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Editorial office address:

National University
of Food Technologies
68 Volodymyrska str.
Kyiv 01601, **Ukraine**

Адреса редакції:

Національний університет
харчових технологій
вул. Володимирська, 68
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e-mail: **ufj_nuft@meta.ua**

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Contents

Effects of protein and carbohydrate ingredients on colour of baked milk products

Olena Mandiuk, Anna Lohinova, Larysa Arsenieva, Oksana Petrusha, Galyna Polishchuk

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Galyna Polishchuk
E-mail: milknuft@i.ua

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Abstract

Introduction. The aim of the present research was to study the influence of the presence of protein and carbohydrate ingredients on intensity of the Maillard reaction in baked dairy products and their color.

Materials and methods. Cream with a fat content of 10%, as well as creams with the addition of whey protein, hydrolysed whey concentrates, and glucose-fructose syrup served as objects of study. The color characteristics of the baked milk cream were examined by the CIE Lab system using a digital colorimeter; the active acidity was determined by the potentiometric method, and the overall sensory quality was estimated by the weighted average of the scores.

Results and discussion. In the CIE Lab system, only the coordinates "a" and "b" should be used to characterize the color change of milk of 2.5% fat and cream of 10% fat during heat treatment at 95–97 ºC for 160–180 min, as the L indicator (light level) is not sufficiently informative. According to the selected coordinates, rational ranges were established as a criterion for the completeness of the Maillard reaction for baked milk and cream, in particular for coordinate "a" in the range from 1.5 to 2.0 units, for coordinate "b" from 11.5 to 13.0 units.

The application of whey protein concentrate, hydrolysed demineralized whey concentrate, and glucose-fructose syrup, which contain monosaccharides and proteins, significantly enhanced the Maillard reaction. The recommended values for color coordinates of cream with milk protein and carbohydrate ingredients were achieved during the simmering process. For cream with whey protein concentrate, this occurs at a minimum of 21 min; for cream with hydrolysed whey concentrate at a minimum of 28 min, and for cream with glucose-fructose syrup and whey protein concentrate at a minimum of 18 min. The samples with whey protein concentrate and glucose-fructose syrup, including those one with whey protein concentrate, showed an excellent level of quality in terms of sensory characteristics after 20 min, while the sample with hydrolysed whey concentrate demonstrated this after 30 min of simmering. These results correlated with the rational duration of cream simmering to achieve the recommended degree of color. A slight decrease in acidity was observed in all cream samples during the heating process. The reduction in the duration of the simmering process of dairy products with simultaneous achievement of recommended color characteristics will contribute to a significant reduction in heat energy consumption.

Conclusions. The duration to achieve desirable color characteristics of baked milk cream can be reduced by the inclusion of ingredients containing carbohydrates and proteins such as glucose-fructose syrup, whey protein concentrate, and hydrolysed demineralized whey concentrate.

Introduction

Baked milk products have an attractive creamy-brown color. Prolonged high-temperature processing at 95-99 ºC causes the Maillard reaction in milk and cream with the formation of melanoidins, while whey proteins become denatured (Lohinova et al., 2023; Nielsen et al., 2022; Zheng, 2023), fat globule membranes are destroyed, and lipids are partially degraded with the formation of aromatic compounds (Jo et al., 2018).

Long-term heat treatment of milk can result in the formation of compounds harmful to human health, such as carboxymethyl lysine and acrylamide (Tamanna et al., 2015). Therefore, the process of milk and cream simmering should be stopped immediately after achieving the desired technological effect, primarily in terms of color characteristics. At the same time, there is currently no method for objective control of the color characteristics of dairy food systems, including cream one.

Information on heat treatment conditions is specified for high- and medium-fat cream only (Newton et al., 2012). Meanwhile, there is a growing demand for fermented cream products with reduced fat content up to 10% (Gouel et al., 2019). Milk whey, by-product from the dairy industry, is widely used in the food industry for the enrichment of various products (Kochubei-Lytvynenko et al., 2022, 2023). Sour cream obtained from low-fat cream has a liquid consistency (Shepard et al., 2013), and thus, the authors propose the addition of whey protein concentrate and liquid hydrolyzed concentrate of demineralized whey to its composition (Mykhalevych et al., 2022). Furthermore, it is possible to improve the sensory characteristics of sour cream with milk protein concentrates by preliminary cream simmering (Wang et al., 2022), since the lactoglobulins and monosaccharides in whey protein concentrate and liquid hydrolysed concentrate of demineralized whey are able to activate the Maillard reaction (Brands et al., 2001; Li et al., 2022).

However, excessive heating of milk and cream can lead to reduction in protein nutritional value and the formation of undesirable compounds (Choudhary et al., 2013, Li et al., 2021). Therefore, strict control of the color, taste, and odour characteristic of baked dairy products is required.

Color changes in heat-treated milk were determined using color difference (ΔE) and yellowness index (YI), which were chosen as the simplest and most reproducible characteristics (Pagliarini et al., 2006), although other alternative methods exist.

There is currently no complete description of the methodology for determining the color of milk and dairy products that would ensure repeatability and comparison of research results (Milovanovic et al., 2020). The authors revealed that among a number of dairy products studied in the CIELab color space (lowercase letters), the color variability for sour cream was the lowest.

Three attributes were used to objectively determine the color of dairy products: hue, brightness, and chroma in the instrumental color spaces CIELab, CIELu*v* and CIEXYZ (Leon et al., 2006). Currently, Hunter Lab and CIELab color spaces are most widely used to analyse colorimetric information, where L* defines the perception of light and dark, a* defines red or green, and b* defines yellow or blue (Pathare et al., 2012; Rossel et al., 2006). The CIELab color space will be used to study the color characteristics of cream as a basis for the production of baked sour cream.

Based on the results of the conducted study, recommendations will be developed for the industry to accelerate the technological process of cream simmering and correspondingly reduce energy consumption, including through the use of protein concentrates and carbohydrate-containing ingredients.

In light of the above, the aim of this study was to examine the patterns of color change in cream during the production of sour cream, with a particular focus on the impact of milk protein and carbohydrate-containing ingredients during the simmering process.

To achieve this goal, the following tasks were formulated: (a) to determine the recommended ranges of color characteristics as a criterion for the completeness of heat treatment of baked milk product, based on the results of the analysis of changes in the nature and degree of color of milk with 2.5% fat and cream with 10% fat during the simmering process; (b) to study the rational simmering duration of cream with 10% fat, enriched with milk protein concentrates and carbohydrate-containing ingredients, according to the specified criteria, to achieve the recommended values of color characteristics; (c) to study the dynamics of changes in acidity and sensory characteristics of cream during the simmering process to compare the identified patterns with the nature of the formation of color characteristics; (d) to formulate conclusions about the influence of individual components on the simmering process.

Materials and methods

Materials

The main raw materials chosen for simmering was cream with a fat content of 10% and milk with a fat content of 2.5.

To enrich the cream with whey proteins, the following protein-containing ingredients were used: whey protein concentrate, containing: solids – 94%, including protein – 80%, carbohydrates – 7%, fat – 7%; liquid hydrolysed concentrate of demineralized whey (Osmak et al., 2021), containing: solids – 40%, including protein – 4.4%, carbohydrates – 33.8%, fat – 0.4-0.6%, ash – 1.0-1.2%. After fermentation, the liquid hydrolysed concentrate of demineralized whey retains about 5% lactose, with the rest of the carbohydrates being monosaccharides.

It was previously determined the recommended content of the selected ingredients in cream ensuring the formation of high-quality finished products: 1% of whey protein concentrate and 30% of liquid hydrolysed concentrate of demineralized whey (Mykhalevych et al., 2022).

For study the influence of monosaccharides on the rate and completeness of the Maillard reaction, glucose-fructose syrup (GFS-42, "Intercorn Corn Processing Industry", Ukraine) was also added to the creams. The amount added syrup ensured an equivalent content of monosaccharides introduced into the cream together with the hydrolysed whey concentrate. The 40% liquid hydrolysed concentrate of demineralized whey contains 29.32% monosaccharides, so its addition to cream at a rate of 30% results in 8.8% monosaccharides in the creams. The glucose-fructose syrup contains 67.2% monosaccharides, so to ensure their equivalent content in the creams, 13.1% of this syrup was added.

Preparation of experimental samples

Milk protein concentrates were dissolved directly in cream at 40°C and holding for 30-40 minutes for preliminary swelling, after which the cream was filtered and subjected to simmering.

The simmering of creams and milk was conducted at a temperature of 96±1°C. Samples were collected at various time points during thermal treatment: 0, 30, 60, 90, 120, 150, and 180 minutes. During the simmering process, the following parameters were determined: degree of coloration, sensory properties, and acidity.

Methods

Color was determined using a digital colorimeter (Colorimeter, model LS173, Linshan, China) according to the CIE Lab system. The following parameters were assessed: L: Lightness level (from 0 for black to 100 for white); Color ranging from green (-) to red; Color ranging from blue (-) to yellow (+) (Ścibisz et al., 2019). Prior to measurement, the device was calibrated using a white standard according to the manufacturer's instructions.

The acidity was determined using the potentiometric method with a pH meter ADWA AD1030 at a temperature of 20±0.5°C (Shepard et al., 2013).

Sensory parameters including consistency, aroma, taste, colour, and appearance were assessed using a 5-point scale (1 – poor, 2 – acceptable, 3 – good, 4 – very good, and 5 – excellent). Taste assessment focused on the pasteurization flavour, sweet, and creamy tastes. Each parameter was weighted accordingly: consistency – 0.2; aroma – 0.2; taste – 0.2; color – 0.3, and appearance – 0.1.

The overall sensory quality was calculated as the weighted average of the scores. Cream samples were categorized based on the calculated overall weighted score as follows: excellent (20.0–25.0 points); good (16.0–19.9 points); satisfactory (11.0–15.9 points); practically unacceptable (6.0–10.9 points); unacceptable (less than 6 points) (Polishchuk et al., 2013).

Results and discussion

Recommended ranges of colour characteristics for baked milk products

At the first stage, the dynamics of changes in color characteristics of milk with 2.5% fat and cream with 10% fat during processing over a period of 3 hours were determined, which is the recommended duration of processing according to current technological instructions.

In the CIE Lab system, the L parameter (lightness) was not sufficiently informative for milk and cream during the 180-minute processing, so only the parameters "a" and "b" were used to characterize colour changes.

The results of measuring these parameters during the processing of milk and cream are shown in Figure 1a, b.

According to Figure 1, cream had numerical values exceeding those typical for milk for the colour coordinates "a" and "b" before processing. In particular, cream exhibited a more yellowish hue in the "b" coordinate compared to milk, which can be explained by its higher fat content with the presence of the colour compound β-carotene.

With the onset of processing, the rate of color change was significantly higher in milk than in cream, which can be attributed to the higher lactose and protein content in milk as the main reactants of the Maillard reaction (Mehta 2015).

Throughout the 180-minute processing, the color intensity increased monotonically for both milk and cream across both color coordinates. A crossing of the values of both color characteristics for milk and cream was observed at the 160th minute. Based on this, ranges of color values for baked milk and baked milk cream were selected as criteria for the recommended degree of their coloration, with minimal values for both colour coordinates corresponding to the indicated intersection point in Figure 1a, b.

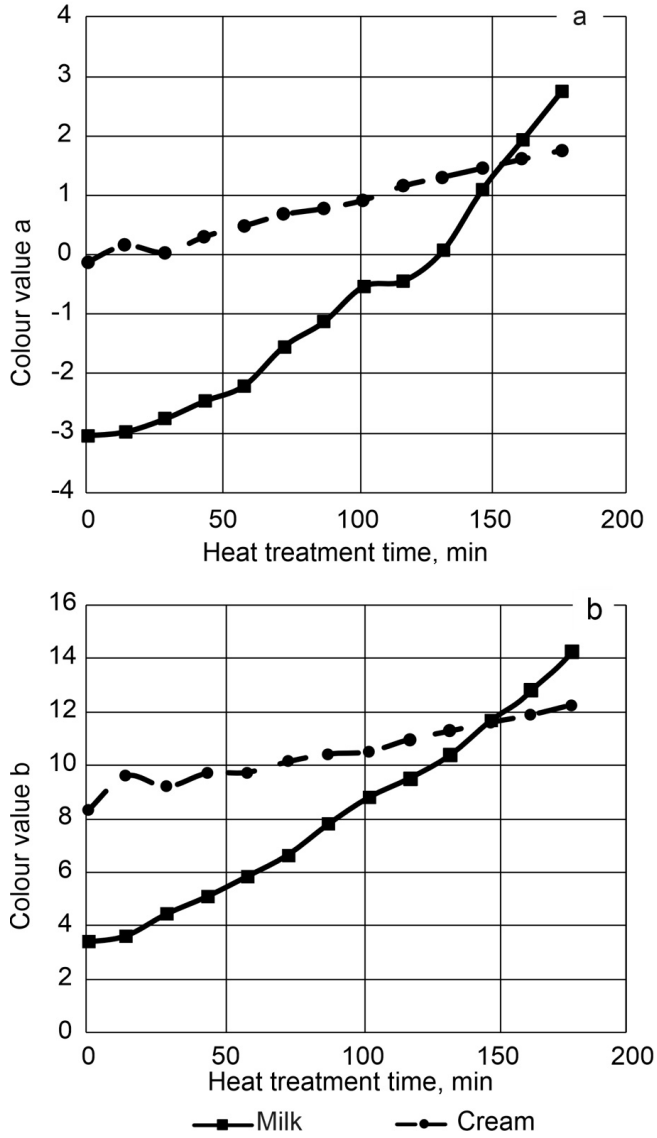

Figure 1. Dependence of changes in color coordinates on simmering time for milk and cream: a – color coordinate "a"; b – color coordinate "b"

Therefore, for the "a" coordinate, the range of values from 1.5 to 2.0 units was chosen as the criterion for achieving the appropriate degree of cream coloration resulting from the Maillard reaction, and for the "b" coordinate, the range from 11.5 to 13.0 units was chosen. If the color characteristics fall within these ranges, it is recommended to stop the cream simmering to save energy costs and prevent possible thermal destabilization of the fat emulsion (Hansen et al., 2020).

Time for cream simmering

The effects of milk-protein and carbohydrate concentrates on the change in color coordinates of 10% fat cream during simmering over 60 minutes was studied. The decision to reduce the simmering time from 180 to 60 minutes for cream with concentrates was based on existing information regarding the potential intensification of the Maillard reaction in the presence of monosaccharides and protein concentrates (Deepika et al., 2013, Spotti et al., 2019), as well as proven by the results of previous studies. The designations for the samples were as follows:

- Control sample (cream);
- Sample 1 – cream with 1% whey protein concentrate;
- Sample 2 – cream with 30% hydrolysed whey protein concentrate;
- Sample 3 – cream with 13.1% glucose-fructose syrup;
- Sample 4 – cream with 1% whey protein concentrate and 13.1% glucose-fructose syrup.

The change in color characteristics of these samples is presented in Figure 2.

According to Figure 2, the cream samples with whey protein concentrate (samples 1 and 4) were characterized by slightly elevated values for the color coordinate "a", which is likely due to an excess of whey proteins. Proteins and carbohydrates in the concentrates in samples 1–4, at 60 minutes of simmering, increased the value of the color coordinate "a" by 8 times (sample 1), 10 times (samples 2 and 4), and 10.4 times (sample 3), compared to the control sample. This effect can be explained by the high ability of monosaccharides and milk proteins to participate in the Maillard reaction (Leiva et al., 2016).

Regarding the color coordinate "b", compared to the control sample, the experimental samples had higher values due to the initial coloration of the added ingredients. The highest colouring of sample 2 – with whey concentrate that after lactose fermentation acquired a typical yellowish-cream shade – is worth noting. Despite the somewhat different nature of the colour change during simmering, at the 60th minute of this process, all investigated samples with protein and carbohydrate ingredients were characterized by fairly close values. However, the presence of monosaccharides as highly reactive compounds (Brands et al., 2001) in samples 3 and 4 allowed to achieve the most intense colouring.

Through the analysis of the research results and comparing their values with the recommended ranges for coordinate "a" from 1.5 to 2.0 units, and for coordinate "b" from 11.5 to 13.0 units determined in the first stage of the study, the recommended simmering duration for cream with protein and carbohydrate ingredients, as shown in Table 1, was found.

Table 1

Time of the cream simmering with protein and carbohydrate ingredients

Colour values CIELab	**Time to achieve color coordinate values for samples, min**			
	Sample 1	Sample 2	Sample 3	Sample 4
a	21	28	18	18
b	20	–	12	5

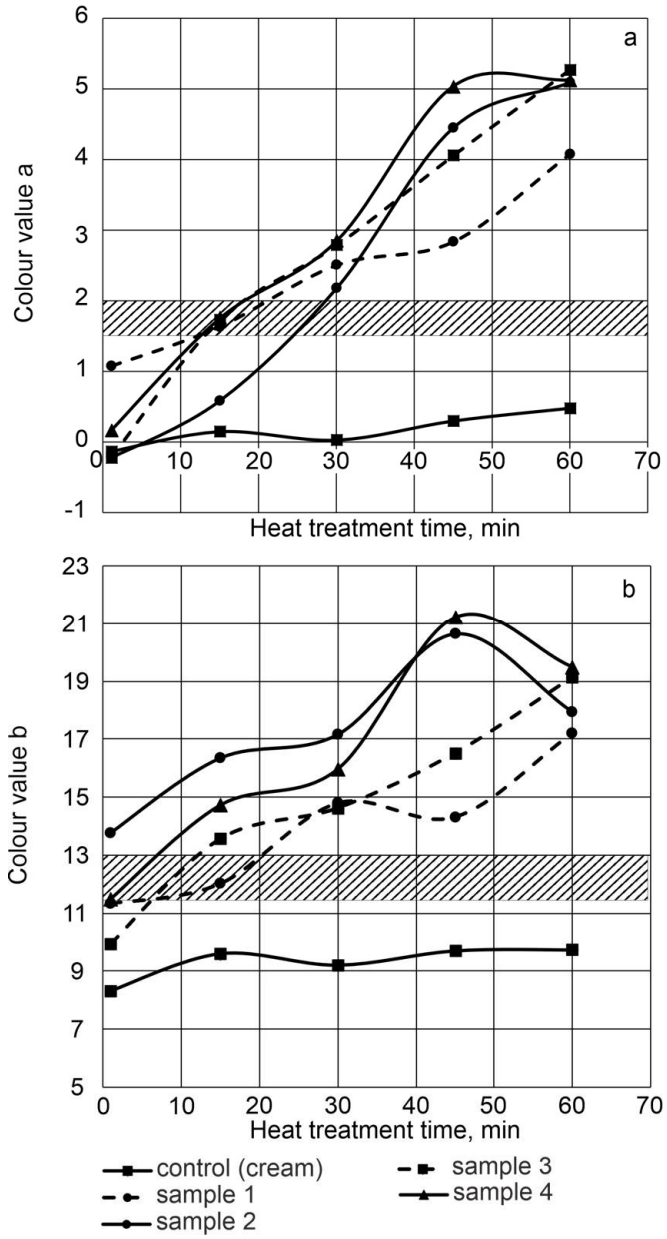

Figure 2. Dependence of color coordinates on simmering time for cream with protein and carbohydrate ingredients

According to Table 1, achieving the recommended degree of coloration using the established standard during the simmering process of 10% fat cream with carbohydrate and protein ingredients was significantly reduced compared to cream without Maillard reaction-activating additives.

For sample 2 achieving coloration based on the colour coordinate "b" occurred immediately after adding the hydrolysed whey concentrate to the cream, which was characterized by intense coloration. The rapid change in color of hydrolysed dairy products and concentrates due to the accumulation of lactose hydrolysis products is also noted by other researchers (Pinto et al., 2021). At the same time, to achieve the recommended degree and type of coloration based on the color coordinate "a", this sample requires the longest simmering time compared to other samples.

If we consider the minimal simmering time for creams to achieve the recommended values of both color coordinates, then for:

- Sample 1, this indicator should be no less than 21 minutes;
- Sample 2, no less than 28 minutes;
- Samples 3 and 4, no less than 18 minutes.

Thus, the simmering time for creams with whey protein concentrate is reduced by 7.6 times, with hydrolysed whey protein concentrate is reduced by 5.7 times, with glucose-fructose syrup, including in combination with whey protein concentrate, is reduced by 8.9 times compared to the control sample. Therefore, the possibility of significantly accelerating the simmering process of cream by introducing protein and carbohydrate concentrates into their composition has been proven. Concentrates containing monosaccharides such as glucose and fructose in glucose-fructose syrup exhibit high reactivity. The exceptional role of monosaccharides in accelerating the Maillard reaction is also confirmed by research results (Zhang et al., 2019).

Acidity and sensory characteristics of cream during simmering

Sensory indicators of the control and experimental samples of clotted creams with protein and carbohydrate components were studied before and during the first 40 minutes of simmering, which does not exceed the recommended simmering duration for all samples. The overall weighted score obtained from the results of the sensory quality assessment of the samples is presented in Figure 3.

According to Figure 3, an excellent quality level (above 20 points) was achieved for samples 1, 3, and 4 after 20 minutes, and for sample 2 after 30 minutes of heating, which correlates well with the recommendations for the rational duration of simmering to achieve the recommended colour characteristics. As for the control sample, it is understandable that the duration of thermal processing for 40 minutes is insufficient to form its excellent quality not only in terms of colour characteristics but also in terms of taste, smell, and consistency.

The acidity of the cream samples during the simmering process was also determined. The research results are presented in Table 2.

According to Table 2, the acidity of cream with milk-protein concentrates is slightly lower, which can be explained by additional binding of free water. Samples 3 and 4 are characterized by lower acidity, attributed to the acidic nature of the glucose-fructose syrup resulting from the acidification of the reaction environment during its production (Xu et al., 2016).

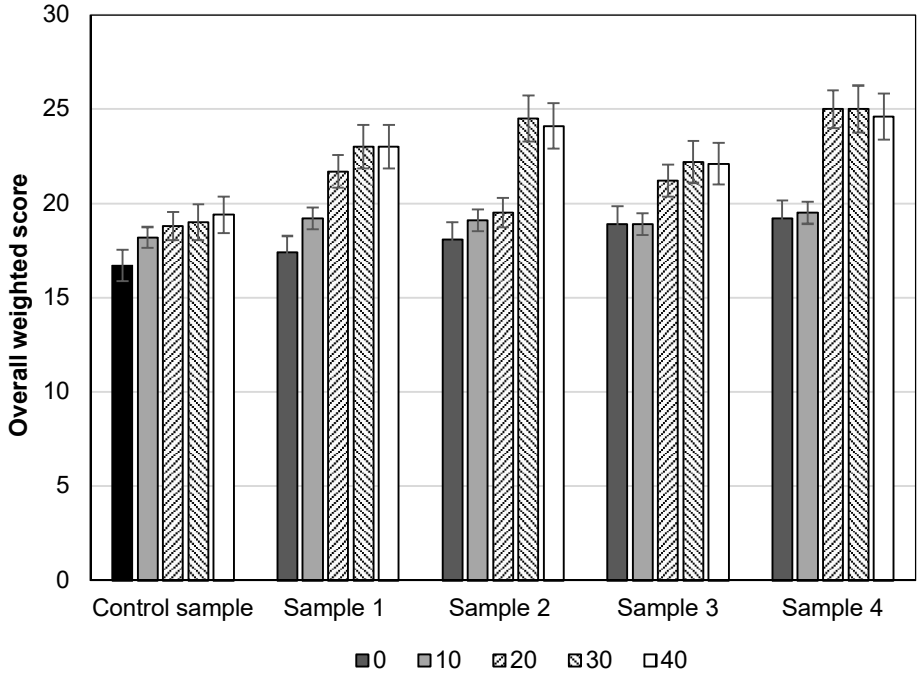

Figure 3. Overall weighted score of sensory evaluation of clotted cream samples

Table 2

Acidity of cream (pH) at different simmering time

Samples	pH of cream at the simmering time, min				
	0	10	20	30	40
Control	6.67±0.19	6.60±0.17	6.59±0.17	6.57±0.11	6.55±0.15
Sample 1	6.65±0.17	6.55±0.15	6.49±0.15	6.42±0.16	6.35±0.13
Sample 2	6.64±0.15	6.38±0.12	6.30±0.12	6.26±0.10	6.21±0.15
Sample 3	6.14±0.16	6.10±0.15	6.08±0.14	6.04±0.15	6.00±0.12
Sample 4	6.12±0.11	6.08±0.12	6.05±0.13	6.01±0.11	5.98±0.11

During heat treatment, there is a slight decrease in the acidity of all samples. Despite the loss of carbon dioxide, cream exhibit processes where soluble calcium and phosphorus transition into insoluble calcium phosphate with the release of acidic phosphates. Sample 2 demonstrates the most dynamic decrease in pH due to the increased content of mineral compounds. The increase in pH levels after prolonged heating of milk, according to data (Jasim, 2014), was not observed in the investigated samples of cream due to their different fat content. Thus, significant changes in the acidity of creams with milk-protein concentrates during simmering were not noticed. Glucose-fructose syrup significantly reduces the acidity of samples at the beginning of heat treatment but without significant changes during simmering.

The decrease in the acidity of cream and the likely change in the structure of protein macromolecules after prolonged heat treatment (Krishna et al., 2021; Li et al., 2021) may affect the microstructure of the protein gel in baked sour cream, which requires further investigation.

Conclusions

1. Recommended color criteria for baked milk and clotted cream in the CIE Lab system are as follows: for coordinate "a" from 1.5 to 2.0 units; for coordinate "b" from 11.5 to 13.0 units. After achieving colour characteristics within these ranges, the process of simmering dairy products can be stopped to reduce energy consumption. The dynamics of colour changes during heat treatment are higher in milk than in cream due to the higher content of highly reactive components.
2. To achieve the recommended colour coordinate values, the simmering process for cream with milk-protein and carbohydrate ingredients should last as follows: for cream with whey protein concentrate – no less than 21 min; for creams with hydrolysed whey protein concentrate – no less than 28 min; for creams with glucose-fructose syrup, including in combination with whey protein concentrate – no less than 18 min. Concentrates containing monosaccharides such as glucose and fructose demonstrate the highest technological efficiency.
3. Excellent quality level for samples with whey protein concentrate, glucose-fructose syrup, and their combination is achieved after 20 min, while for the sample with hydrolysed whey protein concentrate, it's achieved after 30 minutes of simmering, which correlates with recommendations for the optimal simmering duration to achieve the recommended degree of coloration. A slight decrease in the acidity of all cream samples occurs during simmering. The application of glucose-fructose syrup during heat treatment has the greatest impact on acidity due to the increased acidity of this syrup.
4. Further research prospects include studying the color characteristics of fermented clotted cream, including during storage, as well as investigating the microstructure of baked sour cream with milk-protein concentrates and glucose-fructose syrup.

