відпрацьована) у суміші з етанолом концентрація етаполану досягала 13,5–16,0 г/л, а ЕПС-синтезувальна здатність – 3,1–3,7 г ЕПС/г біомаси, що відповідно у 3,2–3,8 та 1,6–1,9 раза вище порівняно з показниками до оптимізації.

Висновки. Встановлено можливість інтенсифікації синтезу етаполану на суміші енергетично надлишкових субстратів (етанол і соняшникова олія) на основі визначення оптимального молярного співвідношення концентрацій моносубстратів у суміші, модифікації складу середовища (заміна нітрату амонію на нітрат калію, підвищення вмісту катіонів магнію, заміна рафінованої олії на змішану відпрацьовану) і дробному внесенню субстратів.

Ключові слова: *Acinetobacter, змішані субстрати, біосинтез, екзополісахарид, етаполан.*

.

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Dear colleagues!

The Editorial Board of scientific periodical
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Requirements to all texts:
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The structure of the article:

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All figures should be made in graphic editor, the font size 14.

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Вимоги до оформлення статей

Мова статей – англійська.

Мінімальний обсяг статті – **10 сторінок** формату А4 (без врахування анотацій і списку літератури)**.**

Для всіх елементів статті шрифт – **Times New Roman**, кегль – **14**, інтервал – 1.

Всі поля сторінки – по 2 см.

Структура статті:

1**.** УДК**.**
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5. Анотація. **Обов’язкова** структура анотації:
   - Вступ (2–3 рядки).
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6. Ключові слова (3–5 слів, але не словосполучень).

Пункти 2–6 виконати англійською і українською мовами.

7. Основний текст статті. Має включати такі обов’язкові розділи:
   - Вступ
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За необхідності можна додавати інші розділи та розбивати їх на підрозділи.

8. Авторська довідка (Прізвище, ім’я та по батькові, вчений ступінь та звання, місце роботи, електронна адреса або телефон).
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Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

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Оформлення цитат у тексті статті:

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1 автор	(Arych, 2019)
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1. Приклад:
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2. Посилання на книгу:

Автори (рік), *Назва книги (курсивом)*, Видавництво, Місто.

Ініціали пишуться після прізвища.

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Приклад:

2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

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(2013), *Svitovi naukovometrychni bazy*, Available at: http://www.nas.gov.ua/publications/q_a /Pages/scopus.aspx

Cheung T. (2011), *World's 50 most delicious drinks*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

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