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Wild and cultivated mushrooms as food, pharmaceutical and industrial products

Olena Stabnikova[1], Viktor Stabnikov[1], Octavio Paredes-López[2]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – National Polytechnic Institute, Guanajuato, Mexico

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Corresponding author:

Olena Stabnikova
E-mail:
stabstab6@gmail.com

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Abstract

Introduction. Mushrooms have been consumed since earliest times and have been recognized for their attractive sensory and culinary attributes; besides, they contain high amounts of bioactive and health-promoting compounds. This review is devoted to wild edible mushrooms and their role in the life of modern man.

Materials and methods. Literature research using scientific publications on the topics related to wild mushrooms as a food resource, their applications in medicine and pharmaceuticals, as well as the methods for mushroom cultivation was conducted.

Results and discussion. Wild edible mushrooms have high nutritional value, contain high-quality proteins, fiber, essential fatty acids, vitamins, including D2, microelements, as well as valuable compounds such as polyphenols, terpenoids, sterols, while having a low energy value, which makes it possible to use them in cooking and in low-calorie diets. Wild edible mushrooms have various specific pharmaceutical properties that can be used in the treatment of various serious diseases. The gathering and use of wild edible mushrooms for food make a significant contribution to both the solution of the global food shortage crisis and economics of different countries around the world, and could be considered in some countries as new sources of income for local people. The increase of interest on mushroom consumption along human history at worldwide level has led to the development of basic and sophisticated techniques for their cultivation. Solid-state and submerged liquid fermentations are nowadays useful methods for cultivation of mushroom in a large-scale for production of volumes of biomass and of valuable specific bioactive metabolites. An interesting and unusual method to grow edible mushrooms of *Ustilago maydis*, which are considered a delicacy produced by the natural infection of the maize ears, is described.

Conclusions. The role that the mushroom kingdom plays in human life is extremely important and varied. In the near future, their role in local economies around the world and as raw materials for food and pharmaceutical products, including industrial cultivation, will be areas of greatest use.

Introduction

Mushrooms are considered to be among the most mysterious forms of life on our planet (Lincoff, 1981). Mushroom belonging to the phylum *Basidiomycota* is the fleshy fruiting body of a certain type of fungus, typically formed over the substrate used for fungi growth. Wild mushrooms have been recognized as a source of food and medicine from ancient times. Thus, it was proved that about 18,700 calendar years ago people ate mushrooms: spores of *Agaricales* and *Boletales* were found in a tooth plaque of a woman buried in the Upper Palaeolithic in northern Spain (Straus et al., 2015).

Legends and superstitions that existed among various peoples associated with mushrooms have come down (Bertelsen, 2013). The Egyptians considered mushrooms to be plants of immortality being a gift from the god Osiris and called them "the flesh of the gods" (Budge, 2017). According to the laws of ancient Egypt, only the pharaoh and his entourage could consume mushrooms, but common people were not allowed to even touch them. In Greek mythology, the growth of mushrooms came from lightning sent to earth by Zeus, as they appeared after thunderstorms. To the ancient Romans mushrooms were "the foods of the Gods" (Niksic et al., 2016), and mushrooms were included in the menu for special occasions (Rahi and Malik, 2016).

The images of mushrooms have been found in prehistoric cave paintings, the oldest of which, found in caves in the Sahara Desert (Tassili, Algeria) and in southern India, which were made by Prehistoric Early Gatherers in 9000-7000 B.P. (Before the Present) and in 1000 B.C. (Before Christ) – 100 A.C. (After Christ), respectively (Samorini, 2001). Depiction of a mushroom in shamanistic scene was found on Mount Bego, France dating at 1800 B.C. (Samorini, 2001).

Some mushrooms have been known for their hallucinogenic effects and early humans used them in spiritual rituals. Fly agaric mushroom (*Amanita muscaria*) has a hallucinogenic effect but can be poisonous to humans (Figure 1). Many legends tell that the Vikings consumed before the battle these mushrooms to induce frenzy and fearlessness and be less sensitive to pain. The native peoples in pre-Columbian Mesoamerican societies used hallucinogenic species of the *Psilocybe* genus in group ceremonies for religious communion (Carod-Artal, 2015). Numerous so-called 'mushroom stones' (sculptures) dating from 1000 – 500 years. B.C. were found in Mexico, Guatemala and Salvador testifying to the existence of mushroom cult in Mesoamerica.

Lingzhi, also known as reishi or *Ganoderma* is a mushroom tightly connected with Taoism. Taoist temples in ancient China were called "the abode of mushrooms", and Lingzhi, "spirits mushroom", used for a concentrated hallucinogenic decoction, was called "the mushroom of immortality". Some mushrooms possess toxicity; among the huge variety of existing mushrooms, about 100 species are poisonous to humans (Graeme, 2014; Li et al., 2021).

Mushrooms have an extremely varied size, shape and appearance; some of them are brightly colored, those belonging to *Phallus indusiatus* are wrapped in a transparent veil, and smell of the cap of Wood witch attracts flies and other insects (Figure 1).

For a long time, mushrooms were not grown, and they could only be collected from their natural habitats. And even today, only a small number of mushroom species are cultivated compared to the total number of edible species. In the modern world, there is an ever-increasing interest in mushrooms as a food product having certain health benefit properties. There are three main areas on the use of mushrooms, namely, wild edible, medicinal and commercially cultivated (Anusiya et al., 2021).

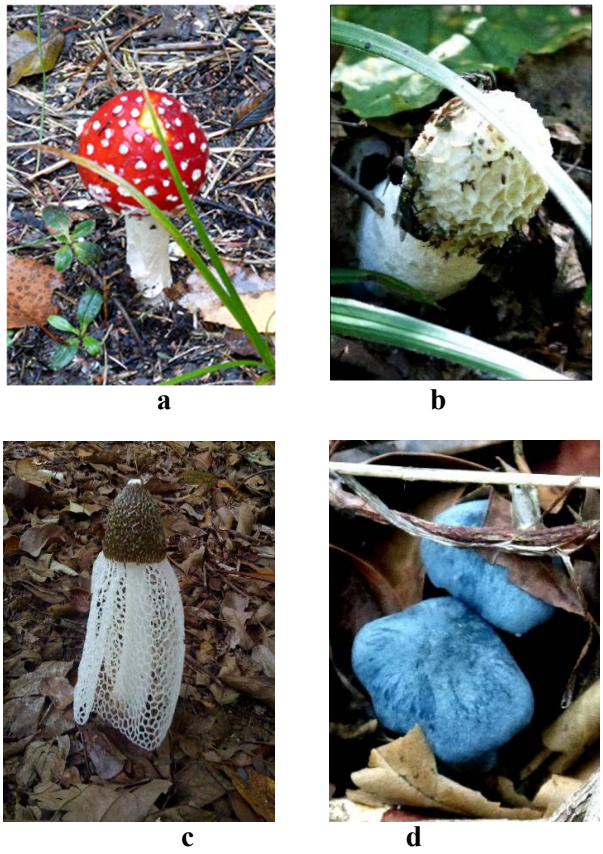


Figure 1. Variety of shapes and colors of mushrooms:
A, Fly agaric (*Amanita muscaria*); B, Wood witch (*Phallus duplicatus*);
C, Bamboo mushrooms (*Phallus indusiatus*); D, Blue mushroom in the tropical forest.

Wild mushrooms as food products

Wild edible mushrooms could serve as a source of ingredients for production of functional food. Mushrooms consist of the fruiting body (stalk, cap, and gills) and mycelium, a root-like structure. Gathering of wild mushrooms for using them for food has been done since ancient times. Fruit bodies of growing mushrooms make up a certain part of the diet of the poor in many rural areas around the world, and at the same time are a favorite delicacy of many gourmets. It was reported based on the current data over 100 countries that more than about 2,100 species of mushroom having different degrees of edibility exist in nature; however the number of them accepted as food does not exceed 25 (Barros et al., 2007; Pérez-Moreno et al., 2021; Zhang et al., 2013). The global market of wild edible fungi exceeded 1, 230,000 tons in 2017 estimated to be worth more than 5 billion USD (Pérez-Moreno et al., 2021). The harvesting of some wild mushrooms takes place on an especially large scale and has become now a really big business (Peintner et al., 2013). This is especially true for chanterelles (*Cantharellus cibarius*), morels (*Morchella esculenta*, *M. deliciosa* and *M. elata*), truffles (*Tuber melanosporum* and *Tuber magnatum*) and matsutake (*Tricholoma matsutake*) (Moore et al., 2020). Truffles, underground mushrooms, are especially appreciated by gourmets. In Europe, truffles are mainly collected in France and Italy. Previously, they were searched for with specially trained pigs, but now dogs are

used for this purpose. Matsutake ("pine mushroom" in Japanese) has a unique taste and aroma and occupies a place in Japanese cuisine similar to the truffle in European cuisine. The price for these mushrooms is extremely high. The cost of truffles depends on season, weather factors (drought or rainfall) and of mushroom size; for example, the price of white truffles in 2022 was around 4,500 euros/kg (Trivelli Tartufi, 2023). A piece of matsutake, one of the most expensive mushrooms, which can only be harvested in the forest, their natural habitats, could be sold for more than 200 USD in the Tokyo market and its overall value is estimated as 4.6 to 7.7 billion USD annually (Moore et al., 2020). The annual world export market of collected chanterelles has been estimated at over 1.5 billion USD (Thorn et al., 2021; Watling, 1997).

It has been reported that the market of wild mushrooms is constantly increasing due to the decline of the traditional forest-based industries; gathering of this product in some countries are now considered as new sources of income (De Frutos Madrazo et al., 2012; Dejene et al., 2017; Román and Boa, 2006; Sileshi et al., 2023; Tibuhwa, 2013). The increased interest for forest mushrooms led to development of "mycosilviculture" aimed at improving productivity and profitability of forest stands, and consequently a better mushroom availability (Corona et al., 2016; Savoie and Largeteau, 2011; Tomao et al., 2017).

Composition of wild mushrooms. Species of wild mushrooms are diverse in taxonomic, ecological, and physiological features; they grow everywhere on a variety of flat and forest soils, and it is influenced, as expected, by local climatic conditions. Among the global mushroom industry, wild species account, according to their applications, for only 8%, while this number for cultivated and medicinal samples consists of 54 and 38%, respectively (Royse et al., 2017). Interestingly, edible wild mushrooms have a high nutritional value containing protein, fibers, essential fatty acids, vitamins, and trace minerals while having low energetic value and are cholesterol-free.

The composition of fruiting bodies of five species of the most popular wild edible mushrooms (Figure 2) are shown in Table 1.

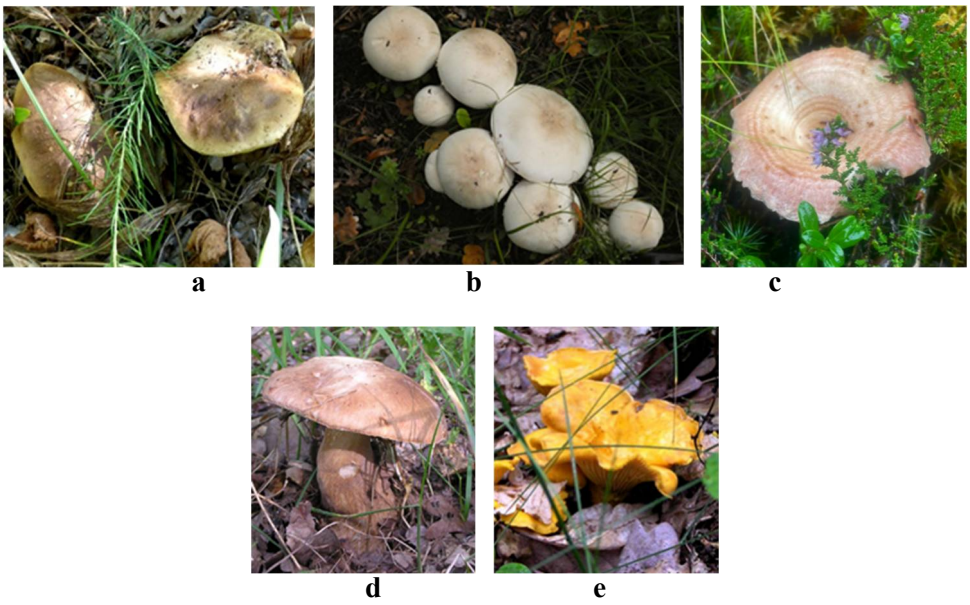


Figure 2. The most popular wild edible mushrooms:
A, Slippery Jack (*Suillus luteus*); B, Horse mushroom (*Agaricus arvensis*); C, Saffron milk cap (*Lactarius deliciosus*); D, Porcini, (*Boletus edulis*); E, Chanterelle (*Cantharellus cibarius*).

Table 1

Composition of mushroom fruiting bodies and their energetic value

Mushroom species	DM, %	g/100 g of dry matter (DM)				kcal/100 g DM	Reference
		Protein	Carbohydrates	Fat	Ash		
Suillus granulatus (Portugal)	n.a.	14.8	73.5	3.7	8.0	387	Reis et al., 2014
S. granulatus (Serbia)	n.a.	7.9	81.4	0.3	10.4	360	Reis et al., 2014
Agaricus arvensis (Portugal)	5.1	56.3	37.5	2.7	3.5	400	Barros et al., 2007
A. bisporus (Portugal)	n.a.	80.9	8.3	0.9	9.9	370	Barros et al., 2008a
Lactarius deliciosus (Portugal)	10.0	29.8	62.9	2.2	5.1	389	Barros et al., 2007
L. deliciosus (China)	8.0	17.2	66.6	4.8	8.6	379	Xu et al., 2019
Boletus edulis (Greece)	12.4	27.2	62.1	2.8	6.3	n.a.	Ouzouni and Riganakos, 2007
B. edulis (Poland)	11.9	20.3	66.0	7.8	5.9	379	Jaworska et al., 2015
B. edulis (Chorvatia)	12.2	36.9	64.3	2.9	5.3	356	Beluhan and Ranogajec, 2011
Cantharellus cibarius (Portugal)	N/A	69.1	14.3	4.5	12.1	376	Barros et al., 2008a
Cantharellus cibarius (Portugal)	7.6	53.8	32.1	2.9	11.5	371	Barros et al., 2008b
C. cibarius (Greece)	17.4	21.6	66.1	2.9	9.4	n.a.	Ouzouni and Riganakos, 2007

n.a. – not applicable

Body of the mushroom contains high levels of water, 86 – 94 g/100 g, so the content of dry matter (DM) is very low. The range of the different compounds present in wild edible species growing in Central and Eastern Europe is extremely varied, in g/100 g DM: protein from 7.9 for *Suillus granulatus* (weeping bolete) to 56.3 for *Agaricus arvensis* (horse mushroom, snowball mushroom) and 80.9 for *A. bisp*orus (button mushroom); fat from 0.3 for *S. granulatus* to 11.5 for *Boletus edulis*; ash from 3.5 for *A. bisporus* to 11.5 for *Cantharellus cibarius* (chanterelle), and carbohydrates from 8.3 for *C. cibarius* to 81.4 for *S. granulatus*. *Boletus edulis* (king mushroom, penny bun, porcino or porcini), one of the most famous edible mushrooms in many countries, contain protein, 27.2; fat, 2.8, and ash, 6.3

(Ouzouni and Riganakos, 2007). It was found that wild species are even richer by protein and have lower fat content in comparison with cultivated ones (Barros et al., 2008). Low energy value, allowed to use mushrooms in low-calorie diets (Barros et al., 2007; 2008a, b; Beluhan and Ranogajec, 2011; Jaworska et al., 2015; Kalač, 2009; Ouzouni and Riganakos, 2007; Xu et al., 2019).

Phenolic acids in wild mushrooms. Wild edible mushrooms are a rich source of phenolic acids, which are known possess antioxidant and anti-inflammatory activities. p-Hydroxybenzoic acid and cinnamic acid are the main compounds present almost in all analyzed wild mushrooms (Table 2).

Table 2

Amount of phenolic acids in wild edible mushrooms, mg/100 g DW

Mushroom species	Protocatechuic acid	p-Hydroxy-benzoic acid	Vanillic acid	Sinapic acid	Cinnamic acid	p-Coumaric acid	Total amount	Reference
S. granulatus (Portugal)	n.a.	0.480	n.a.	n.a.	0.130	n.a.	0.590	Reis et al., 2014
S. granulatus (Serbia)	n.a.	0.130	n.a.	n.a.	0.030	n.a.	0.130	Reis et al., 2014
S. collinitus (Portugal)	0.528	1.414	n.a.	n.a.	0.134	n.d.	2.066	Vaz et al., 2011
S. mediterra-neensis (Portugal)	0.138	0.204	n.a.	n.a.	0.098	n.d.	0.440	Vaz et al., 2011
A. arvensis (Portugal)	n.d.	7.013	n.d.	n.a.	4.910	4.867	16.790	Barros et al., 2009
*A. bisporu*s (Portugal)	n.d.	2.559	n.d.	n.a.	0.872	n.d.	3.431	Barros et al., 2009
L. deliciosus (Portugal)	n.d.	2.266	n.d.	n.a.	1.497	n.d.	3.763	Barros et al., 2009
L. deliciosus (Poland)	0.137	n.d.	n.d.	1.429	0.406	n.a.	1.972	Muszyńska et al., 2013
L. deliciosus (Spain)	1.864	2.140	n.a.	n.a.	n.a	n.d	4.004	Palacios et al., 2011
L. aurantiacus (Portugal)	n.d.	n.d.	n.a.	n.a.	0.918	n.d.	0.918	Vaz et al., 2011
B. edulis (Poland)	0.750	0.194	n.d.	n.d.	n.d.	n.a.	0.944	Muszyńska et al., 2013
C. cibarius (Poland)	0.150	0.230	0.332	0.304	0.129	n.a.	1.149	Muszyńska et al., 2013
C. cibarius (Portugal)	n.d.	n.d.	n.d.	n.d.	1.497	n.a.	1.497	Barros et al., 2009

n.d.– not detected; n.a. – not applicable.

Fatty Acid in wild mushrooms. Lipids of mushrooms contain fatty acids, which are represented by saturated (SFA—without double bonds), monounsaturated (MUFA—with one double bond) and polyunsaturated fatty acids (PUFA—with two or more double bonds). The human body cannot synthesize PUFA, so the presence of them is recommended to be included in commonly used foods (Stabnikova and Paredes-López, 2023). Monounsaturated and polyunsaturated fatty acids are prevalent in mushroom fatty acid composition (Table 3).

Table 3

Fatty acid composition (percent) of the wild edible mushrooms

Mushroom species	Total SFA	Total MUFA	Total PUFA	Reference
S. granulatus	1.32	63.30	35.38	Ribeiro et al., 2009
A. bisporus	22.1	1.5	76.4	Barros et al., 2008a
B. edulis	14.5	40.9	44.6	Barros et al., 2008a
B. edulis	0.96	68.27	30.77	Ribeiro et al., 2009
C. cibarius	12.00	37.50	50.40	Barros et al., 2008a
C.cibarius	3.24	78.89	17.87	Ribeiro et al., 2009
L. deliciosus	22.13	48.37	29.49	Xu et al., 2019

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids;
PUFA – polyunsaturated fatty acids.

The most abundant fatty acids in *Suillus granulatus*, % of total amount, were: oleic acid (C18:1, ω-9), 62.79 and linoleic acid (C18:2, ω-6), 35.35. The most abundant fatty acids in *Agaricus bisporus,* % of total amount, were: palmitic acid (C16:0), 9.97; stearic acid (C18:0), 4.08; linoleic acid (C18:2, ω-6), 75.72, and behenic acid (C22:0), 1.62 (Barros et al., 2008a). The most abundant fatty acids in for *Cantharellus cibarius,* % of total amount, were: palmitic acid (C16:0), 13.08; oleic acid (C18:1, ω-9), 10.78; linoleic acid (C18:2, ω-6), 53.59; α-linolenic acid (C18:3, ω-3), 0.10, and eicosenoic acid (C20:1), 27.98 (Barros et al., 2008a). The most abundant fatty acids in *Boletus edulis*, % of total amount, were: palmitic acid (C16:0), 10.03; oleic acid, (C18:1, ω-9), 39.72, and linoleic acid, (C18:2, ω-6), 44.32 (Barros et al., 2008a). For fifteen species of wild edible mushrooms belonging to the genus *Boletus* grown in Israel, it was determined that oleic acid (18:1, ω-9), 15-42%, linoleic acid (18:2, ω-6), 38–58%, and palmitic acid (16:0), 7–17% were the most abundant (Hanuš et al., 2008). The main fatty acids for *Lactarius deliciosus*, % of total amount, included: palmitic acid (C16:0), 5.17; stearic acid (C18:0), 16.96; oleic acid (C18:1, ω-9), 48.37; linoleic acid (C18:2, ω-6), 29.49 (Xu et al., 2019).

So, oleic, mono-unsaturated omega-9 fatty acid, and linoleic, polyunsaturated omega-6 fatty acid, were present in the highest amount in all mentioned above, wild mushrooms. This is consistent with the finding of Sande and co-authors (2019) who, based on reviewing the literature of *Agaricus bisporus*, *Pleurotus ostreatus*, and *Boletus edulis* mushroom species from different continents noted the predominant presence of the same fatty acids, but their quantitative composition varied significantly: linoleic acid ranges from 0.0–81.1%, oleic acid between 1.0 and 60.3%, and linolenic acid from 0.0–28.8%.

Vitamin D in wild mushrooms. It was shown that mushrooms are a good dietary source of vitamin D, which actively participates in regulation of calcium metabolism and needed to reduce the risk of osteomalacia in adults and rickets in children (Charoenngam et al., 2019). Recommended daily intake of this vitamin is 15 μg/day in Europe, USA, and Canada (Cardwell et al., 2018).

In wild mushrooms exposed to UV radiation, ergosterol present in the cell membrane is converted to D2, one of the forms of vitamin D. Bioavailability of vitamin D2 from mushrooms for humans was demonstrated by the results of clinical studies (Keegan et al., 2013; Mehrotra et al., 2014; Outila et al., 1999). The content of vitamin D2 in some wild mushrooms is shown in Table 4.

Table 4

Content of vitamin D2 in some wild edible mushrooms

Mushroom species	D2, µg/100 g FM	Reference
Agaricus bisporus (Finland)	0.21	Mattila et al., 1994
Agaricus sp. (wild) Denmark	1.50	Kristensen et al., 2012
Boletus edulis (Sweden)	58.7	Teichmann et al., 2007
Boletus edulis (Finland)	2.91	Mattila et al., 1994
Cantharellus cibarius (Finland)	12.80	Mattila et al., 1994
Cantharellus cibarius (Sweden)	10.7	Teichmann et al., 2007
Cantharellus tubaeformis (Sweden)	21.1	Teichmann et al., 2007

FM is fresh matter.

Mushrooms from genera *Cantharellus* have a high amount of vitamin D2 (ranging from 10.7 to 21.1 µg /100 g FM) that is preserved after culinary treatment. Thus, the content of vitamin D2 in canned *Cantharellus cibarius* was 12.1 µg/100 g FM (Teichmann et al., 2007). King mushroom *Boletus edulis* could be a source of vitamin D2, meanwhile the content of D2 in mushrooms from the genera *Agaricus* is low (Teichmann et al., 2007).

Biological active substances in wild mushrooms. Wild mushrooms possess antioxidant activity due to the presence of such bioactive substances as flavonoids, phenolic compounds, tocopherols, ascorbic acid and carotenoids (β-carotene and lycopene) (Table 5).

It should be mentioned that mushrooms also are rich with the vitamins B group such as B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folate), (Dimopoulou et al., 2022; Cağlarirmak, 2011; Muszyńska et al., 2017). Mushrooms usually contain small amounts of vitamin B12 (cobalamin), but relatively high levels of this vitamin was found in golden chanterelle (*Cantharellus cibarius*) from Germany, France and Bulgaria, 1.09 – 1.87 µg/100 g DM, meanwhile B12 in porcini mushrooms (*Boletus* spp.), parasol mushrooms (*Macrolepiota procera*), and black morels (*Morchella conica*) was absent or detected on low levels from 0.01 to 0.09 µg/100 g DM (Watanabe et al., 2012). Higher amounts of B12 were found in commercial shiitaki (*Lentinula edodes*) fruiting bodies, 5.61 µg/100 g DM (Watanabe et al., 2014).

Carbohydrates in wild mushrooms. Carbohydrates of mushrooms include mainly chitin, and also hemicellulose and glycogen. The presence of indigestible chitin apparently limits availability of the nutrients contained in mushrooms (Borthakur and Joshi, 2019) and causes the fact that people with diseases of the gastrointestinal tract are not recommended to consume mushrooms in significant quantities. However, chitin well absorbs toxins and heavy metals, removing them from the body during digestion. The active polysaccharides (β-glucans) contained in mushrooms strengthen the immune system and are considered as a health-promoting factor. Due to the presence in mushrooms carbohydrates such as hemicellulose, chitin, α- and β-glucans, xylans, mannans and galactans also serve as a prebiotic (Jayachandran et al., 2017). It is considered that 100 g of mushrooms supply from 9 to 40% of the daily recommended allowance of dietary fiber (Manzi et al., 2001).

Table 5

Total bioactive compounds of wild mushrooms

Mushroom species	Content, mg/100g DW			Content, µg/100 g DW			Reference
	Total phenols	Flavonoids	Ascorbic acid	β-carotene	Lycopene	Total tocopherols	
Suillus luteus (Poland)	876	n. a	n. a	n. a	n. a	n. a	Witkowska et al., 2011
Suillus luteus (Turkey)	506	n.a	8.2	n.a.	n.a.	n.a.	Keleş et al., 2011
A. arvensis (Portugal)	272	165	2	852	470	n.a.	Barros et al, 2008c
Agaricus bisporus (Portugal) wild On compost	853	367	n. a	n. a	n. a	n. a	Machado-Carvalho et al., 2023
Agaricus bisporus wild (Turkey)	402	n.a.	n.d.	n.a.	n.a,	n.a.	Keleş et al., 2011
Lactarius deliciosus (Poland)	429	n. a	n. a	n. a	n. a	n. a	Witkowska et al., 2011
Lactarius deliciosus (Turkey)	271	n.a,	≤ 20	n.a.	n.a.	n.a.	Keleş et al., 2011
Boletus edulis (Portugal)	503	175	n.d.	273	114	1065	Barros et al., 2008a
Boletus edulis (Poland)	1618	n. a	n. a	n. a	n. a	n. a	Witkowska et al., 2011
Boletus edulis (Poland)	446	32	22.1	1060	68.6	494	Jaworska et al., 2015
Boletus edulis (Portugal)	1096	161	n. a	n. a	n. a	n. a	Machado-Carvalho et al., 2023
Cantharellus cibarius (Portugal)	88	67	86	1356	506	1.8	Barros et al., 2008
Cantharellus cornucopioides (Portugal)	213	171	87	1277	513	187	Barros et al., 2008

n.d – not detected; n.a- not applicable.

Mineral elements in wild mushrooms. Usual content of major mineral elements in wild growing mushrooms is as follows, mg/100 g of dry matter: sodium, 10–40; potassium 2000–4000; calcium, 10–50; magnesium, 80–180; phosphorus, 500–1000; sulfur, 100–300 (Kalač, 2009). The accumulation of a wide variety of minor and trace elements in mushrooms is species- and site-dependent (Alaimo et al., 2019). Taking into account the ability of mushrooms to absorb heavy metals, especially mercury, lead, arsenic and cadmium, it should be noted that they can be collected only in noncontaminated places far from industrial areas (Nowakowski et al., 2021).

Other useful properties of wild mushrooms. Mushrooms do not contain cholesterol, and it has been even reported that they possess cholesterol lowering properties (Berger et al., 2004). It is generally recognized that mushrooms contain such valuable compounds as polyphenols, terpenoids, vitamins including D2, sterols, the unusual amino acid ergothioneine, β-glucans, which are responsible for their anti-inflammatory, antitumor, antiallergic, hepatoprotective, immunomodulating, antioxidant and antimicrobial activities (Kalaras et al., 2017; Lallawmsanga et al., 2016; Muszyńska et al., 2018; Patel and Goyal, 2012; Roncero-Ramos nd Delgado-Andrade, 2017).

It should be noted that although mushrooms of the same species grow under similar conditions, for example chanterelles grow in coniferous forests in mossy areas or in birch forests, but their chemical composition may differ depending on the place of growth, soil and climatic features of the area, and time of harvesting.

Popular species of wild edible mushrooms growing in European forests are shown in Figure 3.

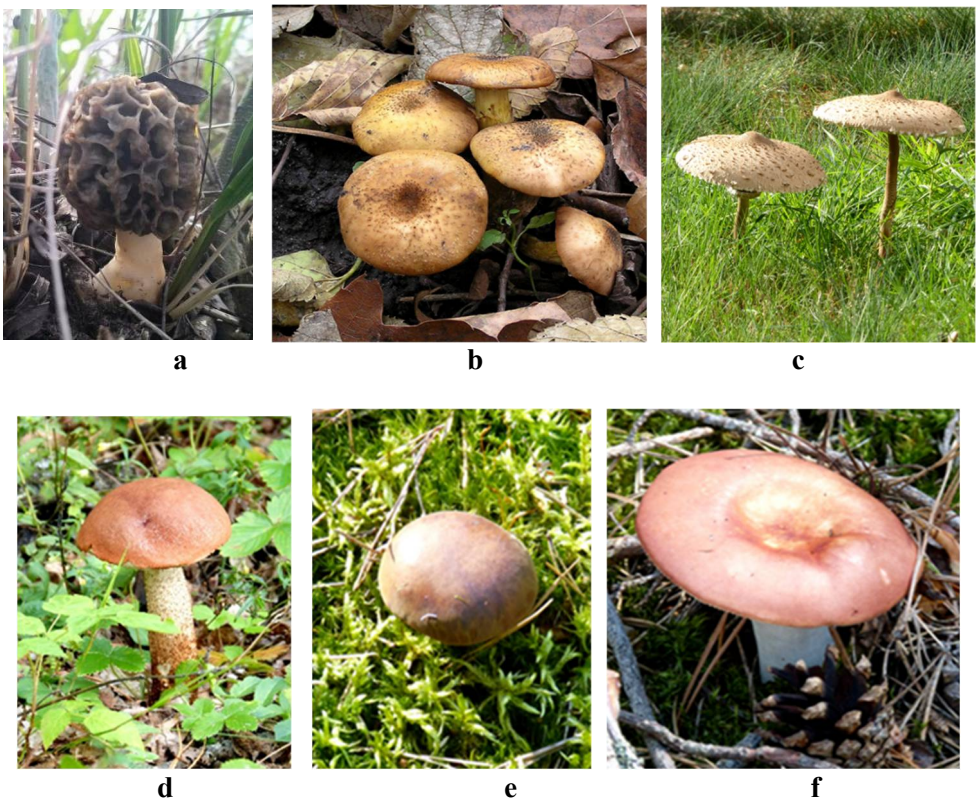


Figure 3. Popular European wild edible mushrooms:
A, Early morel (*Verpa bohemica*); B, Honey mushrooms (*Armillary mellea*);
C, Parasol mushroom (*Macrolepiota procera*); D, Aspen bolete (*Leccinum insigne*);
E, Bay bolete (*Imleria badia*); F, Emetic russula (*Russula rugulosa*);
G, Birch bolete (*Leccinum scabrum*).

Low sodium content, 10 – 40 mg/100 g, in mushrooms independent of their habitat and taxonomic position (Na in whole milk 40-60 mg/100 g, and in meat products 1000-2200 mg/100 g) (Vetter, 2003) makes them advisable supplement to meat to decrease sodium intake (Guinard et al., 2016). Moreover, due to mushroom savory flavor their addition to any dish allows to diminish sodium content in the diet. The substitution of 80% of the meat with ground champignons (*Agaricus bisporus*) in the beef taco blend did enhance its overall flavor and allowed to reduce salt content by 25% (Myrdal Miller et al., 2014).

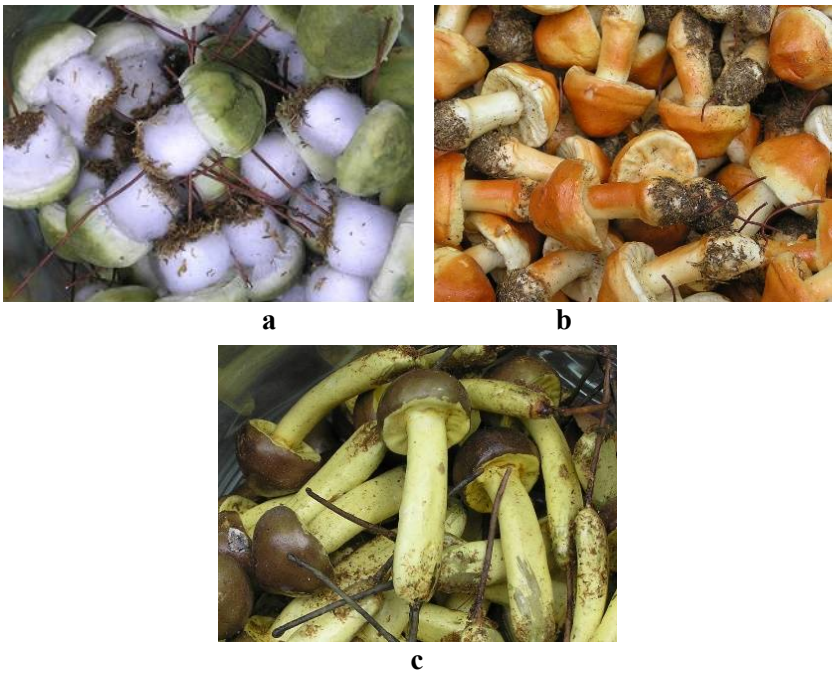
Use of wild mushrooms in food products. It is considered that mushrooms possess a unique taste called umami (Japanese word means "essence of deliciousness"), the fifth taste combining sweet, sour, salty and bitter, which is created by monosodium glutamate-like amino acids and 5'-nucleotides (Bernas, 2017; Zhang et al., 2013). Thus, it was estimated that mushrooms *Agaricus bisporus* contain about 370 mg of monosodium glutamate per 100 g of DM (Bernas, 2017). Therefore, by adding mushrooms to food products, three goals are achieved: increasing the nutritional value, giving the product an exquisite taste, and a certain medical positive effect. However, the cases of food allergy caused by consumption of different mushrooms have been reported (Ito et al., 2020; Kobayashi et al., 2019) and it is considered that around 1–3% of human population have allergy to mushrooms (Anusiya et al., 2021).

The use of edible wild mushrooms for food preparation in catering establishments, food for individual consumption is well known. But there is experience in the use of wild mushrooms in industrial food production. For example, *Cantharellus cibarius* grows from June to October in pine, birch, oak and hornbeam forests and has been used for food preparation in several European countries, Asia, America, and Africa. Only in Europe chanterelle mushrooms are collected for about 188,000 tons per year (Bulam et al., 2021). These mushrooms are used to supplement different dishes like omelets, soups, risotto, pizza, meat and fish dishes. It is a commercially important mushroom, which is present in the world market in fresh, dried, frozen and pickled state.

There are many special dishes from different countries using wild mushrooms. Examples of such dishes popular in European countries can be risotto made with wild mushrooms, a famous Italian dish; Slovak soup "kapustnica", prepared from sauerkraut, smoked meat, sausage, prunes, and mushrooms; Polish bigos (type of stew) made from sauerkraut, different meats, sausage, prune and dried or pickled wild mushrooms (Procházka et al., 2023; Weichselbaum et al., 2009). Fried mushrooms with onions, soup added with mushrooms, mushroom sauces are very popular dishes among Ukrainian population living in wooded areas (Luczaj et al., 2015), and application of wild mushrooms in different food products are appreciated in Lithuania and Germany.

Application of wild mushrooms in preparation of food products not only increases their biological value, but also serves to enhance their sensory properties like aroma and taste, and expands the shelf life of the final products. *Cantharellus cibarius* and *Boletus edulis* decoctions possess antioxidative and antimicrobial activity, and authors proposed to use them instead of the commercial antioxidants in preparation of frankfurters (Novakovic et al., 2019; 2020). Frankfurter sausages prepared with addition of the decoction of the dry powdered mushrooms improve odor, taste, and overall quality of finished products and increase the shelf life under chilled storage due to reducing lipid oxidation several times compared with control.

Due to the popularity of mushrooms worldwide, it may be of interest to note that for a long time hallucinogenic mushrooms were sold in the streets of Amsterdam; but a ban on their sale was introduced on December 1, 2008 (Figure 4).



a **b**

c

Figure 4. Hallucinogenic mushrooms in the streets of Amsterdam.

Wild mushrooms as pharmaceutical products

Wild edible mushrooms as well as cultivated ones possess different pharmaceutical properties including antidiabetic, antiallergic, antioxidative, antiviral, antibacterial, antifungal, immunomodulating, antidepressive, osteoprotective, nephroprotective, hypotensive and hepatoprotective activities and are used in the production of different pharmaceuticals (Anusiya et al., 2021; Thakur and Singh, 2013; Venturella et al., 2021). According to Gargano et al. (2017) mushrooms have more than 130 medicinal functions including also radical scavenging, cardiovascular, cholesterol-lowering, and detoxicative; drugs from mushrooms can be used as s painkillers and analgesics, as well as for prevention of immune disorders and improve quality of life in patients with various types of cancers or who are going under chemotherapy, patients with hepatitis B, C, and D and others. It is also suggested that bioactive compounds in mushrooms have shown neuroprotective effects on Alzheimer disease (Li et al., 2023). However, the pharmacological properties of medicinal mushrooms are studied mainly in *vitro* assays or in *vivo* using animal models, and there are only few clinical trials in humans showing positive effect of orally administered mushroom consumption on health state. So, we have focused only on proven cases of mushroom health benefits for humans. Crude extracts of the whole mushroom fruiting bodies or mycelia and isolated partially purified bioactive substances, for instance lentinan or polysaccharides, are more often used in medical studies (Table 5).

Table 5

Results of clinical trials of mushroom application for medical purposes

Mushroom	Preparation	Effect	Treatment of	Reference
Agaricus blazei	Extract	Reduces of chemotherapy-associated side effects	Gynecological cancer patients undergoing chemotherapy	Ahn et al., 2004
Agaricus sylvaticus	Dried extract	Improves of hematological and immunological parameters, reduce glycemic level	Postsurgical patients with colorectal cancer	Fortes et al., 2009
Agaricus sylvaticus	Dried extract	Increases in the immunity of patients	Patients with colorectal cancer	Fortes et al., 2009
Agaricus sylvaticus	Dried extract	Reduces of chemotherapy-associated side effects	Breast cancer patients undergoing chemotherapy	Valadares et al., 2013
Agaricus blazei	Encapsulated extract from dried mushrooms	Improves insulin resistance	Patients with type 2 diabetes	Hsu et al., 2007
Inonotus obliquus	Extract Befungin	Psoriasis rashes disappeared or weakened. Improves of gastrointestinal tract state	Patients with psoriasis	Frost, 2016
Inonotus obliquus	Extract Befungin	Reduces pain caused by peptic ulcers	Patients with peptic ulcers	Frost, 2016
Ganoderma lucidum	*Ganopoly®, 1880 mg, 3 times daily, 12 weeks	Enhances the immune responses	Patients with advance-stage cancer	Gao et al., 2003a
Ganoderma lucidum	Ganopoly®, 600 mg, 3 times daily, 12 weeks	Enhances the immune responses	Patients with advanced lung cancer	Gao et al., 2003b
Ganoderma lucidum	Spore powder, 1000 mg t3 times daily, for 4 weeks	Reduces cancer-related fatigue and enhances quality of life	Patients with breast cancer undergoing endocrine therapy.	Zhao et al., 2012

Mushroom	Preparation	Effect	Treatment of	Reference
Ganoderma lucidum	Rokkaku Reishi, 3 packs daily, 6 months	No significant anticancer effects	Patients with prostate cancer	Yoshimura et al., 2010
Ganoderma lucidum	Ganopoly®, treatment with 1800 mg, 3 times daily, 8 weeks	Improves of neurasthenia symptoms	Patients with neurasthenia	Tang et al., 2005
Ganoderma lucidum	Ganopoly®, 1800 mg, 3 times daily, 12 weeks	Lowers blood glucose concentrations	Patients with type 2 diabetes mellitus	Gao et al., 2004a
Ganoderma lucidum	Ganopoly®, mushroom polysaccharides	Decreases blood pressure, serum cholesterol levels	Patients with coronary heart disease	Gao et al., 2004b
Ganoderma lucidum	Lingzhi product, 1.44 g daily for 12 weeks	Lowers plasma insulin, normal plasma glucose levels	Patients with mild hypertension and/or hyperlipidemia	Chu et al., 2012
Hericium erinaceus	Mushroom powder	Improves cognitive abilities	Patients mild cognitive impairment	Mori et al., 2009
Lentinula edodes	Lentinan	Survival time extension	Patients with stomach tumors	Oba et al., 2009
Lentinula edodes	Dried mycelia extract, 1800 mg/day for 12 weeks	Decreases the adverse effects from chemotherapy	Patients with advanced cancer	Okuno and Uno, 2011
Antrodia cinnamomea	Aqueous extract, 20 ml daily, 30 days	Improves quality of sleep.	Patients with advanced cancer	Tsai et al., 2016
Poria cocos	Polysaccharidum of *Poria cocos* oral solution	Immune-therapeutics action	Patients with cancers, hepatitis and other diseases	Li et al., 2019
Poria cocos	Preparations from *Poria cocos*	Reduces fasting blood glucose	Patients with type 2 diabetes mellitus	Di et al., 2022
Grifola frondose	Maitake liquid extract 5-7 mg/kg, 2 times daily, 3 weeks	Increases immune activity	Patients with breast cancer	Deng et al., 2009

*Ganopoly®, mushroom polysaccharides

It was reported that such species of mushrooms with anticancer properties as *Lentinula edodes* (22.2% from total studies), *Coriolus versicolor*, and *Ganoderma lucidum* (both 13.9%), followed by *Agaricus bisporus* (*A. blazei* and *A. sylvaticus*) and *Grifola frondosa* (both 11.1%) are more often used in the clinic studies (Panda et al., 2022). Among mushrooms with pharmacological activity there are such species as *Agaricus blazei* (royal sun Agaricus), *Inonotus obliquus* (chaga), and *Ganoderma lucidum* (reishi) (Frost, 2016). These mushrooms are proposed to be used as antitumor agents and immunomodulators (El Enshasy and Hatti-Kaul 2013; Gariboldi et al., 2023).

Agaricus mushroom preparations were studied in clinical trials and it was shown that they could be useful for treatment of patients with cancer to diminish adverse chemotherapy-associated side effects (Ahn et al., 2004; Valadares et al., 2013), and improve health status of patients with colorectal cancer (Fortes et al., 2008; 2009). *Agaricus blazei*, native to Brazil, with a popular name as *Cogumelo do Sol* (sSun mushroom), or Himematsutake in Japan, is one of the more studied medicinal mushrooms. It was shown in randomized, blind, placebo-controlled clinical trials that preparation from *A. blazei* reduced chemotherapy-associated side effects by increasing appetite and emotional stability and diminishing alopecia and general weakness in gynecological cancer patients (Ahn et al., 2004). Dried extract of *A. sylvaticus* increased the immunity of patients with colorectal cancer (Fortes et al., 2008) and improved health status of postsurgical patients with colorectal cancer (Fortes et al., 2009); dietary supplementation with this mushroom (scaly wood mushroom) in form of dried extract reduced adverse side-effects of anticancer drugs improving gastrointestinal functions in patients with breast cancer treated with chemotherapy or radiotherapy (Valadares et al., 2013). Encapsulated extract from dried mushrooms *Agaricus blazei* increased insulin resistance in patients with type 2 diabetes (Hsu et al., 2007).

Inonotus obliquus (chaga, tinder fungus), is a black-brown mushroom that grows on damaged areas of deciduous trees more often on birches and less often on alder, ash, maple, rowan, beech, and elm, but only mushroom grown on birch has useful properties. This mushroom has been used in folk medicine from ancient times. Hippocrates described in the Hippocratic Corpus how to use infusions of this mushroom to wash wounds. And it was published in literature that Vladimir Monomakh, Grand prince of Kyiv, used this mushroom to treat himself for a lip tumor back in the 12th century (Szychowski et al., 2021). There are evidences that the fruiting bodies of *I. obliquus* have been employed in folk medicine in Eastern Europe in the 16 century (Lindequist et al., 2005) and has been used in the treatment of cardiovascular disease, gastrointestinal cancer, and diabetes mellitus until now (Duru et al., 2019).

Extracts from *I. obliquus* contain different biological active substances such as polysaccharides, polyphenols, triterpenoids, melanin, which have anticancer, anti-inflammatory, antiviral, antiparasitic, antioxidant, immunomodulatory, hypoglycemic, hypolipidemic, hepatoprotective, antiviral, hypolipidemic and immunomodulatory activities, and have at the same time the therapeutic potential to counteract the progression of cancer and diabetes (Lu et al., 2021; Szychowski et al., 2020). However, until present there are only few clinical studies confirming the effectiveness of the use of this fungus for medical purposes. There are reports of two clinical trials made in Russia in 1973-1981 (Frost, 2016). It was shown that application of commercially produced extract from chaga "Befungin" helps to normalize the health state of patients with psoriasis or peptic ulcers (Table 5). However, in case of peptic ulcers pain, which was relieved by taking the extract, returned to its previous level after stopping the intake.

Meanwhile, chaga mushrooms are widely used in pharmaceuticals. Despite the lack of controlled studies evaluating the safety of *I. obliquus* preparations from it, like many mushroom supplements, are produced mainly based on the experience of their long-term traditional use (Frost, 2016). The global market for chaga mushroom-based products is estimated at $ 25.8 billion USD in the year 2022, and it will probably reach $ 62.8 billion by 2030 (Report, 2013).

It should be noted that chaga is widely consumed not only as pharmaceutical, but also as herbal tea, syrup, bath agents, or concentrate (Duru et al., 2019). There are many reports of chaga beneficial health effects, but on the contrary there are some cases warning about the need to be careful when using chaga preparations as a pharmaceutical (Lee et al., 2020).

Ganoderma lucidum, wood-degrading basidiomycetes that can be found all over the world, is an edible medicinal mushroom known as Ling Zhi (the mushroom of immortality) in China and Korea, and reishi (the mushroom of spirituality) or Mannentake (10,000-year-old mushroom) in Japan. It has been known from ancient times in folk medicine of China, Japan and other Asian countries to treat stomach diseases, arthritis, and asthma. Studies conducted *in vitro* and *in vivo* showed its anti-inflammatory, antidiabetic, antiviral, and antibacterial activity and various other health benefits (Andrejč et al., 2022). At present, *Ganoderma lucidum* is one of the best studied species of medicinal mushroom. Description of reishi is included in the American Herbal Pharmacopoeia (AHP), and this mushroom is regulated as a dietary supplement in the United States (AHP, 2006). *G. lucidum* is included in the Pharmacopoeia of the People's Republic of China (2000) and approved for the treatment of dizziness, insomnia, palpitations, shortness of breath, cough and asthma (AHP, 2006).

G. lucidum contain about 400 different bioactive compounds (Ahmad, 2018), meanwhile triterpenes, polysaccharides, and peptidoglycans are three major groups of pharmacologically active constituents, which are present in different amounts in the fruiting bodies, mycelium and spores of reishi (Boh et al., 2007; Chan et al., 2021; Ferreira et al., 2015; Martínez-Montemayor et al., 2019). Preclinical studies showed that polysaccharides of this mushroom possess anti-tumor activity due to immunostimulating effects. Clinical study on humans indicated enhanced immune responses in advanced-stage cancer patients treated with 1,880 mg Ganopoly®, the polysaccharide fraction extracted from *G. lucidum*, three times daily for 12 weeks (Guo et al., 2003a). The immunomodulating effects of Ganopoly® taken 600 mg three times daily for 12 weeks by patients with advanced lung cancer was confirmed in a randomized double-blind, placebo-controlled clinical trial (Gao et al., 2003b). The authors concluded that Ganopoly® may have an adjunct role in the treatment of patients with advanced lung cancer. The clinic study showed that patients with breast cancer undergoing endocrine therapy who took spore powder of *G. lucidum* 1000 mg three times a day for 4 weeks became less anxious and depressed than those from the control group who received placebo (Zhao et al., 2012). A randomized, double-blind and placebo-controlled study of the efficiency and safety of Ganopoly® in Chinese patients with neurasthenia showed the improvement of neurasthenia symptoms after 8 weeks treatment with 1800 mg three times a day orally (Tang et al., 2005). It was shown in the clinic study that patients with confirmed type 2 diabetes mellitus after receiving exactly the same treatment had lower blood glucose concentrations than those in the placebo group (Gao et al., 2004a). The double-blind, randomized clinical trials showed a decrease in blood pressure and serum cholesterol levels in patients with confirmed coronary heart disease receiving extracted *G. lucidum* polysaccharides (Ganopoly) for 12 weeks (Gao et al., 2004b).

Currently, a great diversity of commercial *G. lucidum* products are available in forms of powders, dietary supplements, and tea (Chan et al., 2021; Wachtel-Galor et al., 2011). The world trade market value of *G. lucidum* and its derivative products is estimated approximately 4 billion USD and includes over 100 brands (El Sheikha, 2022). Dietary supplements containing reishi or substances from this mushroom could be used to support conventional medicine, as it was demonstrated in various clinical trials, to treat different diseases including cancer. However, further studies for confirmation efficiency and safety of reishi use in medicine should be conducted.

Poria cocos (wolf or fuling), an edible mushroom, which is found all over the world, growing on the dead bark and roots of diverse species of Pinus trees (Li et al., 2019). It has been used as traditional Chinese medicine for more than two thousand years to treat a wide range of human diseases. The most active substance in *Poria cocos* is its polysaccharide fraction, which consists of up to 84% of dried sclerotium weight. Pharmacological effects of *P. cocus* polysaccharides were intensively studied in recent years and as a result a medicine preparation "Polysaccharidum of *Poria cocos* oral solution" was developed and received approval as a drug by Chinese Food and Drug Administration in 2015 (Li et al., 2019). This drug could be used as immune-therapeutics to treat patients with different types of cancers, hepatitis and other diseases, alone or combined with chemo- or radiation therapy for cancer treatment. It was also shown in 73 randomized clinical trials that including of *Poria cocos* to hypoglycemic agent-treatments patients with type 2 diabetes mellitus could benefit reducing their fasting blood glucose (Di et al., 2022). Authors suggested that additional, deeper and careful studies are pending.

Other mushrooms with pharmaceutical properties undergoing clinical trials include *Hericium erinaceus* and *Antrodia cinnamomea*. *H. erinaceus* (Lion's mane mushrooms, yamabushitake) can be used for treatment of people with cognitive impairment. Terpenes and polysaccharides from this species stimulate the growth and differentiation of nerve cells and perform a protective function against exposure to oxidative stress. A double-blind, placebo-controlled clinical trial performed on 50- to 80-year-old Japanese men and women with mild cognitive impairment showed that intake of 250 mg tablets with 96% mushroom powder, three times a day, for 16 weeks improved patient's cognitive abilities (Mori et al., 2009). *A. cinnamomea* or *A. camphorata* is a very rare forest mushroom native to Taiwan that has been used as a traditional medicine for treatment of various human diseases including several types of tumor. However, human clinical trials to study the efficiency of *A. cinnamomea* as medicine are extremely limited. Advanced cancer patients receiving chemotherapy were administered with placebo or 20 mL of a mushroom aqueous extract daily for 30 days in a double-blind, randomized clinical study (Tsai et al., 2016). There was no improvement in the outcome of patients, except that the patients taken mushroom extract showed significantly better quality of sleep.

In another clinical study it was found the incidence decrease of adverse effects from chemotherapy among patients with advanced cancer who received orally dried *Lentinula edodes* mycelia extract, 1800 mg/day for 12 weeks (Okuno and Uno, 2011).

Among other medical mushrooms *Grifola frondosa* (hen-of-the-woods or maitake) is cited which an edible species is growing at the base of oaks or maples in Asia, Europe, and North America. Polysaccharides of its fruiting body and mycelium includes β-glucans and heteroglycans, and extracts of this mushroom demonstrated antitumor and immunomodulatory effects in preclinical studies. An increase in immune activity was

observed in patients with breast cancer who took maitake liquid extract 5-7 mg/kg orally twice a day for 3 weeks (Deng et al., 2009).

Mushrooms contain many biologically active compounds, and a lot of modern studies are devoted to the isolation, study of properties and the possibility of using such substances in medicine, and they accounts for not less than 130 medical functions (Gargano et al., 2017). Polysaccharides, among these compounds, play outstanding functions with antitumor and immunomodulating activities which have been proven in clinical studies. It was demonstrated, for instance, for purified β-glucan lentinan, isolated from Lentinula edodes (shiitake mushroom), its successful application in the combined treatment of cancer diseases in Japan. It was shown, in randomized controlled clinical trials, that advanced gastric cancer patients treated with chemotherapy and lentinan had a much longer survival time compared to patients treated only with chemotherapy (Oba et al., 2009).

A serious drawback in the use of mushrooms for medical purposes is the lack of regulations, standards and protocols for their certified elaboration production and strict testing of mushroom products. This may be the main reason why commercial mushroom products change in composition and consequently in effectivity; even the active components of many commercial mushroom products have not yet been identified (Wasser, 2010, 2011). The latter item is one of the challenges for a more efficient and safety use of mushrooms in medicine.

On the other hand, more and more attention has been recently paid for studying the potential use of mushrooms to prevent Alzheimer's disease (AD) or slow down its symptoms in patients who have been already suffering from AD (Li et al., 2023; Silva et al., 2023). A lot of clinical trials have shown that dietary factors are extremely important in treating and preventing Alzheimer's disease (Bello-Corral et al., 2021; Stefaniak et al., 2022; Yusufov et al., 2017). Results of 10, out of 12, clinical studies demonstrated a protective effect of the Mediterranean plant-based diet on the risk of developing AD. It is believed that to reduce the risk factor for developing this disease, the diet should contain substances with neuroprotective properties, such as antioxidants, B vitamins and polyunsaturated fatty acids, which are present in mushrooms. In addition, these species contain a large number of biologically active compounds involved in mechanisms associated with AD including the potent antioxidants ergothioneine and glutathione as well as vitamin D, which may have neuroprotective properties. In people with mild cognitive impairment being potentially vulnerable to dementia reduced levels of ergothioneine, an unusual thio-histidine betaine amino acid, have been observed (Cheah et al., 2016). Ergothioneine is contained in mushroom fruiting bodies, where it is typically the main antioxidant; it has been found and quantified in various wild and cultivated mushrooms. The highest amounts of ergothioneine is reported in *Pleurotus* ostreatus (oyster), 2.59 mg/g DM, but it was found, mg/g DM, in *Lentinula edodes* (shiitake), 1.98; *Grifola frondosa* (maitake), 1.13; *Agaricus bisporus* (white button), 0.21 (Dubost et al., 2007); *Ganoderma lucidum* (reishi), 0.56; Hericium erinaceus (lion's mane mushroom), 1.12; *Boletus edulis* (porcini), 7.27; *Cantharellus cibarius* (chanterelle), 0.20; and *Morchella esculenta* (morel), 0.47 (Kalaras et al., 2017). In brief, it is considered that increased ergothioneine intake including mushrooms in the diet might possibly promote cognitive health (Feng et al., 2019).

Most studies regarding certain substances found in mushrooms were carried out in *vitro* and on animals, but there are also results from clinical studies on humans. For example, *Termitomyces* species, the termite mushrooms, contain cerebrosides, which are known to have an important role in the treatment of neurodegenerative disorders including AD (Paloi et al., 2023). Erinacine A, cyathin diterpenoid, a compound present in *Hericium erinaceus*

(lion's mane mushroom) was evaluated in clinical trials to relieve AD symptoms and showed neurotrophic and neuroprotective activities (Li et al., 2020).

There are some clinical trials that prove beneficial role of mushroom consumption in improving cognitive performance. The cohort study involving 13,230 elderly Japanese people aged over 65 years showed that frequent consumption of mushrooms (3 times per week or more) reduced by 19% the risk of incident dementia (Zhang et al., 2017). The same finding that mushroom consumption may serve as a preventive measure to slow down neurodegeneration with aging was made by Singaporean researchers, who studied for more than 6 years the association between mushroom consumption and mild cognitive impairment based on data from 663 participants aged 60 years and older (Feng et al., 2019). An analysis of data from U.S. people over 60 years of age found that mushroom consumption is associated with cognitive performance, and if it is consumed in large amounts may reduce the risk of cognitive decline (Ba et al., 2022). Thus, regular intake of mushrooms is a way to potentially reduce the risk of neurodegenerative disorders.

Wild mushrooms as industrial products

Industrial cultivated edible mushrooms are very popular as food products (Rangel-Vargas et al., 2021) widely used for preparation of medicinal drugs (Valverde et al., 2015), and constantly increasing all over the world. Thus, world production of mushrooms has increased more than 30-fold since 1978 (Royse et al., 2017). Total production of mushrooms and truffles worldwide from 2012 to 2021 augmented from 31.78 to 44.21 million metric tons (Statista, 2023); China is a leading country producing about 75% of the word's mushrooms and is the world's largest producer of *Flammulina velutipes* (Royse et al., 2017). The most cultivated edible mushroom genera are *Lentinula* which covers about 22% of the world's production followed by *Pleurotus*, 19% of the world's output; *Auricularia*, 18%; *Agaricus*, 15%; *Flammulina*, 11%; *Volvariella*, 5%; and others, 10% (Royse et al., 2017) (Figure 5).

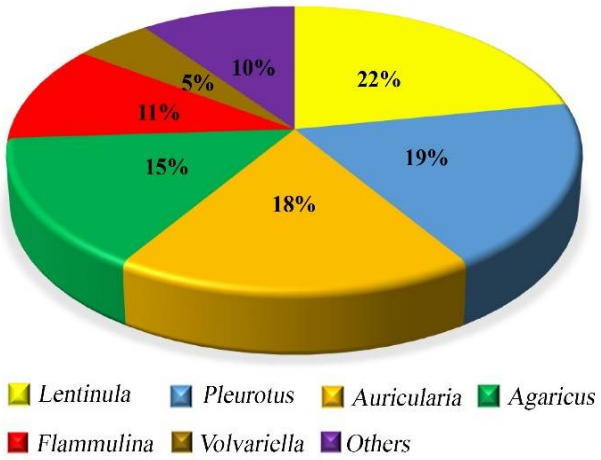


Figure 5. Cultivated mushrooms in percentage of total world's production (Adapted from Royse et al., 2017).

Pleurotus ostreatus (oyster mushroom), *Agaricus campestris*, and *Agaricus bisporus* (button mushroom) are cultivated all over the world, while *Lentinula edodes* (former *Lentinus edodes*) (shiitake), *Auricularia auricula* (wood-ear or tree ear), *Volvariella volvacea* (edible straw mushroom), *Flammulina velutipes* (winter mushroom) and *Ganoderma lucidum* (red reishi) are cultivated mainly in Asia (Stabnikova et al., 2008). Nowadays the mushroom production on a large scale is going in a form of so called solid state fermentation. However, there are two traditional methods, which are still used to harvest mushrooms: (a) cultivation on wood logs and (b) on compost.

Traditional methods used for mushroom cultivation. According to Japanese and Chinese traditions, *Lentinula edodes* (shiitake) was grown on shii (*Castanopsis cuspidata*) logs until the mid-1980s. Growth of shiitake on trees requires from one to two years before the first crop of fruiting bodies could be harvested. It is possible to expedite the growth of shiitake by growing the mushrooms aseptically in plastic bags or in trays on sterilized substrate containing saw dust, bagasse, straw, paper chips and supplemented with nutrients such as starch, yeast, sugars and protein (Figure 6a). Application of this method allows their harvesting in three-four months after inoculation; the residual compost could be used as a soil fertilizer (Stabnikova et al., 2008).

The composition of medium for shiitake cultivation could be different, but the basis of the substrate should be hardwood chips and sawdust and/or cereal straw. To increase the nutritional value, medium is supplemented with bran or grain, straw of cereals, waste from the food industry and agriculture. 2-3 mm is an optimal particle size of sawdust and shavings of hardwood trees, and cereal straw is crushed to a size of 1-2 cm. Chalk or gypsum is added to improve the physical properties of the substrate. Cultivation is carried out in special chambers with the maintenance of desirable microclimate under sterile conditions or in conditions of a high degree of purity. Under the cited conditions, a yield of fruiting bodies from 15 to 30-50% from the weight of used substrate may be obtained.

Pleurotus (oyster mushroom) are much easily cultivated and the corresponding costs are lower compared to others mushroom species because these mushrooms have a shortest time to produce fruit bodies; additionally they can be grown using various lignocellulosic agricultural wastes due to their ability to produce enzyme laccase, which plays a key role in lignin degradation (Li et al., 2022). Using different waste products as substrate for cultivation of oyster mushroom species (*Pleurotus ostreatus, P. cystidiosus, P. pulmonarius*) allows to reduce their price to a reasonable level. It was reported that oyster mushrooms can be grown on sawdust, wood chips, cereal straw, banana leaves, peanut hull, corn leaves, wheat and rice straw, mango fruits and seeds, sugarcane leaves, hazelnut branches and husk, rice husk, spent coffee grounds, coffee pulp, grass, weed plants, olive cake, tomato tuff, pine needles, and cotton wastes (Akcay et al., 2023; Das et al., 2007; Hernández et al., 2003; Jatwa et al., 2016; Li et al., 2022; Raman et al., 2020). For instance, lignocellulosic biomass mixture from grounded leaves of date palms (*Phoenix dactylifera*), wheat straw, saw dust, and boobialla (*Myoporum serratum*) supplemented with wheat bran and corn meal at 5% of the substrate dry weight and gypsum, 5% (Alananbeh et al., 2014). Oyster cultivation is conducted in plastic bags (Figure 6b) or trays.

Different species of *Pleurotus* can grow under different temperature conditions using pasteurized composed substrate or sterile non-composted one, meanwhile their fruiting bodies are relatively rarely exposed to diseases or pests. However, *Pleurotus* fruit bodies have short life that makes them less competitive than *Agaricus bisporus* and *Lentinula edodes* (shiitake) (Raman et al., 2020).

a b

Figure 6. Shiitake (A) and oysters (B) cultivation in plastic bags

After harvesting of oyster mushroom fruiting bodies, "spent compost" could be used as soil conditioner to enhance soil structure; biofertilizer; animal feed due to enrichment composting material with protein by mushroom mycelium, or to use in bioremediation of soil polluted with toxic organic substances (Moore et al., 2020; Sadiq et al., 2019). Because *Pleurotus* has the ability for easy accumulation of heavy metals, only waste free from this contaminant could be used in mushroom production.

Auricularia auricular has different common names such as black fungus due to its dark brown to black color and wood ear or ear mushroom because of its shape. In nature this mushroom grows mainly on deciduous older trees or dead and decaying branches. It is currently the third most produced mushroom in the world and is widely cultivated in Asian countries for food and medicinal applications. China is a major producer of *A. auricular,* which accounts for more than 90% of the total global production and amounted 7 018 million tons in 2019 (Hao et al., 2022; Zhao et al., 2019).

This fungus possesses cellulases, xylanase, laccase and polyphenol oxidase enzymatic activity, so it is able to decompose lignin and cellulose and can be grown on lignin containing agricultural wastes. So, methods of its cultivation using compost to produce fruit bodies for food is similar to *Pleurotus ostreatus*. Major advantage is that cultivation of *A. auricular* could be done using submerged fermentation on simple liquid media for further isolation of medicinal valuable substances (Sun et al., 2016).

Cultivation of wild mushrooms in Europe was started in the 1600s when growers in Paris grew species known as *Agaricus bisporus* in fields, but the European mushroom industry started in the limestone caves beneath Paris at the end of the nineteenth century. This underground mushroom cultivation method has been used until our days.

Cultivation of mushrooms includes two major steps: preparation of the compost or solid medium and mycelium growth until fructification. For production of compost or medium for mushrooms cultivation, different organic and lignocellulosic materials can be used, such as wood chips, sawdust, hay, maize waste, paddy straw, cassava bagasse, waste paper, cotton seed hulls, water hyacinth, apple pomace, grape pomace oil palm bunch, husk rice, banana leaves cheese whey, horse manure, chicken manure, and others (Ivanov et al., 2021; Moore et al., 2020; Stabnikova et al., 2008).

Button mushrooms *A. bisporus* is one of the most cultivated species in the world, and it is the dominant fungus cultivated in Europe. In the USA, about 98% of the mushroom

production comes from button mushrooms. The fruit bodies of white button of this mushroom are produced in large amounts for human consumption on a medium consisting of wheat straw, straw-bedded horse manure, chicken manure and gypsum (Straatsma et al., 2000). The materials are mixed, the ingredients are wetted, and composted for 8–9 days while temperatures will rise to 80°C during the period of uncontrolled self-heating. Then compost is packed in boxes and pasteurized for 8 h at 56–60°C and continues at 45°C for up to 7 days to remove volatile NH_3 and to produce pathogen free substrate. After inoculation, compost is covered with a mixture of soil, peat, and chalk. The optimal temperature for mycelium growth is 24◦C, and the optimal temperature for fruiting body production is from 14 to 18◦C. The yield of mushrooms consists of around 1 kg from 1 kg of compost dry matter. Composition of compost could be changed depending on local availability of casing materials (Baysal et al., 2007).

Volvariella volvacea (paddy straw mushroom) also known as Chinese mushroom is also one of the most cultivated mushrooms of the world. It is grown mainly on rice straw, although other agricultural waste could be used. Generally, it prefers substrates with high content of cellulose and low content of lignin. Among the plant materials used for *V. volvacea* cultivation there are water hyacinth, oil palm bunch and pericarp waste, banana leaves, saw dust, cotton waste, and sugarcane bagasse (Ahlawat & Bindvi, 2016). Paddy straw is tied into bundles and immersed in water for 24 – 48 h. After removing excess water the straw is piled into heaps which are inoculated with *V. volvacea* pure cultures or spent compost from previous mushroom cultivation. The first crop of egg-like sporophores could be harvested in less than 1 month. The main disadvantage of *V. volvacea* production is very short shelf life even in cold storage because of autolysis. These mushrooms could be stored just for 3 days at 10 – 15°C. Therefore, straw mushrooms are best used in canned, pickled or dried form.

Ganoderma lucidum, a wood-destroying mushroom, having different pharmaceutical properties, is grown primarily for medicinal use, not as food. The common method for its cultivation is provided on wood logs or sawdust or wheat straw added with wheat bran, tea leaves and cotton husk in plastic bags or bottles. For cultivation on sawdust, addition of sucrose, 1%, and calcium carbonate, 1%, is recommended. For cultivation on wood, short pieces of wooden logs (15 cm or less in diameter and 15-24 cm in length) with moisture content of 35-40% are inoculated with mushroom mycelium, covered with soil, and then with chopped straw to ensure needed moisture and temperature. Short-log cultivation takes 4–5 months for mycelial incubation and fruiting bodies are harvested approximately after 25 days from primordia formation (Boh et al., 2007). Modern biotechnology gives the opportunity to produce mycelium biomass by their submerged cultivation in bioreactors. Cultivation of *G. lucidum* in synthetic liquid media is proposed for production of different valuable substances for medical use (Abdullah et al., 2020; Supramani et al., 2023; Zhao et al., 2011).

Flammulina velutipes, commonly known as golden needle mushroom or winter mushroom, is wood decaying mushroom growing in nature on the stumps of the Chinese hackberry tree, called enoki in Japanese, but also on some other trees such as aspens, willows, elms, mulberry and persimmon. Wild species have a brown color and short stem, while artificially grown ones are white with long thin stems. *F. velutipes* is the fifth largest edible mushroom in global production, which is especially popular in Asian countries, China, Japan, Republic of Korea, and Taiwan. *F. velutipes* could be cultivated on wood logs or on sawdust added with rice or wheat bran in polypropylene bags or plastic bottles (Harith et al., 2014; Sengar et al., 2019).

Solid-state and submerged liquid cultivation of mushrooms. Nowadays, for mushroom cultivation in industrial scale solid-state and submerged liquid fermentations could be used. Solid-state fermentation (SSF) involves the growth of mushrooms on a solid substrate, mainly an agro-industrial waste, meanwhile submerged cultivation is the growth of mushrooms in a liquid medium with dissolved nutrients under agitation and air supply. Application of solid substrate with low moisture content is advisable to produce mushroom fruit bodies for food or medicine, meanwhile cultivation of mushrooms in liquid media in bioreactors is a promising way to obtain mycelial biomass as an animal feed or for the production of metabolites (Letti et al., 2018).

SSF has such advantages as possibility of using a wide range of agro-industrial wastes as substrates for mushroom cultivation, relatively low energy consumption because of a lack of mechanical agitation/mixing and aeration, use of a residual substrate for bioremediation, animal feed, biofuel production, and biosynthesis of mushroom metabolites. However, when fermentation is carried out on a large scale, it is not so difficult but expensive to control the parameters of the cultivation process, such as pH, moisture content, homogeneity of substrate, concentration of oxygen, heat and mass transfer, and in addition, there is the possibility of contamination. On the other hand, SSF could be successfully used for production of certain biological active substances, for example, lignocellulolytic enzymes (Wang et al., 2019). Thus, the efficiency of SSF for the production of enzymes such as laccases, among others, is evident due to their wide industrial and technological applications; these SSF enzymatic potentialities may catalyze the oxidation of various phenolic compounds and a number of aromatic amines during the production of edible mushrooms of the genera *Pleurotus* (Han et al., 2020) or *Ganoderma* (Postemsky et al., 2017; Sharma et al., 2019).

Meanwhile, submerged cultivation is a very effective way to produce mushrooms in the form of mycelium biomass, especially for manufacturing of different valuable substances with health benefits. Liquid cultivation of mushrooms gives opportunity to control the fermentation process and final product quality and avoid contamination. The process takes place under a sterile environment in liquid medium at conditions optimal for the growth of selected mushrooms such as temperature, pH, mixing, and level of aeration (Bakratsas et al., 2021). Among the advantages of using submerged fermentation is the possibility of obtaining mushroom biomass or high-value bioactive substances in a short cultivation time for large-scale industrial applications. There are research studies devoted to production of truffles biomass using submerged fermentation, which is a promising way for business of this fungus (Tang et al., 2015). Application of liquid fermentation was proposed to produce bioactive metabolites such as triterpenoids, polysaccharides, antrodin, from medicinal mushroom *Antrodia cinnamomea* (Zhang et al., 2019). Cultivation of *Auricularia auricular* by this type of was proposed for melanin production (Sun et al., 2016). To synthesize exopolysaccharides by mycelial culture of *Inonotus obliquus* has also been done in liquid agitated conditions (Xiang et al., 2012).

Submerged mushroom cultivation is possible to use for production of mycoproteins, which possess high nutritional value and can be used in manufacturing of functional foods, for example in meat analogues production. Thus, cultivation of edible mushroom *Pleurotus ostreatus* LGAM was conducted in a stirred-tank bioreactor using aspen wood chips hydrolysate. The specific growth rate of mushroom was 1.8±0.4 d^{-1}, biomass concentration was 25.0±3.4 g/l, and protein yield consisted of 54.5 g per 100 g of sugars (Bakratsas et al., 2023).

Unusual method for mushroom cultivation. An interesting and unusual method is used to grow edible mushrooms of *Ustilago maydis* (huitlacoche or cuitlacoche) in Mexico and in some areas of Central America, where it has been traditionally produced, and of the USA, where huitlacoche has been recently used, as human food. There are clear evidences in different regions of Mexico that this fungus was in the common diet of the pre-Hispanic population. The maize, *Zea mays,* smut termed huitlacoche is characterized by the formation of galls or tumors in maize ears (Figure 7).

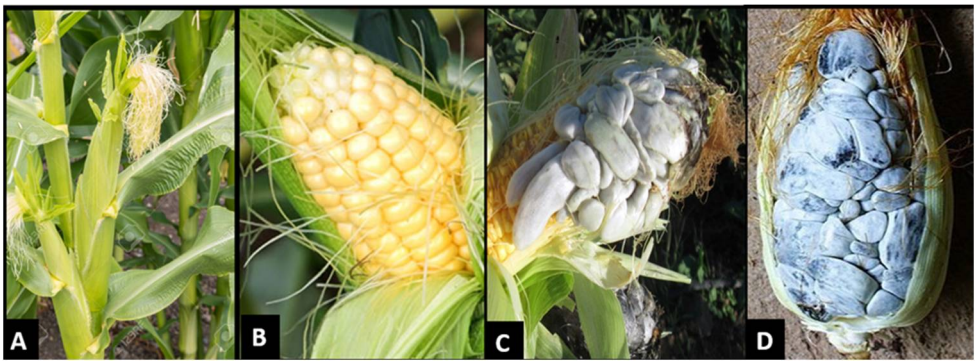


Figure 7. Ustilago maydis is responsible for corn smut:
A, maize plant; B, maize cob; c, maize infected by U. maydis; D, formation of galls of tumors

Ear galls are considered an edible delicacy produced by the natural infection of the maize ears with the fungus *Ustilago maydis*. These smut galls are also called as maize mushrooms, Mexican truffles, or maizteca mushrooms and serve as an important food source in this country. Nowadays huitlacoche is a culinary delight for international chefs in selected places in the USA, France, Spain, Japan and others. In addition to its unique flavor, huitlacoche has been identified as a high- quality functional food and could be included into the daily diet for its attractive characteristics, selected nutrients, valuable compounds, and nutraceutical potential.

Valdez-Morales et al (2010) found that chemical composition of huitlacoche may change due to maize genotype, stage of development, plant environment, and cooking process. This composition includes, in g/100 g DM: protein, 11.5-16.4; fat, 1.6-2.3; ash, 5.2-7.0; fiber, 16.0-23.5, and carbohydrates, 55.1-66.5 (Valverde et al., 1995). Amino acid lysine is present in huitlacoche in high amounts, 6.3 to 7.3 g/100 g protein; thus, it is an important source of this essential aminoacid, especially for consumers where cereal is the main component of the daily diet. Huitlacoche contains also high levels of polyunsaturated fatty acids with balanced ω-6/ω-3 ratio. The main fatty acid is the polyunsaturated omega-6 linoleic acid (18:2ω-6), 38.7-48.4 % from total amount of fatty acids, followed by another polyunsaturated omega-3 linolenic acid (18:3ω-3), 25.2-34.1%; saturated palmitic acid (16:0), 13.0-14.6%, and other residual fatty acids, 11.8-14.4% (Valverde and Paredes-López, 1993; Valverde et al., 1995). Optimal ω-6/ω-3 ratio in foods should be less than 4:1 (Stabnikova and Paredes-López, 2023); interestingly this ratio in huitlacoche varies from 1.1 to 1.9. Content of monosaccharides in huitlacoche, in mg/g of dry weight, is as follows: glucose, 140-180; fructose, 60-100; mannitol, 3.2; sorbitol, 4.5 (Juárez-Montiel et al., 2011; Valdez-Morales et al., 2010). Huitlacoche contains high amounts of phosphorus, 342.07 mg/kg, and magnesium, 262.69 mg/kg, as well as total phenolic compounds, 113.11 mg

GAE/kg, and flavonoids, 28.51 mg CE/kg (Aydoğdu & Gölükçü, 2017). It was reported that huitlacoche possesses important functional properties, namely, antioxidant, hypocholesterolemia, immunomodulatory, anticancer, anti-inflammatory, antidiabetic and antihypertensive (Juárez-Montiel et al., 2011; López-Martínez et al., 2022). Huitlacoche is sold in Mexico in domestic markets all over the country; and the commercial importance of this product is now increasing in high-income places of the U.S. and in some few other countries.

Conclusions

The role that the mushroom kingdom plays in human life is remarkable, and at the same time very important to be assessed. Simultaneously with the development of humanity, the function of mushrooms in human life has intensified and expanded. It started in the initial historical times, before the beginning of agriculture, as an additional source of nutrition, to become in the last decades as the object of large-scale industrial production using modern biotechnological procedures. The participation of mushrooms in human consumption and treatment has evolved from their use by healers and shamans to serious and careful clinical trials.

Wild edible mushrooms have a high nutritional value containing high-quality proteins, fibers, essential fatty acids, vitamins including D2, trace minerals, and such valuable compounds as polyphenols, terpenoids, sterols, while having low energetic value that allows them to be used in low-calorie diets.

Wild edible mushrooms contain many bioactive nutraceutical compounds and possess different specific pharmaceutical properties, which can be used in treatment of various serious diseases. However, more clinical trials on humans showing positive effect of orally administered mushroom consumption on health state are needed as well as regulations for food supplements with mushrooms, standards and protocols for the production and testing of mushroom products in general.

The gathering and use of wild edible mushrooms for food make a significant contribution to both the solution of the global food shortage crisis and economics of different countries around the world, and could be considered in some countries as new sources of income for local people.

The increase of interest on fungus consumption along human history at worldwide level has led to the development of basic and sophisticated techniques for their cultivation. Solid-state and submerged liquid fermentations are nowadays useful methods for cultivation of mushroom in a large-scale for their production including volumes of biomass and of very valuable specific bioactive metabolites. In brief, nowadays wild and cultivated procedures for the small and large scale production of mushrooms, and of fungal metabolites are playing, and much more will do it in the near future, outstanding roles in the use of raw materials and in the economy of populations of local people around the world; food, and pharmaceutical products and including industrial uses are their fields of utmost influence.

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Changes in vitamin content and sensory characteristics of frozen wild berries during storage

Galyna Simakhina, Nataliia Naumenko, Svitlana Kaminska

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Galyna Simakhina
E-mail: lyutik.0101@gmail.com

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Abstract

Introduction. The aim of the study was to evaluate changes in the content of ascorbic acid, bioflavonoids, and β-carotene, as well as the organoleptic properties of frozen wild berries under the influence of cryoprotectants after long-term storage.

Materials and methods. Wild edible berries (fresh, frozen, and defrosted) of chokeberry, blackberry, raspberry, blueberry, and guelder rose were used in the study. The berries were frozen under the protection of a cryoprotectant (a 10% aqueous solution of sucrose together with a 1% solution of citric acid). Defrosting of frozen berries after 9 months of storage was performed by four different methods to determine the most effective one.

Results and discussion. The berries contain ascorbic acid ranging from 49.7 (guelder rose) to 139.5 (chokeberry) mg/100 g of fresh weight (FW), and β-carotene ranging from 1.94 (blueberry) to 3.76 (chokeberry) mg/100 g FW. Wild berries had high content of bioflavonoids, ranging from 785 mg/100 g FW (guelder rose) to 1654 mg/100 g FW (blueberry). Even after 9 months of storage, frozen berries under the protection of cryoprotectants lost no more than 11.6% of ascorbic acid that is the most labile bio-component; sensory characteristics of the berries showed almost no significant difference with fresh raw material by all indicators.

The methods of defrosting frozen berries affect sensory characteristics and the indicator of cell juice loss of the resulting product; the most effective method was found to be defrosting berries in a refrigerator at a temperature of 0 °C for 30–33 minutes. This method preserved the tissue strength of the berry surface completely, resulting in null loss of cell juice. The best indicators were obtained after thawing the berries in air at a temperature of 18–22 °C for 130-135 minutes: the loss of cell juice was 19.8%, and the surface of the berries had cracks, so the sensory characteristics were 4.1 points, and 3.7 points for the color. Frozen berries proved to be a reliable source of vitamins for dietary needs: 50 g of berries provide the human body with 24.9–65.7% of the Recommended Dietary Allowances for ascorbic acid, 19.0–36.6% for β-carotene, and 156.4–332.2% for bioflavonoids (priority was given to blueberries).

Conclusions. Berries, processed with a water solution of cryoprotectant before freezing, after 9 months of storage, showed almost no significant difference with fresh raw materials in terms of vitamin content and sensory characteristics. The optimal conditions for defrosting of berries was temperature 0 °C for 30–33 minutes.

Introduction

To overcome the vitamin deficiency in the population diets, great importance is given to berries that accumulate essential concentrations of biologically valuable active substances, primarily antioxidants and vitamins (Becker et al., 2004; Stabnikova et al., 2024; Vahapoglu et al., 2021). However, the berries do not have a protective peel, are not able to retain water for a long time and are highly perishable, so it is necessary to develop special conditions for their storage. Optimum storage conditions for strawberries (7–10 days), blueberries (2–4 weeks), raspberries, and blackberries (2–5 days) are 0 °C and 90–95% of relative humidity (Błaszczyk et al., 2022; Horvitz, 2017).

Since berries are a seasonal product, they must be preserved to be used effectively throughout the year. The most effective method for this is freezing (Arteaga et al., 2021). Frozen berries retain their biological value and attract consumers due to their high sensory characteristics and the preservation of fresh material properties even during long-term storage (Goyal et al., 2000; Rickman et al., 2007). Therefore, there is a growing demand for frozen fruits worldwide, with their turnover increasing annually by 4–6% (Frozen fruit, 2018).

Cultivated and wild berries occupy a special place among plant raw materials, increasingly gaining importance in therapeutic, preventive, and dietary nutrition (Paredes-López et al., 2010), particularly in extreme living conditions (Simakhina et al., 2021). Berries serve as suppliers of bioactive secondary metabolites (polyphenols, aromatic compounds, volatile acids) that contribute to their aroma, fragrance, and taste (Gu et al., 2022). The antioxidant substances of various chemical natures (bioflavonoids, ascorbic acid, carotenoids) contained in fruits and vegetables foodstuffs in different concentrations are believed to resist the expansion of free-radical processes (Ishiguro et al., 2007; Pap et al., 2021; Toor et al., 2006). Unlike the synthetic pharmacological remedies, antioxidants of biological origin are easily and organically involved in metabolic processes in the organism and, in turn, do not cause undesirable side effects (Gundesli et al., 2019). Polyphenolic compounds are secondary metabolites of plants and constitute the largest group of phytochemicals that promote health. These compounds are known to be important antioxidants, exhibiting antiglycemic, antiviral, anticancer, and anti-inflammatory activities, as well as antiallergic and antimicrobial properties (Manach et al., 2004).

However, berries pose the most challenging object for freezing due to their high water content (up to 90%), extremely delicate surface tissue, and low storage stability. This necessitates the search for innovative solutions in developing freezing technologies for berry crops. One of the most promising methods is the combination of artificial cold with the use of cryoprotectant compounds (Neri et al., 2020). Only recently has this direction become the subject of research in the field of food technologies. Scientific developments in cryobiology can be adapted to the processes of freezing berry raw materials (Simakhina et al., 2019) and minimize losses of the most labile component (ascorbic acid) to 5–7%. This is achieved because the development of extra- and intracellular crystallization, which is the main destructive factor for the cells and tissues of frozen objects, is significantly inhibited in berries treated with aqueous solutions of cryoprotectants (Neri et al., 2020).

The subject of research for most authors is the freezing of cultivated berries: cherry (Kutlu et al., 2022), strawberry (Da Silva et al., 2022), blueberry and raspberry (Neri et al., 2020). Therefore, the study of the biological value of wild berries remains relevant, considering their ability to produce vitamins more effectively during vegetation, as well as the fact that no other plant sources have such a balanced quantitative content of essential nutrients. Methods of wild berries freezing and conditions for their storage should be oriented towards ensuring that the final products, after prolonged storage, practically do not differ in

quality and sensory characteristics from fresh raw materials and serve as a rich source of vitamins in the population's diet during the inter-seasonal period for berry gathering. One realistic approach to solving such tasks is the pretreatment of berries with aqueous cryoprotectant solutions before freezing. There are only a few articles in the periodicals on this subject (Neri et al., 2020), hence the research on the impact of cryoprotection on the preservation of vitamins, sensory characteristics of berries at the stages of freezing, storage, and thawing remains relevant.

The aim of the present study was to determine the content of ascorbic acid, bioflavonoids, and β-carotene in fresh and cryoprotectant-covered frozen wild berries after prolonged storage and to provide a comparative assessment of their sensory characteristics.

Materials and methods

Materials

Selection of berries for research

The criteria for selecting berries for research are based on their vitamin value (content of ascorbic acid, bioflavonoids, and β-carotene), correlation between the content of ascorbic acid and bioflavonoids, presence of natural cryoprotectants (mono- and disaccharides) that enhance the stabilization of intracellular berry structures during freezing, ability of berry components to participate in cold adaptation, structural strength of berry surface tissues, and minimal difference in sensory indicators between fresh and frozen berries.

Fresh and frozen wild berries golden rose (*Viburnum opulus* L.), blackberries (*Rubus caesius* L.), raspberries (*Rubus ideaus* L.), blueberries (*Vaccinium myrtillus* L.), and chokeberries (*Aronia melanocarpa* L.) were used in the study. The berries were collected at their individual optimal maturity stage, in the mixed forests of the central part of Zhytomyr Oblast (province), Ukraine, in July-September, 2023.

Pre-treatment of berries with cryoprotectants

For the pretreatment of berries prior to freezing, a water solution of a mixture of sucrose and citric acid was applied (Simakhina et al., 2019. Solution of cryoprotectant containing sucrose, 10%, and citric acid, 1%, was prepared using distilled water, crystalline sucrose and crystalline citric acid at a temperature of 18–22 °C, and thoroughly mixed until complete dissolution of the components.

A single layer of berries was placed into a broad-bottomed container, completely covered with the cryoprotectant solution, held for 60 minutes at a temperature of 18–22 °C with periodical stirring to ensure even treatment of their surface. Each subsequent batch of berries was treated with a freshly prepared cryoprotectant.

Obtaining frozen berries

After treatment with cryoprotectants, the berries were dried to remove excess moisture and then frozen in a blast freezer spread out at a temperature of –34 °C for 25 minutes, corresponding to the parameters of rapid freezing (Zlabur et al., 2021). The process continues until the temperature in the center of the berries reaches –18 ± 1 °C.

The frozen berries were packed into 500-gram packets made of thermoplastic polymer materials suitable for low-temperature storage (–18 °C), ensuring the integrity and hermetic sealing of the packaging. The containers with the frozen berries were packed into boxes made of triple-layer corrugated cardboard weighing 6 kg and stored in a refrigerated chamber for 12 months (maximum term) at a temperature of –18 °C and relative humidity not exceeding 95%. Prior to research, the berries were thawed.

Thawing

Thawing was conducted using several methods: (a) air thawing at temperatures of 18–22 °C and 37–42 °C; (b) volumetric thawing in a microwave oven, and (c) in a refrigerated chamber at 0 °C. Thawing continued until the recommended consumption temperature of the thawed berries reached –5 °C. The duration of thawing depended on the size of the berries, the structure of their tissues, and the density of the pulp. On average, the time spent on thawing berries through air thawing at temperatures of 18–20 °C was 130–135 minutes, at temperatures of 37–42 °C it was 45–50 minutes; for thawing in a microwave oven, it was 3–5 minutes, and in a refrigerated chamber, it was 30-33 minutes.

Cellular juice loss

Cellular juice loss during thawing was determined as a percentage based on the relative change in mass of the frozen berry sample before and after thawing.

Determination of ascorbic acid content

Ascorbic acid content was determined by titrometric method based on extraction of ascorbic acid from the test sample with a solution of acid (hydrochloric, metaphosphoric or a mixture of acetic and metaphosphoric), followed by titration visually or potentiometrically with a solution of 2,6–dichlorophenoline sodium phenolate (Majidi et al., 2016).

Determination of bioflavonoid content

The content of bioflavonoids was determined using a method involving the Folin-Ciocalteu reagent by spectrophotometric analysis (Viña et al., 2006).

Determination of β-carotene content

The content of carotenoids was determined using a commonly employed method based on the extraction of carotene using organic solvents (hexane) and measuring the optical density of the solution using a spectrophotometer at a wavelength of 450 nm (Juntachote et al., 2005).

Sensory evaluation

A panel of 7 members (staff from the Department of Technology of Healthy Foods in National University of Food Technologies) was selected on their ability to perceive the indicators of appearance, surface state, color, aroma and taste of fresh and frozen berries and to verbalize those perceptions. Panelists were semi-trained.

Table 1 presents a 5-point evaluation system for the sensory indicators of fresh berries, while Table 2 shows the evaluation of frozen berries under cryoprotectants after 9 months of storage.

Statistical analysis

The data represents the mean of a minimum three replicates ± standard deviation (S.D.). Graphical presentation of experimental data was performed using the program Microsoft Excel 2010.

Table 1

Assessment of sensory characteristics of fresh berries

Index	Score points	Estimation of fresh berries quality by score points
Appearance	5	Fresh, whole, without defects and microbial damages, homogenous.
	4	Fresh, whole, practically without defects.
	3	Whole, partly withered, slightly damaged.
	2	The significant share of withered and damaged berries.
	1	Inhomogeneous, with defects and microbial damages.
Taste and smell	5	Typical for fresh berries, without strange taste and smell.
	4	Slight strange taste and smell.
	3	Stable and obvious strange taste and / or smell.
	2	Stable and expressed, atypical strange taste and / or smell.
	1	Strong rotting stench and atypical taste.
Color	5	Typical for a certain type of ripen berries, saturated, homogenous.
	4	Meets the requirements of the special type, slightly lighter.
	3	Low-intensive due to anthocyanin decomposition.
	2	Transforms from natural to brown.
	1	Dull and unpleasant.
Maturity grade	5	Berries are homogenous in maturity grade, well shaped.
	4	Berries are sometimes inhomogeneous in maturity grade, well shaped.
	3	Berries are slightly inhomogeneous in maturity grade, mostly well shaped.
	2	Berries are practically inhomogeneous in maturity grade, different in shape.
	1	Berries are different in maturity grade, non-calibrated.

Note: berries with quality estimated as 1 or 2 points were not recommended for further procession.

Table 2

Assessment of sensory characteristics of frozen berries

Index	Score points	Estimation of frozen berries quality by score points
Appearance	5	Frozen, with bluish coating, well-shaped
	4	Frozen, about 5 percent of berries with slightly damaged skin
	3	About 8 percent deformed, slight losses of juice
	2	Frozen, over 15 percent damaged, losses of juice, unsuitable for storage
	1	Frozen, wrinkled turgor, over 25 percent deformed, significant losses of juice
Taste and smell	5	Typical for special sort of berries, without strange taste and smell; sour, sweet, spicy, astringent tastes or their combinations; smell may get stronger due to cold stress
	4	Typical for special sort of berries, slightly strange taste and smell due to cold stress
	3	Expressed strange taste with stable bitter aftertaste and smell atypical for special sort of berries
	2	Stable expressed strange taste atypical for special sort of berries; strange smell due to destructive biochemical processes
	1	Unpleasant strange taste and smell due to destructive biochemical processes
Color	5	Typical for special sort of berries, may get stronger due to cold stress
	4	Typical for special sort of berries, slightly less intensive and saturated
	3	Berries almost discolored, upper layer attained the brown hue
	2	Berries attain the brown hue due to the anthocyanin decomposition
	1	Dull, dark-brown, unpleasant due to microbiological damages
Surface state	5	Clean, slightly moisturized, with natural turgor, without skin damages; berries are suitable for long-term (under 12 months) storage
	4	Clean, without skin damages and losses of juice; berries are suitable for long-term (under 12 months) storage
	3	Slightly deformed and crushed (about 5 percent) berries, slight cracks with signs of juice losses
	2	Significantly deformed, crushed (about 15 percent) berries, with cracks and juice losses
	1	Serious surface defects in significant amount of berries (about 25 percent), skin damages with significant juice losses

Results and discussion

Wild berries viburnum (*Viburnum opulus* L.), blackberries (*Rubus caesius L.*), raspberries (*Rubus ideaus* L.), blueberries (*Vaccinium myrtillus* L.), and chokeberries (*Aronia melanocarpa* L.) were chosen for the present research. Golden rose (also known as Viburnum) is widespread in Europe, Northern and Central Asia (Ersoy et al., 2019). Fruits are used for jam, jellies, marmalades production, could be an ingredient for different drinks, and are used in the traditional cuisine of various nations (Polka et al., 2019; Soylak et al.,

2002). They are an excellent source of ascorbic acid containing from 28 to 48 mg per 100 g of fresh berries, as well as high amounts of phenolic compounds (Stabnikova et al., 2024). Raspberries are recognized by consumers as a tasty and beneficial berry, rich in bioflavonoids and carotenoids (Ponder et al., 2019), which act as antioxidants in food products. It is used in the canning industry for making jams, preserves, and jellies. Blackberries contain antioxidants, vitamins, pectin substances, mineral elements, and serve as a natural anti-inflammatory agent, used in dietary nutrition, including for diabetes prevention (Kaume et al., 2012). Blueberries are part of the functional food sector, known today as superfoods due to their high content of flavonoids, particularly anthocyanins (Kalt et al., 2020). Chokeberry is an important source of biologically active compounds (Borowska et al., 2016); this is why it is widely used in the food and pharmaceutical industries (Lyubych et al., 2022).

Vitamin content in fresh and frozen wild berries after long-term storage

The content of ascorbic acid, bioflavonoids, and β-carotene in fresh (mg/100 g fresh berries) and frozen (mg/100 g frozen berries) berries after storage for 3 and 9 months were determined. The results are shown in Figures 3-6.

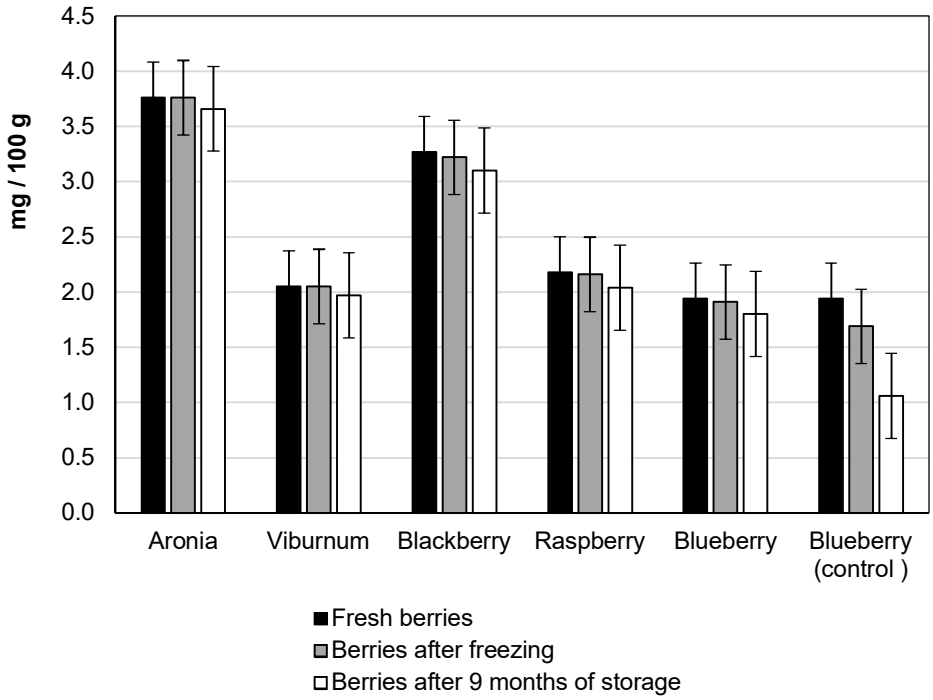

Figure 3. Content of β-carotene in fresh (mg/100g of fresh berries) and frozen (mg/100g of frozen berries) berries

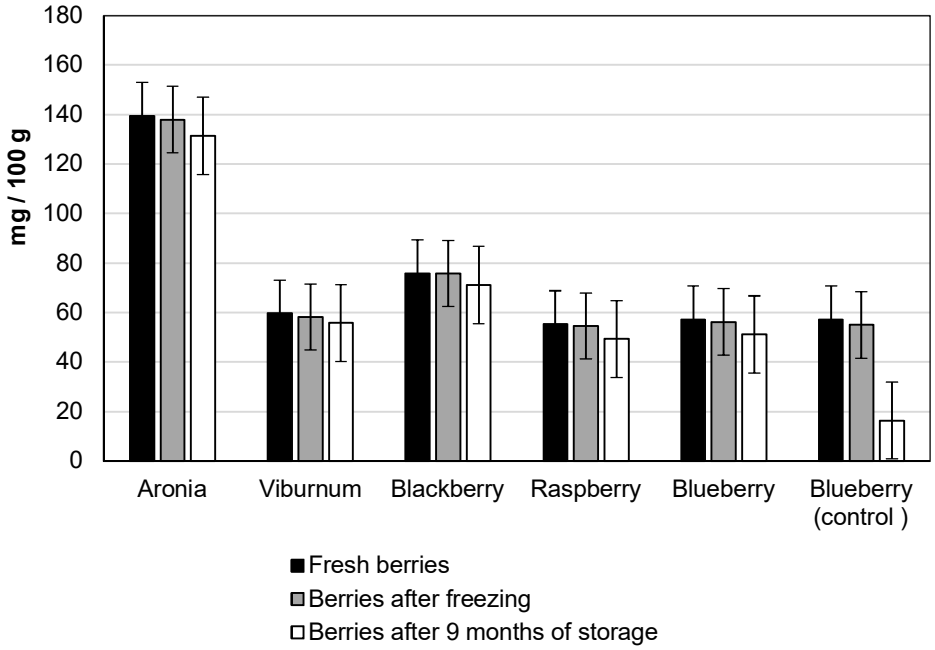

Figure 4. Content of ascorbic acid in fresh berries (mg/100g of fresh berries) and frozen berries (mg/100g of frozen berries) after prolonged storage

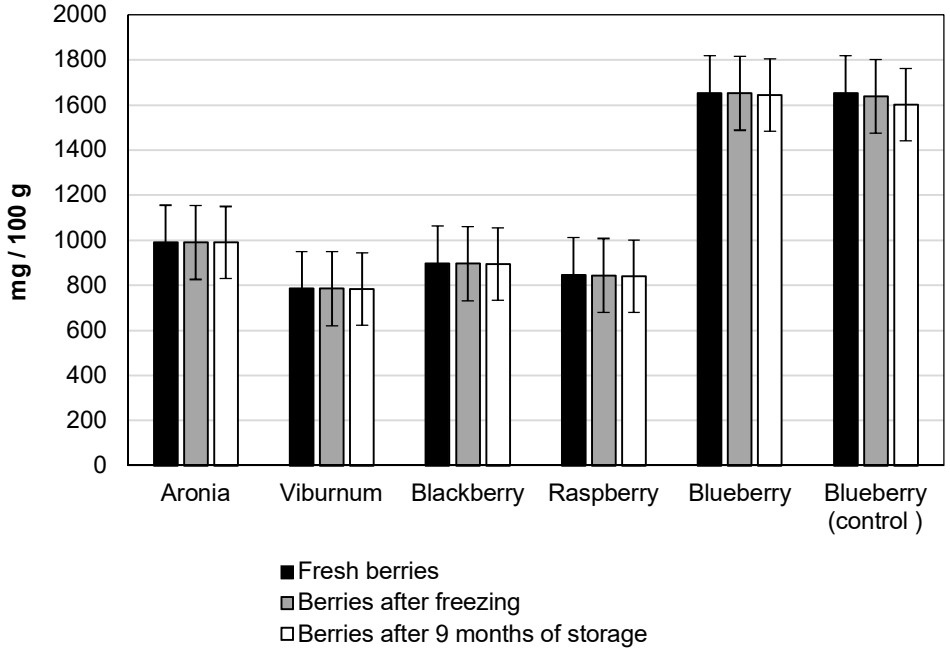

Figure 5. The content of bioflavonoids in fresh berries (mg/100g of fresh berries) and frozen berries (mg/100g of frozen berries) after prolonged storage

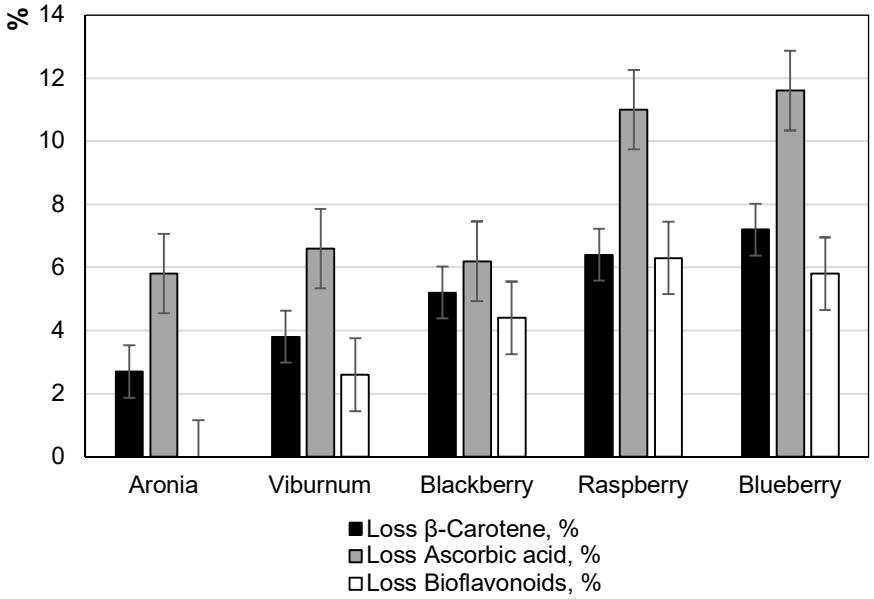

Figure 6. Vitamin losses in frozen berries after 9 months of storage, % compared to the content in fresh berries

Analysis of the data presented in the diagrams (Figures 3, 4, 5, 6) showed that freshly frozen berries, pretreated with cryoprotectants, have almost lost no vitamins, or these losses are insignificant (1–2%). Even after 9 months of storage at –18 °C, the maximum losses of β-carotene concerning fresh berries were 7.2%, and the maximum losses of bioflavonoids were 6.3% (both indicators are presented for blueberries). Along with that, blueberries and raspberries have slightly higher losses of ascorbic acid (11.6 and 11%) compared to other berries. This confirms the status of ascorbic acid as the compound most labile to any external influences, and at the same time explains that blueberries and raspberries have more delicate surface tissues than other berries, which makes them less resistant to the influence of low temperatures. In particular, some cells lose their integrity, the cytoplasmic membrane ruptures, leading to direct contact of the vitamins contained inside the cell with oxidoreductases, primarily with ascorbate oxidase, which, in process, causes the destruction of a certain amount of ascorbic acid molecules.

The results can be properly evaluated by comparing them with data obtained from freezing berries using the traditional method (without cryoprotectants). These data are presented in Figure 6 as a comparative study of vitamin losses in blueberries frozen with cryoprotectants and with traditional technology. Having compared the obtained data, we attained a convincing evidence of the effectiveness of freezing technologies with the use of cryoprotectants: losses of β-carotene with this technology amounted to 7.2% of its content in fresh blueberries, whereas for the traditional method this index counts 45.4%; respectively, losses of ascorbic acid were 11.6 and 71%; bioflavonoids – 5.8 and 31.6%. Significant losses of vitamins in berries frozen with traditional technology can be explained by the formation of large ice crystals in the cells and intercellular spaces during slow freezing, which destroy cell membranes and subcellular structures. As a result, oxidoreductases gain access to biologically active substances concentrated in the cells and initiate biochemical processes of vitamin oxidation, leading to their loss.

Sensory characteristics of fresh and frozen berries

To obtain a comparative characteristic of fresh berries and berries frozen under cryoprotectants, an evaluation of their sensory indicators was conducted, using blackberries as an example. The results are presented in Table 3.

Table 3

Sensory evaluation of fresh and frozen blackberries after 9 months of storage

Indicators	Validity coefficient	Scores	Characteristics	
			Fresh berries	Frozen berries
Appearance	0.35	5	Clean, fresh, free from defects and microbiological damage, uniform, with elastic turgor	The berries are uniform, evenly frozen, with a bluish hue, elastic turgor, undamaged, with retained shape.
Taste	0.2	5	Characteristic of a particular variety, free from foreign flavors; sweet	Characteristic of a specific variety, without any foreign taste, identical to natural fresh berries, sweet
Color	0.1	5	Characteristic of this particular ripe material, intense, rich	Corresponds to the ripeness of fresh berries, no deviations from the natural color; color intensity slightly higher due to anthocyanin synthesis as a response to cold stress.
Surface state	0.2	5	Clean, free from defects and damage by pests, without cracks or spots, glossy or matte	Clean, slightly moist, with natural turgor, undamaged surface tissue, without loss of cellular juice
Aroma	0.15	5	Absence of foreign odor, delicate aroma characteristic of raspberries, rich, pronounced	Characteristic of fresh berries; more intense than the natural aroma due to the synthesis of aroma-forming compounds during cold stress, which gives the berries additional positive qualities.
Conclusion			Top grade	Top grade, suitable for long-term storage

The maximum validity coefficient, 0.35, was accepted for berry appearance since it is considered to be a comprehensive that includes shape, size, maturity grade, freshness, and color. Moreover, in case of berries' discrepancy with the established requirements for the appearance, the use of other evaluation criteria is considered inappropriate.

The results showed that blackberries frozen using cryoprotectants received the maximum score of 5 for all organoleptic indicators, which confirms the high quality of frozen berries that can be used as a source of vitamins in the off-season.

Thawing of frozen berries

Before using frozen berries, it is necessary to thaw them. Frozen berries, stored for 9 months at a temperature of –18 °C and relative humidity not exceeding 95%, were thawed using various methods. The choice of defrosting method was based on maintaining the structural integrity of the berries' surface (sensory characteristic) and, as a result, the loss of cellular juice and berry bio-components dissolved in it. A comparative characteristics of different thawing methods are presented in Table 4.

Table 4

Evaluation of frozen berry thawing methods

Thawing method	Thawing temperature, °C	Thawing duration, minutes	Loss of cellular juice, %	Sensory parameters on a 5-point scale	
				Surface state	Color
Air	18–22	130–135	19.8	4.1	3.7
Air	37–42	45–55	11.2	4.5	4.2
Microwave oven (power 400 W)	50–55	3–5	2.6	5	4.8
Refrigerator	0	30–33	0	5	5

The most effective methods of defrosting frozen berries are thawing in the refrigerator (temperature 0 °C, duration 30–33 min), and thawing in the microwave oven (temperature 50–55 °C, duration 3–5 min, power 400 W). Under these parameters, the structural integrity of the berry tissues is fully preserved (surface state rated at 5 points, and color at 4.8–5 points), preventing the loss of cellular juice and dissolved biocomponents (cellular juice loss was 0% and 2.6%, respectively). The worst indicators were observed after thawing berries in the open air (temperature 18–22 °C, duration 130–135 min), which resulted in cellular juice loss reaching 19.8%, some berries being deformed, and cracks appearing on their surface – with a rating of 4.1 for surface state and 3.7 for berry color.

Providing daily vitamin requirements through consumption of 50 g of frozen berries

Berries, especially wild ones, occupy an important place among plant-based raw materials, given their status as natural vitamin sources, characterized by various health-promoting, preventative, and therapeutic properties. The balance and quantitative content of essential vitamins are such that cannot be found in other types of plant-based raw materials. Therefore, at this stage of the research, the ability of frozen berries to meet a certain portion

of the human body's daily needs for ascorbic acid, bioflavonoids, and β-carotene was calculated using a computational method, according to the Norms of physiological needs of the population of Ukraine for basic nutrients and energy (2017): the daily requirement for ascorbic acid is 100 mg, for bioflavonoids is 250 mg, for β-carotene is 5 mg. The results are presented in Figure 7.

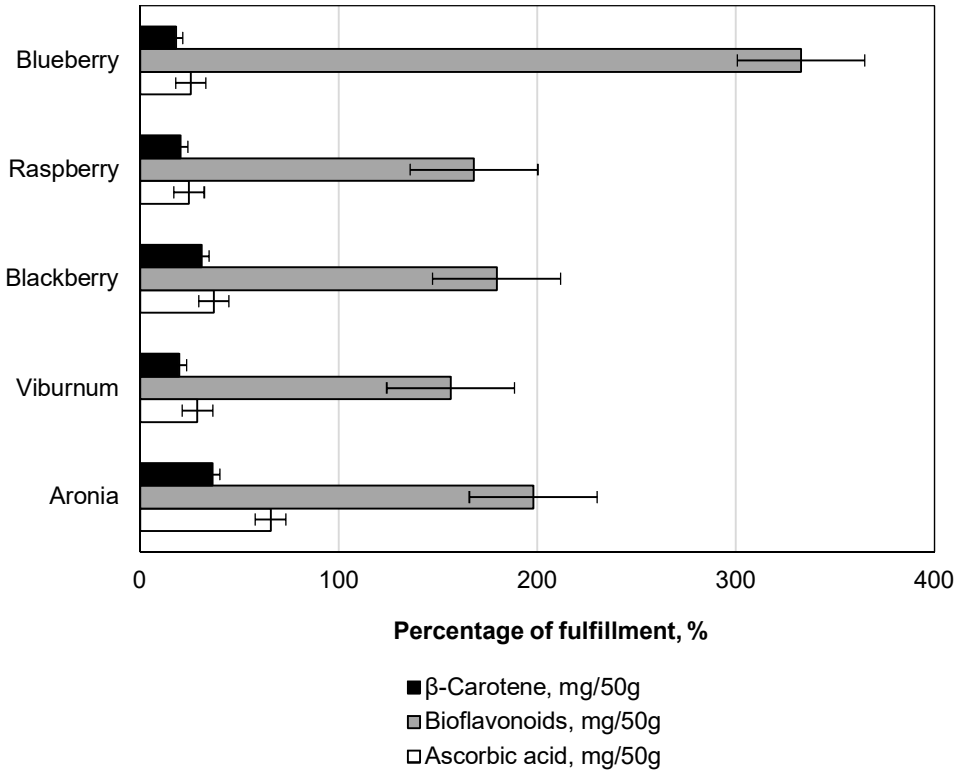

Figure 7. Meeting the average daily requirements of the human body for essential vitamins due to the consumption of 50 g of frozen berries (%)

According to the data from the diagram, 50 g of frozen berries provide a sufficiently high level of daily human vitamin requirements. For the studied berries, this portion's contribution to meeting the daily requirement for ascorbic acid ranges from 24.7% (raspberries) to 65.7% (aronia), for bioflavonoids – from 156.4% (viburnum) to 332.8% (blueberries), and for β-carotene – from 18% (blueberries) to 36.6% (aronia). For all berries, the portion providing the body's daily requirement for bioflavonoids exceeds 100%; however, this does not pose a danger or lead to overdose since bioflavonoids belong to water-soluble compounds and are excreted from the body rather quickly, meaning they do not have cumulative properties.

In European countries, the list of vitamins and their daily requirements differs slightly (The Nutrition Source, 2023). For example, women need 70 mg of ascorbic acid, and men 80 mg; β-carotene is not listed separately, but only vitamin A. The content of bioflavonoids is also not regulated, although they are recognized as potent bio-antioxidants today.

Conclusions

1. Wild berries are valuable sources of antioxidants due to significant amounts of ascorbic acid, bioflavonoids, and β-carotene.
2. The shelf life of fresh berries does not exceed 15 days, so, for long storage they are should be frozen and kept at a temperature of –18 °C. Advanced freezing technology involves combining artificial cold with the use of cryoprotectants that protect the surface and cellular structure of the berries from the damaging effects of ice crystals, thereinafter preventing losses of cellular juice during thawing and the vitamins containing in it.
3. The examined berries, frozen under the cover of a cryoprotectant, even after 9 months of storage under optimal conditions, hardly differ in quality indicators from fresh raw materials: for the content of β-carotene (losses for different types of berries ranged from 2.7 to 7.2%), ascorbic acid (losses from 5.8 to 11.6%), bioflavonoids (losses from 0 to 6.3%), and sensory indicators were rated at 5 points on a 5-point scale. Losses of vitamins in berries frozen by traditional methods without the use of cryoprotectants amounted to 45.4% for β-carotene content, 71% for ascorbic acid content, and 31.6% for bioflavonoid content, respectively, compared to these indicators in fresh raw materials.
4. 50 g of frozen berries, depending on their type, meet the daily human requirements for β-carotene by 19–36.6%, for ascorbic acid by 24.7–65.7%, and for bioflavonoids by 156.4–332.8%.
5. The search for new raw material sources of high biological value, the improvement of methods for their freezing and storage, is a relevant and promising direction for the development of the health food industry, fully meeting human nutritional needs and global market trends.

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Effect of pumpkin seed flour, chestnut flour, and rosehip flour on wheat bread staling rate

Rosen Chochkov[1], Denka Zlateva[2], Dana Stefanova[2], Petya Ivanova[1]

1 – University of Food Technologies, Plovdiv, Bulgaria
2 – University of Economics, Varna, Bulgaria

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Corresponding author:

Rosen Chochkov
E-mail:
rosen4o4kov@abv.bg

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Abstract

Introduction. The aim of this study was to analyse the impact of chestnut flour, rosehip flour, and pumpkin seed flour (in the amount of 5% or 10% on the basis of wheat flour) on the staling rate of wheat bread.

Materials and methods. The bread was made from type 500 wheat flour with the addition of pumpkin seed, chestnut or rosehip flour (5% or 10%). Bread staling was determined by measuring the deformation characteristics of the bread crumb. The total, plastic and elastic deformation of the bread crumb in penetrometric units (p. u.) was measured on 3; 24; 48; 72, and 96 h after baking.

Results and discussion. It was found that as the storage time increases, the values characterizing the total, plastic, and elastic deformation of the bread crumb decrease for all the samples. After three hours after baking, the highest total deformation was determined in the control sample (144 p. u.). At longer storage times (72 h and 96 h), the crumb of the wheat bread hardened progressively, and the samples with 5% rosehip flour, as well as 5% and 10% pumpkin seed flour, preserved their softness.

The duration of storage affects differently depending on the type and quantity of the supplement. At the end of storage, the sample with 10% rosehip flour and with 10% pumpkin seed flour had the lowest rate of change of plastic deformation (32% and 37%, respectively). The elastic properties of the bread crumb deteriorated at significantly slower rates in the enriched samples. In the case of bread with 5% rosehip flour after storage for 96 h, the elastic deformation decreased by 38%, and in the case of bread with 10% pumpkin seed flour – by 35.5%, while for the control sample, the elastic deformation decreased by 70%.

Conclusions. The appropriate dosing of the chestnut, rosehip and pumpkin seed flour in the wheat bread formulation results in preserving the softness and elasticity of the crumb and can retard bread staling. The most significant delay in staling was achieved when using 5% rosehip flour and 10% pumpkin seed flour.

Introduction

Staling of bakery products is accompanied by changes in the physical, chemical, and sensory properties of bread, it reflects on consumer preferences and willingness to buy and consume the product and causes economic losses because of the waste of large quantities of bread (Demirkesen et al., 2014). Typically, dough formulations include different components, each of which undergoes changes during the baking process of the bread, as well as during storage of the bread, making staleness an extremely difficult phenomenon to describe (Gray and BeMiller, 2003).

Bread staling is a process that occurs during storage and involves redistribution of moisture, change in the structural properties of the crumb and the crust and deterioration of the taste, smell and other sensory characteristics of bread. It has been clearly proven that staling is a result of complex reasons, such as changes in the starch fraction (more specifically – amylopectin retrogradation), migration and redistribution of moisture, and changes in gluten structure (Amigo et al., 2019). It is believed that approximately 3% of global bakery products fail to be realised on the market because of the staling process (Gómez et al., 2008).

Even though this process has been first studied and described as early as in 1852 (Boussingault, 1852), a number of authors have tried to clarify its mechanism at a later stage. Some of them (Goesaert et al., 2009; Hallberg and Chinachoti, 2002) highlight that amylopectin retrogradation is the main reason for the changes in the physical and chemical properties of the bread crumb during storage. The process of retrogradation starts when the amylopectin chains transform, and the amorphous molecules turn into into a denser and stable crystal form (Slade and Levine, 1991). By using light microscopy, Hug-Iten et al. (1999) studied the microstructure of starch in bread that has undergone staling. According to the authors, air gaps and partial separation of the starch from the protein phase are observed, which is the reason for the crumbliness of the bread crumb.

More recent studies show that gluten, non-gluten proteins, lipids and pentosans also play an important role in the staling process (Blaszczak et al., 2004; Hallberg et al., 2002; Smith and Johansson, 2004). According to other authors, the migration of water molecules plays a significant role in bread staling (Baik and Chinachoti, 2000; He and Hoseney, 1990). Water migrates from gluten to starch, which results in a decrease in the crumb plasticity. On the other hand, water is redistributed and incorporated in the amylopectin crystal formed during retrogradation and causes hardening of the crumb during storage.

It could be suggested that all components of the bread's chemical composition (more or less) have an impact on the speed of the process. Actually, the kinetics of staling depends on a complex set of factors: the raw materials used (the ingredients of the dough, yeast, enzymes), the technological process (kneading, fermentation, baking, cooling) and the storage conditions (humidity and temperature).

Bread staling has a negative impact on the quality of the product and significantly reduces its consumer acceptability, while causing great economic losses. Finding possibilities for retarding the process is the subject of many studies. Various attempts have been made to improve the shelf life of bakery products through additives, formulation changes (Curti et al., 2016); changes in the technological process (Bosmans et al., 2013) or in storage conditions (Rasminssen and Hansen, 2001), as well as the type of packaging (Akhtar et al., 2008). In this regard, the use of various additives to improve shelf life is a widespread approach in the bakery industry.

Different researchers have focused their interest on the selection of additives that could delay bread staling (Fadda et al., 2014; Tebben et al., 2018). In this context, efforts focus on

the use of additives of natural origin with high nutritional value, which also include flours from alternative raw materials.

There are only some isolated studies on the impact of chestnut, rosehip and pumpkin seed flour on the structural properties of bread crumb and wheat bread staling. Dall'Asta et al. (2013) replaced 50% of wheat flour with chestnut flour in the bread formulation and found that the staling process was delayed in the enriched bread. Man et al. (2012) found that the input of 5%, 10% or 15% chestnut flour had a clear favourable effect on the elasticity of the bread crumb, with a well-defined linear correlation ($r = 0.7980$). Addition to the high dietary fibre content, pumpkin seeds are also distinguished by the fact that they are a very good source of protein, which makes them suitable for inclusion in the bread formulation (Costa et al., 2018; Stabnikova et al., 2023). The authors highlight that the elasticity of bread enriched with 30% pumpkin seed flour is lower as compared to the control sample (made of wheat flour only), however, the differences are not statistically significant. Gomez et al. (2004) explained this by the increased fibre content and the reduced gas-retention capability of dough. The increased quantity of dietary fibres and proteins in the enriched samples of bread resulted in increased hardness of the crumb (9.64 N for the enriched sample as compared to 6.07 N for the control sample).

Halvorsen et al. (2002) pointed out that rosehip had the highest antioxidant activity compared to other berries in the *Rosaceae* family. Rosehip powder has been used in bread formulation to enhance bioactivity of bread, to increase total phenolic content and dietary fibre content (Zhou et al., 2023). However, data on the effect of rosehip flour on bread staling are scarce.

The objective of this study was to analyse the impact of replacement of 5% or 10% of wheat flour with chestnut flour, rosehip flour and pumpkin seed flour on the staling rate of wheat bread.

Materials and methods

Materials

The bread samples were made of the following raw materials:

- **Wheat flour –** with average chemical composition, as follows: moisture – 12.36%, acidity – 2.10 °H, ash content – 0.50% of dry matter, wet gluten – 27.60%, fat 0.9 g/100 g, of which saturated fat 0.3 g; carbohydrates 70.3 g/100 g, of which sugars 3.4 g, dietary fibres 4.0 g/100 g; proteins 10.8 g/100 g;
- **Water** – meeting the requirements of Regulation No. 9 of the Ministry of Health of 16.03.2001 on the quality of water intended for drinking and household purposes (SG, issue No. 30 of 2001);
- **Bread yeast, compressed –** Lesafmaya (Lesaffre) according to the manufacturer's technological documentation;
- **Salt** – meeting the requirements of Regulation (Decree of the Council of Ministers No. 23/2001) about the requirements for the composition and characteristic of food grade salt (SG, issue No. 11/2001).

Types of flour, unconventional for breadmaking:

Pumpkin seed flour (Figure 1) with average chemical composition: fat 10 g/100 g, of which saturated fat 1 g; carbohydrates 34 g/100 g, of which sugars 0 g, dietary fibres 16 g/100; proteins 56 g/100 g;

Figure 1. Pumpkin seed flour

Chestnut flour with average chemical composition: fat 3.7 g/100 g, of which saturated fat 0.7 g; carbohydrates 70.9 g/100 g, of which sugars 29.5 g, dietary fibres 10.8 g/100 g; proteins 6.4 g/100 g (Figure 2).

Figure 2. Chestnut flour

Rosehip flour with average chemical composition: fat 0 g/100 g, of which saturated fat 0 g; carbohydrates 38 g/100 g, of which sugars 3 g, dietary fibres 24 g/100; proteins 2 g/100 g (Figure 3).

The types of flour so described are available in the retail network in Bulgaria.

Figure 3. Rosehip flour

Preparation of the bread samples

To identify the impact of pumpkin seed, chestnut and rosehip flour on wheat bread staling, the following samples of bread have been studied:

- Control bread sample made of wheat flour (CS);
- Bread sample containing 5% pumpkin seed flour (PSF 5%);
- Bread sample containing 10% pumpkin seed flour (PSF 10%);
- Bread sample containing 5% chestnut flour (CF 5%);
- Bread sample containing 10% chestnut flour (CF 10%);
- Bread sample containing 5% rosehip flour (RF 5%);
- Bread sample containing 10% rosehip flour (RF 10%).

The formulations of the bread samples described above are presented in Table 1.

Table 1

Formulation of the bread samples

Raw materials	Bread samples						
	CS	PSF 5%	PSF 10%	CF 5%	CF 10%	RF 5%	RF 10%
Wheat flour (type 500),%	100	95	90	95	90	95	90
Water,%	56.0	56.0	56.0	56.0	56.0	56.0	56.0
Compressed yeast,%	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt,%	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Pumpkin seed flour,%	–	5	10	–	–	–	–
Chestnut flour,%	–	–	–	5	10	–	–
Rosehip flour,%	–	–	–	–	–	5	10

The bread was made by type 500 wheat flour based on a two-stage method of dough making. Initially, dough was made with 40% of the total quantity of flour and water (flour to water ratio of 1:1) and the entire quantity of yeast in a kneading machine (Labomix 1000,

Hungary). The control sample was made of wheat flour only and the additional ingredients indicated in Table 1. For the enriched samples, 5% or 10% (on the basis of wheat flour) unconventional for the baking industry flours (pumpkin seed flour, chestnut flour or rosehip flour) were added to the water for kneading and were homogenised very well, followed by adding the necessary quantity of wheat flour. The dough was fermented for 60 min at a temperature of 33 °C. This was followed by kneading the bread dough with the remaining raw materials (salt and the remaining quantity of the flour and water) until homogenised. The bread dough was divided, shaped and subjected to final fermentation for 60 – 70 min at a temperature of 35 °C (Tecnopast CRN 45–12, Novacel ROVIMPEX Novaledo, Italy). After the final fermentation, the dough was baked in an electrical oven (Salva E-25, Spain), pre-heated to 220 – 230 °C. Baking time was 24 min. After baking, the bread was left to cool down for 3 hours at room temperature.

Methods

Bread staling was determined by measuring the deformation characteristics of the crumb. An automated penetrometer type AP-4/2 was used. The total, plastic and elastic deformation of the bread crumb (in p. u.) was measured 3; 24; 48; 72 and 96 h after baking. The measurement was made on a piece of the bread crumb (from the central part of the loaf) with a thickness of 40 mm. The deformation characteristics were identified in the following way: on a flat surface, the bread crumb was subjected to the impact of a body with a certain mass, which was left to fall freely for a certain time (5 s). This caused penetration of the body and immersing in the bread crumb and the degree of its immersing in the crumb determined its compression or total deformation (Dt). Then, the immersing system was removed and the bread crumb partially recovered its height as a result of its elasticity properties, based on which the plastic deformation was determined (Dp). The difference between total and plastic deformation constitutes the elastic deformation (De), based on which the bread freshness is identified. Depending on the size of the studied piece, the measurement was performed on three (or five) spots at least 30 mm away from the crust.

Statistical analysis

Analysis of variance (ANOVA) was performed to determine whether storage time and bread formulations significantly ($p \leq 0.05$) affected staling of breads.

Results and discussion

Impact of pumpkin seed, chestnut and rosehip flour on total deformation of bread crumb

The results regarding the impact of pumpkin seed, chestnut and rosehip flour on the total deformation of the bread crumb during storage are presented in Figure 4.

It was found that with the increase in storage time, the values characterising total deformation decreased in all samples studied, i.e. the crumb became harder. Three hours after baking, the values in the bread samples containing 5% non-traditional flours were close each to the other and varied from 131 to 138 p.u. The highest total deformation was measured in the control sample (144 p.u.) indicating that the wheat bread had the softest crumb.

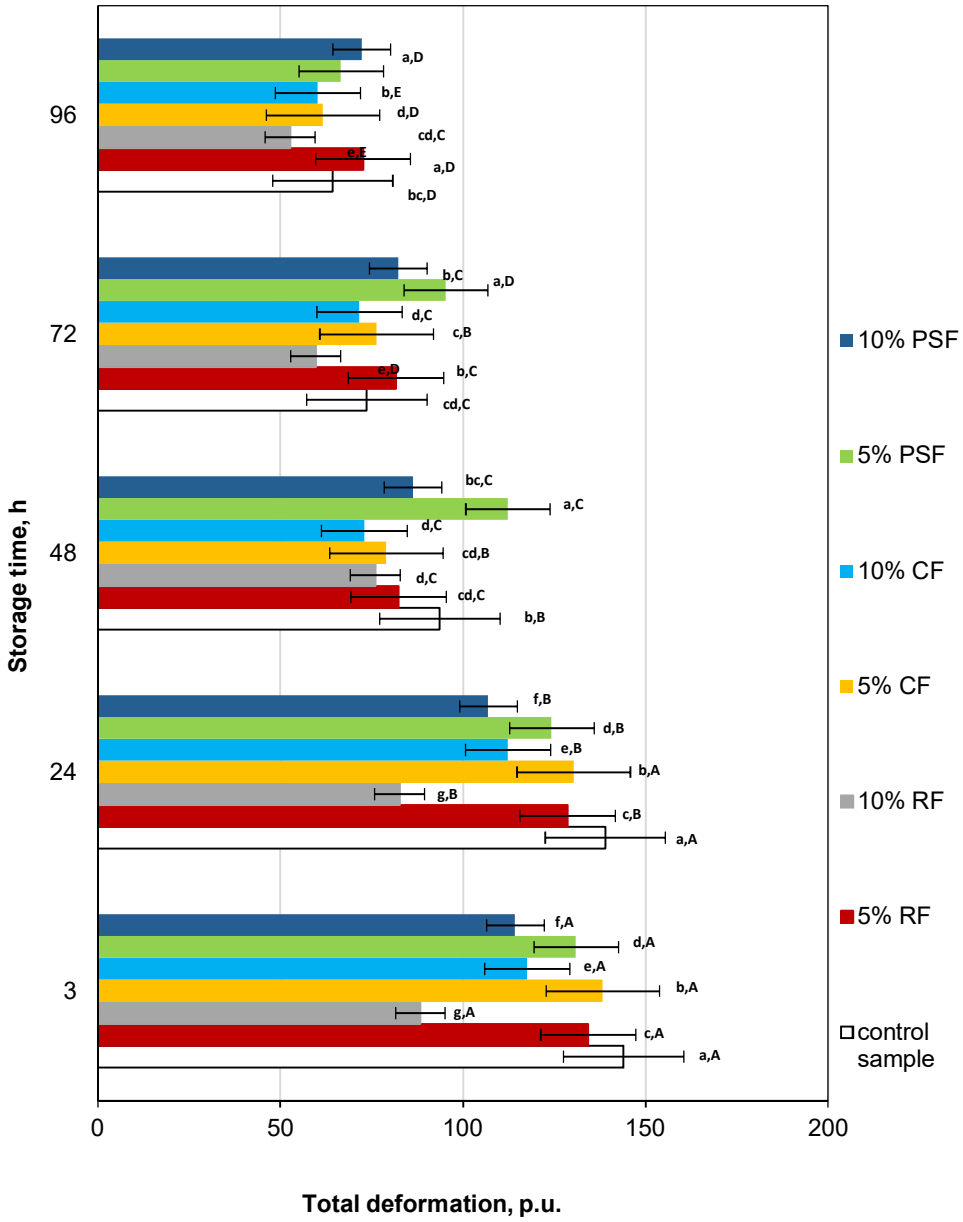

Figure 4. Effect of pumpkin seed flour, chestnut flour and rosehip flour on the total deformation of wheat bread crumb during storage:
[a–g] Means of the seven samples at the corresponding time of the measurement without a common letter differ significantly ($p < 0.05$).
[A–E] Means of one sample measured at 3; 24; 48; 72 and 96 h after baking without a common letter differ significantly ($p < 0.05$)

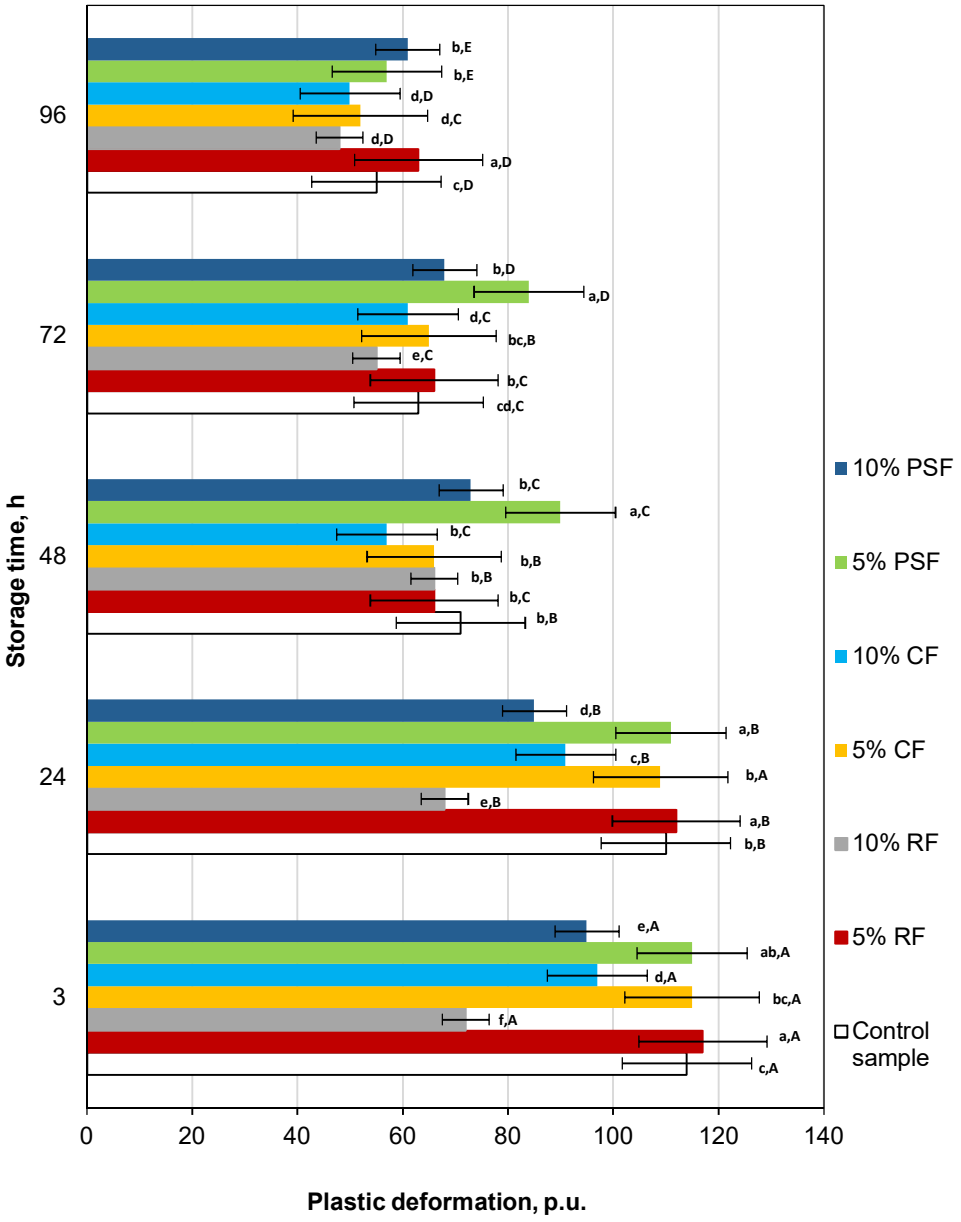

Figure 5. Effect of pumpkin seed flour, chestnut flour and rosehip flour on the plastic deformation of wheat bread crumb during storage:
[a–g] Means of the seven samples at the corresponding time of the measurement without a common letter differ significantly ($p < 0.05$).
[A–E] Means of one sample measured at 3; 24; 48; 72 and 96 h after baking without a common letter differ significantly ($p < 0.05$)

All the samples containing alternative flours in quantities of up to 5% have higher plasticity of the crumb as compared to the bread prepared from wheat flour only. At the beginning of the storage (3 h after baking), the lowest plasticity was observed in the sample with 10% rosehip flour – 72 p. u., whereas the highest values were measured in the samples with 5% chestnut, rosehip and pumpkin seed flour – 117; 115 and 117 p. u., respectively. These exceed, albeit insignificantly, the plastic deformation of the control sample – 114 p. u. After storage for 24 h, the plastic properties of the bread crumb were almost the same to the initial ones in the different samples. After 48-hour storage, the crumb of the bread with 5% pumpkin seed flour retained its high plasticity (the value decreased by 19.3% as compared to the one measured after 3 h), while the other samples showed clear deterioration of the plasticity properties. For instance, this decrease was by 35% as compared to the result after 3 h of storage in the bread with 10% chestnut flour. Rinaldi et al. (2014) also concluded that the input of high quantities of chestnut flour in the formulation of wheat bread (20%) resulted in significantly higher hardness of the crumb as compared to the control sample. The partial replacement of wheat flour with chestnut flour decreases gluten content and hinders the formation of a three-dimensional spatial structure and thus causes increased hardness of the bread (Rodriguez-Sandoval et al, 2017). Demirkesen et al. (2010) also found that adding chestnut flour to the recipe increased the crumb hardness. Authors attributed this to the increased amount of fiber in the enriched samples. Gómez et al. (2003) pointed out that the addition of fiber increases bread firmness, probably due to the thickening of the walls around the air bubbles in the bread crumb. This probably also explains the low values of plastic deformation, obtained in the sample with 10% rosehip flour. Vartolomei et al. (2021) found that the addition of rosehip flour to wheat flour resulted in a decrease in protein, wet gluten and moisture content. The same results are confirmed by another study, in which the influence of rosehip flour on the composition of wheat mixtures was investigated (Vartolomei et al., 2020).

According to other authors, adding 2% chestnut flour results in improvement of the plastic properties (Wang et al., 2023). In their study, Dall`Asta et al. (2013) found that the crumb hardness of bread with the addition of 20% chestnut flour was lower than that of wheat bread. However, a higher amount of the additive (50%) leads to an increase in hardness, also to a smaller volume and a darker color. This leads to the conclusion that the impact on the plastic properties of bread depends on the amount of additive included in the recipe. In this regard, the ratio between amylose and amylopectin in wheat and chestnut starch also matters. As pointed by Chang et al. (2021) starches isolated from different botanical sources have different ratios of amylose to amylopectin, which has a strong impact on the starch properties. After being plasticized during gelatinization, amylose has a better rearrangement ability than amylopectin, because its linear structure requires relatively little space for rearrangement and resettlement. In contrast, amylopectin has a large number of branches, and its chain distributions are disordered after gelatinization, which makes it much more difficult to rearrange in ordered structure. Amylopectin retrogradation requires a longer time than for amylose (Vamadevan and Bertoft, 2018). Therefore, the short-term retrogradation of starch (from the first few hours to several tens of hours) is generally attributed to the re-arrangement of amylose, while the long-term retrogradation is attributed to the re-arrangement of amylopectin (Chen et al., 2015).

In the control sample plastic deformation after 96 h of storage decreased by more than 50% as compared to the one after 3 h. The slowest rates of change were observed in the sample containing 10% rosehip flour (a decrease of 32%) and 10% pumpkin seed flour (a decrease of 37%).

At the end of the storage time (96 h after baking), the sample with 5% rosehip flour had significantly better plasticity as compared to other samples. Ascorbic acid, normally considered an antioxidant, is used to achieve greater dough stability and bread volume. Enrichment of bread dough with additives rich in ascorbic acid represents a strategy for producing bread with a higher degree of oxidative stability and better overall quality during storage (Osuna et al., 2018). As pointed by Tebben, Shen and Li (2018) ascorbic acid is a reducing agent, but is oxidized to the dehydroascorbic acid during mixing, and this form acts as an oxidant in dough. Authors concluded that reducing compounds from wheat bran may counteract the effects of oxidants in whole wheat dough. Therefore, oxidizing agents are less effective in whole wheat systems compared to those in refined wheat flour (Boz and Karaoglu, 2013) as used in the present study.

Impact of pumpkin seed, chestnut and rosehip flour on elastic deformation of bread crumb

The results regarding the impact of pumpkin seed, chestnut and rosehip flour on the elastic deformation of the bread crumb during storage are presented in Table 2.

Table 2

Effect of pumpkin seed flour, chestnut flour and rosehip flour on the elastic deformation of wheat bread crumb during storage

Samples	Storage time, h				
	3	**24**	**48**	**72**	**96**
Control sample	$30.00 \pm 0.82^{a,A}$	$29.67 \pm 0.47^{a,A}$	$20.67 \pm 0.47^{a,B}$	$10.00 \pm 0.82^{b,C}$	$9.00 \pm 1.63^{ab,C}$
5% RF	$16.67 \pm 0.94^{c,A}$	$16.33 \pm 0.47^{c,A}$	$14.00 \pm 2.16^{cd,A}$	$15.00 \pm 1.63^{a,A}$	$10.33 \pm 2.05^{ab,B}$
10% RF	$14.67 \pm 1.70^{c,A}$	$15.00 \pm 0.82^{d,A}$	$10.67 \pm 1.70^{ef,B}$	$3.67 \pm 1.25^{c,C}$	$2.67 \pm 0.94^{b,C}$
5% CF	$23.00 \pm 0.00^{b,A}$	$20.67 \pm 0.47^{b,B}$	$13.00 \pm 0.00^{cde,C}$	$11.00 \pm 1.63^{b,CD}$	$9.33 \pm 1.25^{ab,D}$
10% CF	$21.00 \pm 0.82^{b,A}$	$21.00 \pm 0.82^{b,A}$	$9.67 \pm 2.05^{f,B}$	$9.33 \pm 1.25^{b,B}$	$8.67 \pm 1.70^{ab,B}$
5% PSF	$14.00 \pm 1.63^{c,B}$	$12.67 \pm 0.47^{e,BC}$	$18.00 \pm 1.63^{ab,A}$	$9.67 \pm 1.25^{b,CD}$	$8.00 \pm 1.63^{ab,D}$
10% PSF	$20.67 \pm 1.89^{b,A}$	$20.67 \pm 0.47^{b,A}$	$15.33 \pm 2.62^{bc,B}$	$14.67 \pm 1.25^{a,B}$	$13.33 \pm 0.47^{a,B}$

[a–f] Means of the seven samples at the corresponding time of the measurement (at each column) without a common letter differ significantly ($p < 0.05$).

[A–D] Means of one sample measured at 3; 24; 48; 72 and 96 h after baking (at each row) without a common letter differ significantly ($p < 0.05$)

The elastic properties of bread crumb are mainly characterised by the quantity and physical properties of gluten, but also by the changes that take place in the bread during staling. At the beginning of storage (3 h after baking), the control sample of bread had significantly better elastic properties of the crumb, which was proven by the highest reported value (30 p. u.). Wheat bread had the highest elasticity even after storage for a period of 24

h (27.33 p. u.) and 48 h (20.67 p. u.), which was followed by sharp deterioration – 96 h after baking, the value characterising elastic deformation decreased by 70%. Crumb elasticity in the enriched samples deteriorated at a significantly slower rate – the elastic deformation decreased by 38% in the bread with 5% rosehip flour and by 35.5% in the sample with 10% pumpkin seed flour. Paciulli et al. (2016) also pointed out that incorporation of chestnut flour in wheat bread formulation results in a delay of staling. According to Černiauskienė et al. (2014) pumpkin seed is a rich source of dietary fiber, including a mixture of plant carbohydrate polymers, both oligosaccharides and polysaccharides (e.g. cellulose, hemicelluloses, pectic substances, gums, resistant starch, inulin), that may be associated with lignin, and other non-carbohydrate components. In five different cultivars *Cucurbita maxima* grown in Lithuania, content of neutral detergent fiber (cellulose, hemicellulose, lignin) ranges from 15.50±0.14 to 26.50±0.00% dry matter. The influence of hemicelluloses on bread staling has been a subject of research in recent years. A penetrometric test revealed that the supplementation of wheat bread recipe of 0.3, 0.5, or 0.7% hemicelluloses increased the penetration depth of the crumb after 72 h of storage, thus delaying crumb hardening, and resulted in bread with a higher specific volume than the control during a 3-days storage period (Hromádková et al., 2007). Jacobs et al. (2008) also confirmed that incorporation of fiber-rich fractions in bread formulation affects bread staling. The authors proposed that the beneficial effects found could be ascribed either to the higher water retention capacity and a possible inhibition of the amylopectin retrogradation, or to the increase of the total area of gas cells (Fadda et al., 2014). Gomez et al. (2003) pointed out that this effect can be attributed to the already mentioned water-binding capacity of the fibers, which in turn reduces the loss of water during storage as well as the possible interaction between fiber and starch, resulting in a delay in starch retrogradation.

After a longer storage period (72 h and 96 h) the elasticity of the enriched samples is higher or close to the one of the control sample. The only exception was the bread with 10% rosehip flour. This was also found by Boz et al. (2013), who observed that adding rosehip flour results in deterioration of the elastic properties and more rapid staling of wheat bread. The reason is the reduced gluten content. Namely gluten forms a viscoelastic network that is responsible for slowing down water migration and retaining gas produced from yeast fermentation (Demirkesen et al., 2013).

In the studied samples, there was more intensive decrease in elasticity at the beginning of storage (3 to 48 h after baking), which was followed by more gradual change. In the control sample, the elastic deformation decreased by 3.33 times. In the bread samples supplemented with 5% alternative types of flour, the change was as follows: for the sample with 5% rosehip flour – 1.61 times, sample with 5% chestnut flour – 2.50 times and sample with 5% pumpkin seed flour – 1.80 times.

Conclusions

1. The staling rate of wheat bread enriched with types of flour, non-traditional for breadmaking (chestnut, rosehip and pumpkin seed flour) depends on both the type and the amount of the additive.
2. Regarding the total deformation – a different intensity of hardening of the bread crumb during storage was found. The most intensive changes were observed in the control sample and the bread with 5% chestnut flour – after 96 hours of storage, the softness decreased by 55%, compared to the value measured after 3 hours of storage. The slowest hardening was observed in the bread with 10% pumpkin seed flour.

3. The plastic deformation of the crumb in wheat bread at the end of the storage (96 h) decreased by more than 50% as compared to the one after 3 h of storage. The slowest rates of change were observed in the sample containing 10% rosehip flour (a decrease of 32%).
4. Up to 48 hours of storage, the control sample of bread had significantly better elastic properties of the crumb, which was followed by sharp deterioration – 96 h after baking, elastic deformation decreased by 70%. Crumb elasticity in the enriched samples deteriorated at a significantly slower rate. The most pronounced delay in the staling process was achieved when using 5% rosehip flour and 10% pumpkin seed flour.
5. The appropriate dosing of rosehip and pumpkin seed flour in the wheat bread formulation resulted in preserving the physical properties (softness, plasticity, elasticity) of the crumb for a longer time and thus – in retarding the wheat bread staling.

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Technology of lager and dark beers with chicory roots

**Yurii Bulii, Roman Mukoid,
Anastasia Parkhomenko, Anatolii Kuts**

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Yurii Bulii
E-mail:
yvbuliy@
gmail.com

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Abstract

Introduction. Chicory root (*Cichorium intybus* L.) contains valuable components such as inulin, inulides, bitter substances, pectin, and fibers and is a promising non-traditional raw material for the production of new beer varieties and reduction of their cost.

Materials and methods. Dried and roasted chicory roots, lager and caramel barley malt, enzyme preparation inulinase, granulated hops of "Agnus" variety, yeast *Saccharomyces cerevisiae* (race RH) were used. Inulin content was determined by spectrophotometric method, amine nitrogen was measured by Pope and Stevenson method, content of reducing substances was estimated by Wilstetter-Schudl method. Standard methods accepted in brewing were used to determine other beer characteristics, sensory parameters were evaluated using profile methods.

Results and discussion. The technology for light beer production was proposed, according to which light malt and dried crushed chicory roots taken in the amount of 4% of malt are used as raw materials, and at the stage of mashing the enzyme preparation inulinase with activity of 14 units/g was introduced into the mixture. For the hydrolysis of inulin at the mashing stage, an inulase pause was provided at a temperature of 55–56 °C for 20–30 minutes. The method allows increasing the content of reducing substances in the wort by 1.6%, the apparent and actual fermentation degree by 3.9%, the content of alcohol and carbon dioxide in the beer by 3.2 and 10%, respectively. The finished beer had increased foam resistance and higher foam height, while the introduction of chicory did not impart excessive and extraneous bitterness. The innovative technology of dark beer provides mixing of aqueous extract of roasted chicory with malt wort cooled to the temperature of 85–90 °C. It was found that the optimal mode of the extraction process was the temperature 85–90 °C, hydromodule 1:6, and duration 90 min. Beer with chicory content of 3% was the best in terms of physical, chemical, and sensory properties. The improved method allows to increase the content of reducing substances in wort by 1.5%, apparent and actual fermentation degree by 2.2 and 3%, respectively, to increase the content of alcohol in beer by 2.3%, and carbon dioxide by 3.2%. It was proved that partial replacement of malt by chicory allows to reduce consumption of bitter hops for light beers by 20% (from 14.8 to 12.0 g/dal) and for dark beers by 10% (from 10.3 to 9.3 g/dal).

Conclusions. The addition of dried and fried chicory roots makes it possible to obtain new varieties of high-quality beer with their cost reduction.

Introduction

To obtain new types of beer and reduce the cost of their production, brewing has recently increasingly used non-traditional unmalted raw materials of plant origin, the addition of which to malt will improve the quality characteristics of beer (taste, aroma, foaming) and enrich the drink with biologically active substances (Shoaib et al., 2016).

Chicory root (*Cichorium intubus* L.) is a promising raw material for the production of lager and dark beers. Its most valuable components are inulin and bitter substances (Perović et al., 2021). Inulin is a fructan-type polysaccharide that has health-promoting properties and demonstrates several potential therapeutic benefits. Inulin is considered to be a prebiotic dietary fiber. It could stimulate activities beneficial to human health gut lactic acid bacteria and bifidobacteria (Waqas and Summer, 2017). Being low in calories, inulin can be used as a fat and sugar replacer in manufacturing of dairy products such as ice-cream, yogurt, and cheese (Krishna et al., 2020; Stabnikova and Paredes-Lopez, 2024).

Content of inulin in fresh chicory root crops is in the range from 60.8 to 65.0%, and in dried roots varies from 51.7 to 59.7% in terms of dry matter (DM) (Bais et al., 2001). Inulin, like starch, is not fermented by yeast, but it is hydrolysed when heated with water under the action of the enzyme inulinase (Madrigal et al., 2007). The products of its complete hydrolysis are the monosaccharides: fructose, 97%, and glucose 3%, which are fermented by brewer's yeast (Ricca et al., 2007).

Among bitter substances of chicory roots, glycoside intibin, lactucin, and lactucopicrin have been identified. Chicory also contains bitter cell sap, the bitterness index of which is 1:600. Biologically active substances of chicory include inulides, pectin, fiber, organic acids, amino acids, vitamins, macro- and microelements (Dubova et al., 2022; Massoud et al., 2009).

There are methods of brewing dark beers with chicory, which involve the addition of fried chicory extract in the amount of 5–10% to the wort at the stage of mashing grain products during the protein pause at a mash temperature of 52 °C or during the boiling of wort with hops 30 minutes before the end of the boiling process (Koshova et al., 2018). The disadvantages of described methods are the increase in the cost of finished beer due to the use of expensive concentrated extract, as well as the partial loss of valuable bitter and aromatic substances of chicory under the influence of high temperatures at the wort boiling stage.

Taking into account the fact that coloring substances of roasted chicory roots and caramel malt lie in the same region of the spectrum, and volatile substances of chicory give beer the aroma of rye bread, it is reasonable to use roasted chicory roots as a substitute for valuable coloring malts (caramel, dark, burnt) to produce dark beer (Liscomb et al., 2015; Van Arkel et al., 2012). There is a known method according to which the crushed roasted roots of vegetables were introduced into the filtration apparatus, where, simultaneously with the filtration of mash and washing of crushed root vegetables, extraction of water-soluble substances of chicory took place (Patent SU for invention 1666528. Method of wort preparation for dark beer). This method does not require additional equipment, but has some disadvantages, the main of which are the loss of part of inulin, valuable bitter, and aromatic substances of chicory at the stage of boiling wort with hops (Shoaib et al., 2016).

To exclude the above disadvantages, an innovative method of dark beer brewing has been developed, which involves mixing aqueous extract of roasted chicory roots with partially cooled hoped wort (Patent UA for invention 114994. Method of preparation of wort for dark beer).

There is no information in the literature about the use of dried chicory root vegetables for brewing lager beers, which requires research. For this purpose an innovative method was developed, which provides mashing of a mixture of malt and dried chicory and differs from the classical one by carrying out inulase pause at temperature 55–56 °C for 20–30 min and introduction of enzyme preparation "Inuloavamorin P10X" for activation of inulin hydrolysis (Patent UA for invention 115398. Method of preparation of low-calorie dietary beer).

Assessing the effectiveness of innovative brewing methods that use partial replacement of malt with dried or roasted chicory, and choosing optimal technological modes is relevant for both craft and large breweries.

The aim of the present study was to improve the technology for light and dark beer by the use of dried or roasted chicory roots to obtain new varieties of beer of enhanced quality at lower costs.

Materials and methods

Materials

The following materials were used for the research: dried chicory roots; fried chicory roots; light barley malt "Best Pilsen Malt" (Germany); caramel malt "Best Caramel Malt Dark" (Germany); enzyme preparation "Inuloavamorin P10X", activity 14 units/g (Ukraine); granulated hops of the Czech variety "Agnus" (α-acid content of 5.2% in terms of air-dry matter, moisture content of 10.3%); bottom fermentation yeast *Saccharomyces cerevisiae*, race RH.

Research methods

Analytical, chemical, physicochemical, organoleptic, profile and calculation methods were used to evaluate the research results obtained using devices and research methods used in brewing.

Determination of reducing substances in wort

The content of reducing substances in wort was determined by the Wilstetter-Schudl method based on the oxidation of aldoses with iodine (Kunze, 2007).

Determination of amine nitrogen content in wort

Amine nitrogen content in wort was determined by an iodometric (copper) method according to Pope and Stevens (Narzib, 2007). The method is based on the ability of amino acids to form soluble complexes with copper. Excess copper is filtered off, acetic acid is added to the filtrate, which detaches copper from the complex compound to form copper acetate, after which potassium iodide is added. When the latter interacts with acetic acid copper, free iodine is released, the amount of which is proportional to the amount of copper and, accordingly, the amount of amine nitrogen. Free iodine is titrated with sodium thiosulfate solution.

Physico-chemical properties of the finished beer

Physico-chemical parameters in the finished beer (acidity, ml of 1 M NaOH per 100 ml of wort; color, ml of 0.1M I_2 per 100 ml of water; carbon dioxide content,%) were determined using an Anton Paar analyzer (Ciocan et al., 2020).

Determination of the dry matter concentration

The method is based on the determination of the content of extractive substances in beer by relative density (Kunze, 2007).

The beer free from carbon dioxide was poured into a cylinder, which was placed on a flat surface, the temperature was measured and an areometer was immersed. The upper meniscus was used to read the areometer and determine the concentration of dry matter (DM), taking into account the correction for temperature.

Determination of the alcohol concentration

The method is based on the distillation of alcohol from a weighed sample of beer, followed by the determination of the mass fraction of alcohol by refractometric method and the solids content by areometric method (Kunze, 2007).

In a dry distillation flask 200 ml of beer freed from carbon dioxide were taken, the flask was connected to a refrigerator through a droplet eliminator and the beer was distilled. After distillation of 1/3 of the sample volume, the rest of the distillation flask was brought to the original volume with water, mixed thoroughly, cooled to the temperature of 20 ºC and the concentration of DM (actual content of the extract) was determined by areometric method. The distillate in the receiving flask was brought with water to the initial volume, mixed thoroughly and the mass fraction of alcohol in the sample was determined at 20 ºC by a dip refractometer using alcohol tables.

Determination of inulin and fructooligosaccharides

High Performance Liquid Chromatography coupled with the Refractive Index Detector (HPLC RID) method for determination of inulin and fructooligosaccharides was used (Petkova et al., 2014, 2015). The essence of the method is the chromatographic separation of chicory extract filtrate on a Shodex® Sugar SP0810 column with a Pb2+ protective column (50 × 9.2 mm i.d.) and an analytical column (300 mm × 8.0 mm i.d.) with a movable phase – ionized water. The movable phase provided long retention time, resolution, and satisfactory chromatogram peak profiles. The column was placed in a thermostat The column was placed in a LCO 102 thermostat (ECOM, Czech Republic). The preparation of the extract involved precipitation of proteins by adding Carrese I (K_4 $Fe(CN)_6$ x $3H_2O$) and Carrese II ($Zn(CH_3COO)_2$ x $2H_2O$) reagents in an amount of 5 ml each. The sample was filtered through a 0.45 μm paper filter, transferred to a 50 mL flask, and made up to the mark with deionized water (Bugner et al., 1992). Prior to injection into the HPLC column, samples were passed through a 0.2 μm cellulose acetate filter (Sartorius AG, Göttingen, Germany). The extract containing inulin was dissolved in hot water to a concentration of 10 mg/ml. From the standard solution prepared in this way, working standard solutions containing 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, and 10 mg/ml of inulin were prepared. The operating temperature of the column was 85 °C and the movable phase velocity was 1 ml/min. The linearity of the method was in the range of 0.1-10 mg/ml, and the correlation coefficient R_2 exceeded 0.997.

Determination of sensory properties

The finished beer was evaluated for clarity, color, taste, aroma, hop bitterness, carbon dioxide saturation, foaming, and foam stability. The maximum tasting score of the prototypes was 25 points and was determined as the sum of the scores for each indicator: clarity, 3; color, 3; taste, 5; hop bitterness, 5; aroma, 4, and foam stability, 5 (Table 1).

Table 1

Overall beer quality assessment

Evaluation	Total score
Excellent	22-25
Good	19-21
Satisfactory	13-18
Unsatisfactory	12 and less

The tasting committee consisted of five experts from the Department of Biotechnology of Fermentation Products and Winemaking of the National University of Food Technologies. Special cylindrical glasses made of colorless glass with a capacity of 150–200 ml and a diameter of 50-60 mm were used for beer tasting. The beer temperature was 12±2 °C. The order of tasting provided for the evaluation of lager beers first, with initial wort concentration from lower to higher, and then dark beers. The profile method (Bocharova et al., 2017) was used to evaluate the aroma and taste of beer. Comparison of the profiles of the experimental samples allowed to determine their differences and draw conclusions about the quality of beer.

Processing of research results

Determination of physical and chemical parameters of light barley malt, chicory, wort, young and matured beer was carried out in triplicates. The results are shown as mean ± standard deviation.

Setting up the experiment

During the experiment, the quality parameters of wort and beer were investigated and compared with control samples.

Experimental samples of light wort and beer (11% DM)

- sample 1 – 100% barley light malt (control);
- sample 2 – 98% barley malt, 2% dried chicory;
- sample 3 – 96% barley malt, 4% dried chicory;
- sample 4 – 94% barley malt, 6% dried chicory.

Experimental samples of dark wort and beer (13% DM)

- sample 5 – 95% barley malt, 5% caramel malt (control);
- sample 6 – 97% barley malt, 3% fried chicory;
- sample 7 – 95% barley malt, 5% fried chicory;
- sample 8 – 93% barley malt, 7% fried chicory.

At the first step, the effectiveness of the chicory lager beer technology was studied. Chopped dried roots were added to the mash tun along with light barley malt at the rate of 2, 4, and 6% of the malt amount. The extractivity of dried chicory coincided with the extractivity of light malt and amounted to 78–80%. This made it possible to obtain experimental samples of wort with chicory and control wort of the same concentration (11% DM).

To obtain the control sample, barley light malt was mashed without chicory. The infusion method was chosen for mashing (Figure 1).

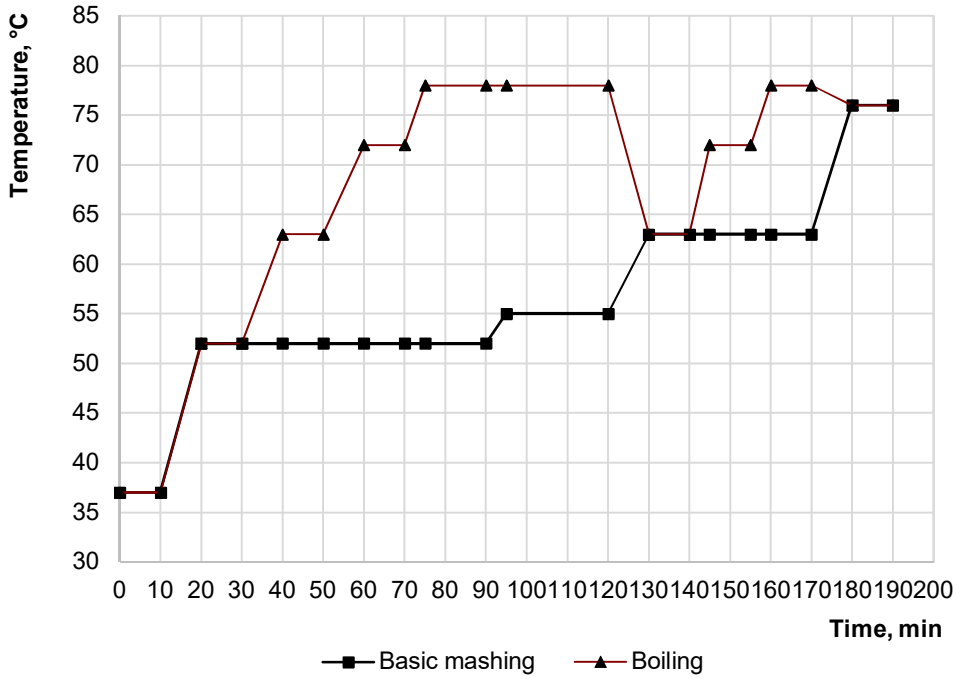

Figure 1. Technological modes of mashing malt with the addition of dried chicory

The mashing process was carried out as follows. The light brewing malt was crushed and mixed with water at a temperature of 37–42 °C in a ratio of 1:4 in a mash tun and kept for 20–30 min (cytolytic pause), then the mash was heated to a temperature of 52 °C and kept for 20–30 min with stirring to hydrolyze proteins (protein pause). After that, the temperature of the mash was gradually raised to 55–56 °C. At this temperature, the enzyme preparation inulinase (Inuloavamorin P10X) was added to the stirrer to perform the inulase pause. At this temperature, inulinase had maximum activity. During the inulase pause, enzymatic hydrolysis of inulin occurred with the formation of fructose and a small amount of glucose.

After 20-30 min, the mash temperature was increased to 63 °C with a heating rate of 1 °C per 1 min to continue the enzymatic hydrolysis of inulin and malt starch, the mash was kept at this temperature for 30 min (maltose pause), after which the temperature was increased to 72 °C, and the mash was kept until the starch was completely saccharified and inulin was hydrolysed. The saccharified mash was then heated to 76 °C and filtered. The resulting wort was boiled with hops for 90 minutes. Due to the addition of chicory bitter substances, the consumption of bitter hops at the wort boiling stage was reduced from 20 to

18-14 g/dal (by 10–30%), depending on the amount of chicory. After clarification of the wort from protein precipitate and its cooling to a temperature of 8 °C, the wort was fermented at this temperature for 7 days. After the end of the main fermentation, the young beer was cooled to a temperature of 1–2 °C, the yeast was removed from the sediment and the young beer was fermented for 14 days.

At the second step, the effectiveness of the technology of dark beer with chicory was studied. The proposed method involved the preparation of an aqueous extract of fried chicory and it's mixing with wort cooled to a temperature of 85–90 °C. To prepare the chicory extract, chopped roasted roots in an amount of 3, 5, and 7% of the amount of light malt were poured into an extractor, mixed with water in a ratio of 1:6 at a temperature of 85–90 °C, and soluble substances were extracted for 90 min. Beer wort with a concentration of 13% DM was prepared by the classical method. For its hopping, the consumption of bitter hops was reduced from 20 to 18–14 g/dal (by 10–30%). Fermentation of wort and fermentation of young beer was carried out according to the above modes. As a control, the beer was brewed from light malt with the addition of caramel malt in the amount of 5% at the mashing stage.

At the third step, the optimal consumption of hops for producing lager and dark beers with chicory was determined and the cost of the finished beer was calculated.

To achieve the required bitterness of wort according to the recipe for lager beer (0.82 g/dal in terms of DM), the standard amount of air-dried hops when added to the hot wort was 14.8 g/dal. On this basis, for the preparation of lager beer (control sample 1), granulated hops were added at the wort boiling stage in the amount of 14.8 g/dal. Due to the addition of bitter substances of dried chicory, the consumption of hops was reduced by 10, 20, and 30%. Thus, different amounts of hops were added to the experimental samples of wort: 13.3, 12 and 10.4 g/dal.

To achieve the required bitterness of wort according to the recipe for dark beer (0.57 g/dal in terms of DM), the standard amount of air-dried hops when added to the hot wort was 10.3 g/dal. Based on this, for the preparation of dark beer (control sample 5), granulated hops were added during the wort boiling in the amount of 10.3 g/dal. Due to the addition of bitter substances of fried chicory, the consumption of hops was reduced by 10, 20, and 30% to produce dark beer with chicory. Thus, hops were added to the wort in the amount of 9.3, 8.2 and 7.2 g/dal.

The optimal hop addition was determined by sensory evaluation of lager and dark beer with chicory.

Results and discussion

Physical and chemical properties of wort for lager beer

The physical and chemical properties of wort for lager beer are shown in Table 2.

The content of dried chicory in the samples was: sample 1 (control) – 0%, sample 2 – 2%; sample 3 – 4%, sample 4 – 6% of the malt. All samples almost did not differ from the control in color and acidity. With an increase in the amount of chicory to 4%, the concentration of reducing substances in the wort increased by 1.6%, and the content of amine nitrogen decreased by 0.5% compared to the control. With further increase of chicory, the concentration of reducing substances decreased due to incomplete hydrolysis of inulin at the mashing stage.

Table 2

Physical and chemical properties of wort for lager beer

Indicator	Sample 1 (control)	Samples of wort with dried chicory		
		2	3	4
Content of DM in the initial wort,%	11.0±0.2	11.0±0.2	11.0±0.2	11.0±0.2
Color, ml 0.1 M I_2/100 ml of water	0.6±0.2	0.6±0.2	0.6±0.2	0.7±0.2
Acidity, ml NaOH/100 ml of wort	2.1±0.2	2.1±0.2	2.1±0.2	2.2±0.2
Content of reducing substances, g/100 ml of wort	6.2±0.3	6.2±0.3	6.3±0.3	6.2±0.3
Amine nitrogen content, mg/100 ml of wort	21.8±0.5	21.7±0.5	21.7±0.5	21.6±0.5

Visible extract during the main fermentation of wort for lager beer

During the main fermentation in the experimental samples, the change in the concentration of DM in the wort was analysed (Figure 2).

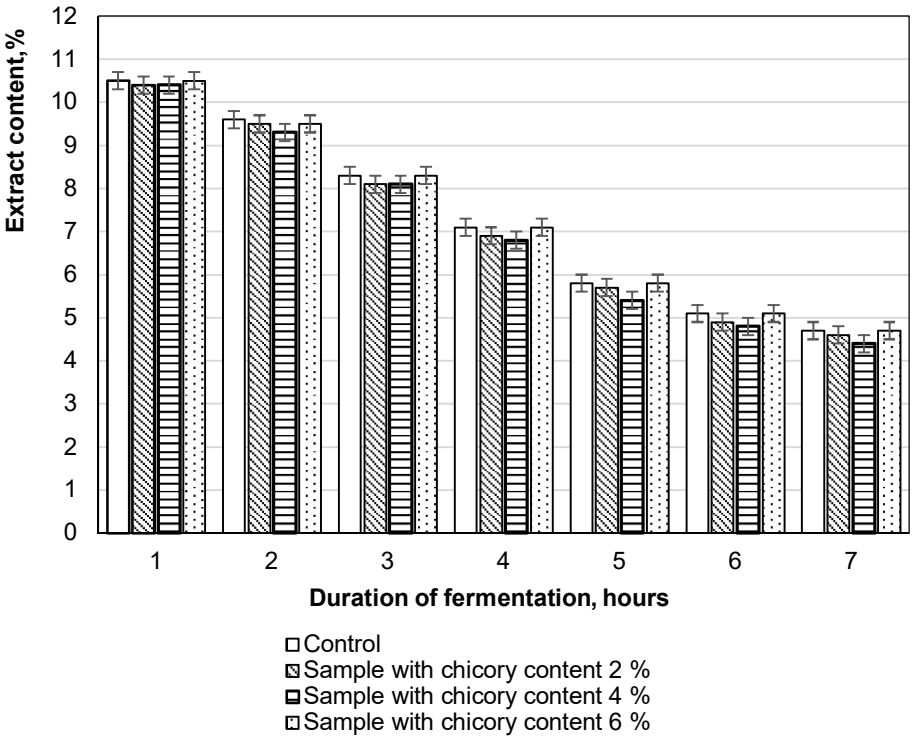

Figure 2. Dynamics of changes in visible extract during the fermentation of wort to produce lager beer

The analysis of the results showed that the sample with a content of dried chicory of 4% fermented faster and more completely than the others. Thus, on the fifth day of the main fermentation, the content of visible extract in the sample with a content of dried chicory of 4% was 5.4% (in the control – 5.7%); on the seventh day, its content in the control was 4.7%, in the experimental samples with a content of dried chicory of 2% – 4.6%, with a content of dried chicory of 4% – 4.4%, with a content of dried chicory of 6% – 4.7%.

This is explained by the fact that in the sample with a content of dried chicory of 4%, the content of reducing substances was 1.6% higher compared to the control.

Determination of physical, chemical and sensory properties of lager beer

After fermentation, physical, chemical and sensory properties of lager beer were determined (Table 3).

Table 3

Physical and chemical properties of lager beer

Indicator	Sample 1 (control)	Samples of lager beer with dried chicory		
		2	3	4
The actual extract,%	5.1±0.2	5.0±0.2	4.8±0.2	5.1±0.2
Visible extract,%	3.7±0.2	3.6±0.2	3.4±0.2	3.7±0.2
Alcohol content,% vol.	3.4±0.2	3.4±0.2	3.5±0.2	3.4±0.2
The degree of fermentation: - visible,%	66.4±0.2	67.2±0.2	68.9±0.2	66.4±0.2
- actual,%	53.8±0.2	54.4±0.2	55.9±0.2	53.8±0.2
Active acidity (pH)	4.3±0.2	4.4±0.2	4.5±0.2	4.5±0.2
Titrated acidity, ml 1M NaOH/100 ml of beer	2.1±0.2	2.1±0.2	2.2±0.2	2.2±0.2
Color, ml of 0.1 M I_2/100 ml of water	0.4±0.2	0.4±0.2	0.5±0.2	0.5±0.2
Content of carbon dioxide,%, not less than	0.30±0.5	0.30±0.5	0.33±0.5	0.30±0.5

The sample with 4% dried chicory content had a 3.2% higher alcohol content and 10% higher carbon dioxide content than the control sample. The apparent and actual degree of fermentation of this sample was 3.9% higher than that of the control. This is explained by the fact that the content of reducing substances in this sample was the highest.

According to the sensory evaluation, the best sample was the one with a dried chicory content of 4%. This sample had the highest foam stability and foam height. This is explained by the increased concentration of carbon dioxide in it compared to other samples. All samples were characterized by a rich malt flavor, clean hop aroma, and foam height that met the standard requirements. Beer samples with chicory content of 2% and 4% had a pleasant hop bitterness. The sample with 6% dried chicory content had a pronounced excessive bitterness, which is not typical for this type of beer. Thus, the sample with a 4% dried chicory content was the best in terms of physical, chemical and sensory properties of wort and finished beer.

Physical and chemical properties of wort for dark beer

The physical and chemical properties of the wort for dark beer are shown in Table 4.

Table 4

Physical and chemical properties of wort for dark beer

Indicator	Sample 5 (control)	Samples of wort with fried chicory		
		6	7	8
Content of DM in the initial wort,%	13.0±0.2	13.0±0.2	13.0±0.2	13.0±0.2
Color, ml of 0.1 M I_2/100 ml of water	4.5±0.2	4.4±0.2	4.5±0.2	4.7±0.2
Acidity, ml of 1M NaOH/100 ml of wort	2.3±0.2	2.2±0.2	2.3±0.2	2.5±0.,2
Reducing substances content, g/100 ml of wort	6.8±0.3	6.9±0.3	6.8±0.3	6.5±0.3
Amine nitrogen content, mg/100 ml of wort	19.6±0.5	19.4±0.5	19.1±0.5	18.7±0.5

The content of fried chicory in the wort was,% from the amount of light malt: 0, sample 5 (control); 3, sample 6; 5, sample 7; 7, sample 8. The color and acidity of the wort increased with the increase of the amount of fried chicory. The highest content of reducing substances was in the sample with chicory content of 3% (exceeded their content in the control by 1.5%). In samples with chicory content of 5 and 7%, their content decreased, which was explained by an increase in the content of the polysaccharide inulin and melanoidins in the wort, which are not fermented by yeast (Dack et al., 2017). The highest content of amine nitrogen was in control sample 5. As the amount of chicory in the wort increased, the content of amine nitrogen decreased. This is explained by the fact that, compared to barley malt, chicory contained a small amount of protein substances, and part of the sugars was used for the formation of melanoids (Massoud et al., 2009; Narzib, 2007). Due to the increase in the content of melanoidins, which have an acidic character and bitter taste, the acidity of the experimental samples increased (Koshova et al., 2018).

Visible extract during the main fermentation of wort for dark beer

The dynamics of change of visible extract during the main fermentation of wort for dark beer is shown in the diagram (Figure 3).

The diagram shows that compared to the control, the wort with the content of roasted chicory of 3% fermented better and faster: On the sixth day of the main fermentation, the visible extract content in this beer sample was 6.0%, while in the control it was 6.2%; on the seventh day, the extract content in the control was 5.4%, and in the experimental samples with chicory content of 3% – 5.3%, with chicory content of 5% – 5.5%, with chicory content of 7% – 5.5%.

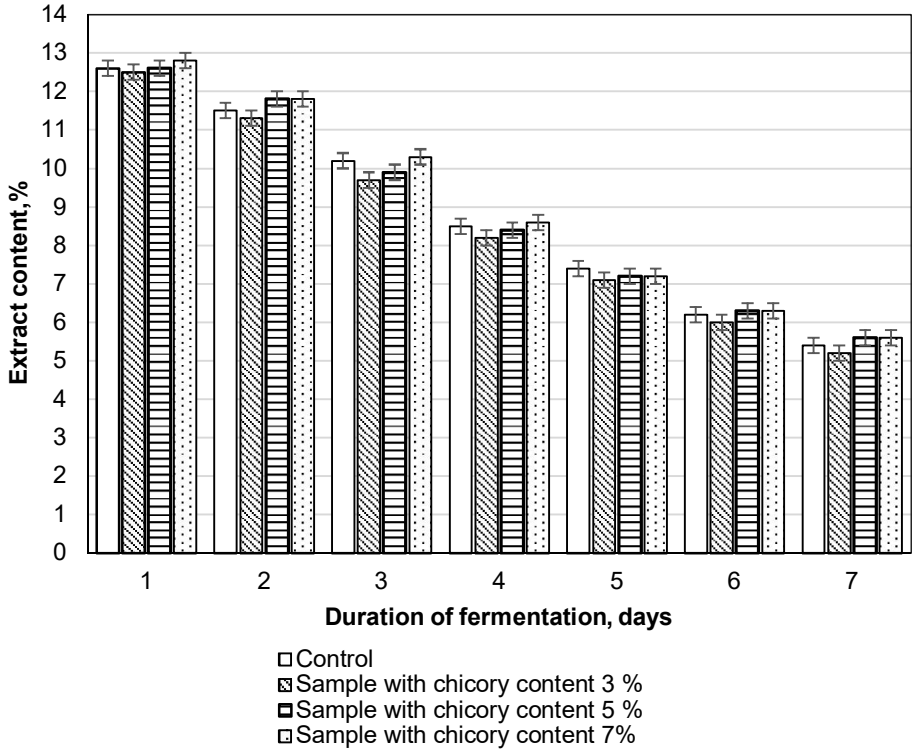

Figure 3. Dynamics of changes in visible extract during the fermentation of wort for dark beer

With the increase of chicory content to 5 and 7%, the rate of fermentation of the experimental wort samples decreased, which was explained by a decrease in the concentration of reducing substances in the wort, which were added with chicory, and an increase in the content of inulin, which was not fermented by yeast (Massoud et al., 2009; Narzib, 2007).

Determination of physical, chemical, and sensory properties of dark beer

The physical, chemical and sensory properties of the dark beer are shown in Table 5.

The beer sample with a chicory content of 3% had by 2.3% higher alcohol content and 3.2% higher carbon dioxide content than control. The visible and actual degree of fermentation of this sample was by 2.2 and 3.0%, respectively, higher than in the control. With the increase in the content of fried chicory due to the addition of coloring substances, the color of the finished beer increased, and the titratable acidity increased. At the same time, the apparent and actual degree of fermentation decreased. This is explained by the fact that an increase in the content of coloring substances led to a decrease in the activity of brewer's yeast (Dack et al., 2017; Kunze, 2007). The acidity of the sample with a roasted chicory content of 7% was higher compared to control. In addition, this beer had the highest color. This is explained by the increased content of melanoids, which have an acidic character (Dack et al., 2017; Langner et al., 2014).

Table 5

Physical, chemical, and sensory properties of dark beer

Indicator	Sample 5 (control)	Samples of dark beer with fried chicory		
		6	7	8
Content: – of the actual extract,% – of the visible extract,% – of alcohol,% vol.	5.9±0.2 4.2±0.2 4.3±0.2	5.8±0.2 4.1±0.2 4.4±0.2	5.6±0.2 3.9±0.2 4.3±0.2	5.9±0.2 4.2±0.2 4.2±0.2
The degree of fermentation: – visible,% – actual,%	57.5±0.2 46.6±0.2	58.8±0.2 48.0±0.2	57.5±0.2 46.7±0.2	56.8±0.2 46.0±0.2
Acidity, ml of 1M NaOH/100 ml of beer	2.8±0.2	2.6±0.2	2.7±0.2	2.9±0.2
Color, ml of 0.1 M I_2/100 ml of water	4.6±0.2	4.5±0.2	4.6±0.2	4.8±0.2
Content of carbon dioxide,%, not less than	0.31±0.5	0.32±0.5	0.31±0.5	0.30±0.5

According to the sensory evaluation, the beer with a fried chicory content of 3% had the best taste and aroma (tasting score of 24 points). The beer prepared with the addition of chicory had a balanced pleasant hop bitterness. Beer with chicory content of 5 and 7% had an excessive and unpleasant bitterness. At the same time, all the beer with chicory were well saturated with carbon dioxide, had a thick, compact, fine-grained and stable foam. The beer with a fried chicory content of 3% was the best in terms of physical, chemical and sensory properties of wort and finished beer.

Effects of hop addition on the sensory properties of lager beer with chicory content of 4%

For the preparation of lager beer and the hopping of pure malt wort (control), bitter hops were added in the amount of 14.8 g/dal. Since the beer sample with a dried chicory content of 4% was chosen as the best in terms of physical, chemical and sensory properties, the amount of bitter hops added at the wort boiling stage was reduced by 10, 20, and 30%. The consumption of hops was 14.8 g/dal for pure malt wort, and 13.3, 12.0, 10.4 g/dal for the experimental samples. After the end of the fermentation process of young beer, the control and chicory beer samples were evaluated by sensory properties (Table 6).

According to the results of the sensory evaluation, beer with bitter hop content of 13.3 and 12 g/dal had better taste and aroma compared to others. The finished beer was characterized by a purely mild, balanced hop bitterness, which was inherent in the control.

It was found that for the preparation of lager beer, the selected mashing method and technological modes can reduce the specific consumption of valuable hops by 20% due to the introduction of bitter substances of dried chicory without deterioration of the quality characteristics of beer.

Table 6

Sensory properties of lager beer with chicory content of 4%

Amount of hops, g/dal	Taste	Flavor	Appearance	Hop bitterness	Foam
14.8	harmonious, malty, without off-flavors	clean, fresh, pronounced malty	a clear liquid with a shine, without suspensions	purely hop, soft, balanced, consistent with this type of beer	thick, stable, compact, fine-grained
13.3	harmonious, malty, without off-flavors	clean, fresh, pronounced malty	a clear liquid with a shine, without suspensions	purely hop, soft, balanced, consistent with this type of beer	thick, stable, compact, fine-grained
12.0	harmonious, malty, without off-flavors	clean, fresh, pronounced malty	a clear liquid with a shine, without suspensions	purely hop, soft, balanced, consistent with this type of beer	thick, stable, compact, fine-grained
10.4	not very harmonious, malty, without off-flavors	clean, fresh, slightly pronounced malt	a clear liquid with a shine, without suspensions	purely hop, weak, which does not correspond to this type of beer	thick, stable, compact, fine-grained

Effects of hop addition on the sensory properties of dark beer with chicory content of 3%

For the preparation of dark beer and the hopping of the control wort sample containing 95% barley pale malt and 5% caramel malt, bitter hops were added in the amount of 10.3 g/dal. Since the sample with the content of fried chicory of 3% was chosen as the best in terms of physicochemical and organoleptic parameters, the amount of bitter hops added at the wort boiling stage was reduced by 10, 20, and 30%. The hop addition was 10.3 g/dal for control, and 9.3, 8.2, 7.2 g/dal for the wort with chicory.

After the end of the fermentation process of young beer, control and chicory beer samples were sensory evaluated (Table 7).

Table 7

Sensory properties of dark beer with chicory content of 3%

Amount of hops, g/dal	Taste	Flavor	Appearance	Hop bitterness	Foam
10.3	harmonious, clean, with tones of caramel malt without off-flavor	pure hop, malt, with caramel aroma	clear liquid with a shine, without suspensions	purely hop, soft, balanced, consistent with this type of beer	thick, stable, compact, fine-grained
9.3	harmonious, clean, with tones of caramel malt without off-flavor	pure hop, malt with rye bread aroma	clear liquid with a shine, without suspensions	purely hop, moderately mild, balanced, consistent with this type of beer	thick, stable, compact, fine-grained
8.2	harmonious, malty, without off-flavor	clean, slightly hopped, malty with a rye bread aroma	clear liquid with a shine, without suspensions	purely hop, soft, weak, not very balanced, not suitable for this type of beer	thick, stable, compact, fine-grained
7.2	not very harmonious, malty, without off-flavor	clean, very slightly hopped, malty with a rye bread aroma	clear liquid with a shine, without suspensions	purely hop, soft, weak, not balanced, not appropriate for this type of beer	thick, stable, compact, fine-grained

According to the sensory evaluation, the best samples were those with a hop content of 10.3 and 9.3 g/dal. These samples were characterized by a harmonious, clean taste with tones of caramel malt present without off-flavor, clean, balanced hop bitterness that corresponded to this type of beer, and a clean, hop aroma with a rye bread flavor. Other samples did not meet the standard requirements due to insufficient bitterness for finished beer.

It was found that the selected technological methods and modes of brewing dark beer can reduce hop consumption by 10% due to the addition of bitter substances of fried chicory without deteriorating the quality of beer.

Beer sensory properties

After determining the physical and chemical properties of the finished beer and the optimal consumption of hops, the best experimental samples of light and dark beer were evaluated by their sensory properties and compared with control (Table 8).

Table 8

Sensory properties of the lager and dark beer

Indicator	Lager beer		Dark beer	
	Experimental sample*	Control*	Experimental sample**	Control**
Appearance	9	8	11	9
Flavor	3	3	4	3
Taste	8	7	10	8

*Experimental sample of lager beer: content of dried chicory 4%, hops – 12 g/dal. Control sample of lager beer: light malt content 100%, hops – 14.8 g/dal.

**Experimental sample of dark beer: content of fried chicory 3%, hops – 9.3 g/dal. Control sample of lager beer: pale malt content 95%, caramel malt content 5%, hops content 10.3 g/dal.

It was found that in terms of quality indicators, the samples of lager beer with chicory almost did not differ from the control, and the indicators of dark beer received higher tasting scores compared to the control. Based on the results of the sensory evaluation of the samples, profilograms of beer aroma and taste were constructed, which are presented in Figures 4, 5, 6, 7.

When comparing the profilograms of light beer with chicory and the control sample (Figures 4, 5), it was found that the experimental samples with an initial wort concentration of 11% by weight were characterized by a rich malt flavor and a clean hop aroma.

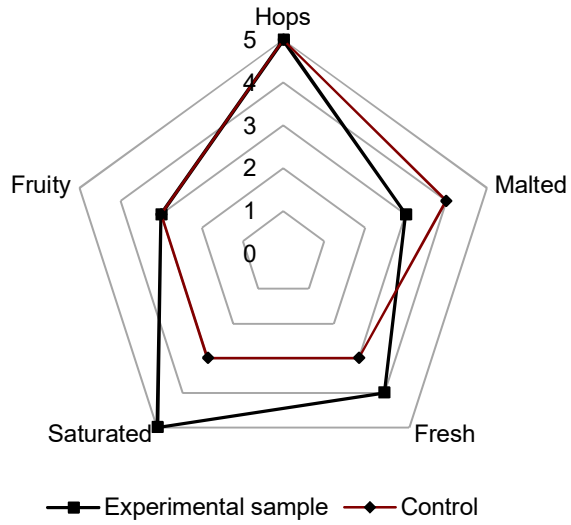

Figure 4. Aroma profile of lager beer

The control sample had a better malt flavor. Beer with chicory had a more pronounced fresh and rich flavor. The addition of chicory bittering substances did not impart excessive and extraneous bitterness to the finished beer. In the experimental sample, there was more acidity due to the addition of dried chicory, the active acidity of which was 4.3, which affected the aftertaste and sweetness of the beer. The resulting lager beer was characterized by better balance, fullness of flavor and less aftertaste.

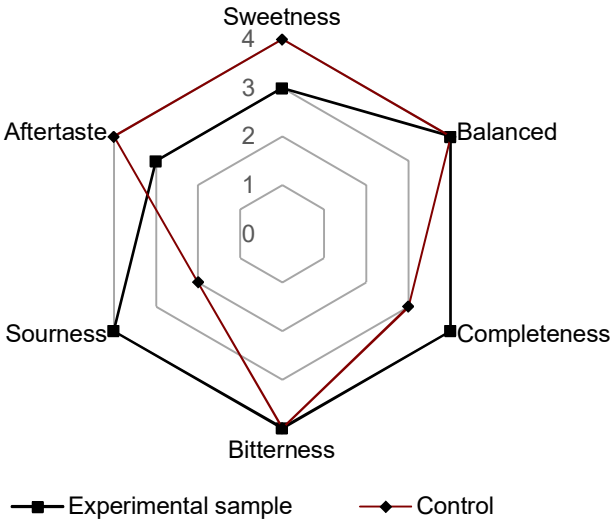

Figure 5. Taste profile of lager beer

When comparing the profilograms of dark beer with fried chicory and the control sample with caramel malt (Figures 6, 7), it was found that the hop aroma in the experimental sample was less than in the control, which is explained by the reduced content of hop aromatics in beer. At the same time, dark beer with chicory was characterized by a richer and fresher aroma due to the addition of chicoryol essential oil and aromatic substances of fried roots, which have the aroma of rye bread.

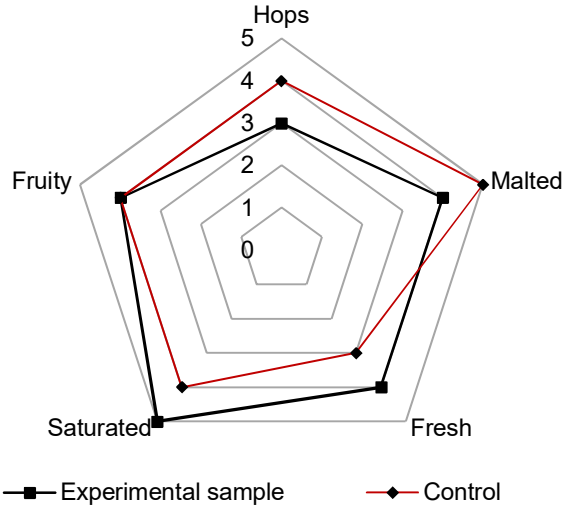

Figure 6. Aroma profile of dark beer

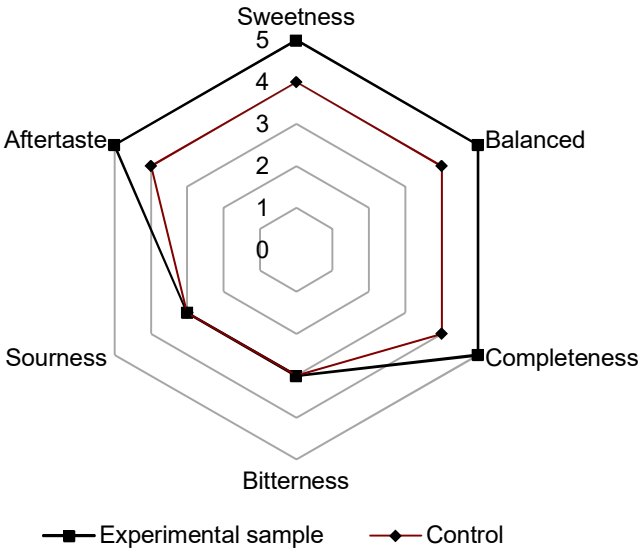

Figure 7. Taste profile of dark beer

The chicory beer sample was characterized by higher sweetness, better aftertaste, and fullness. This is due to the fact that the test sample contained more fructose and inulin. Melanoids from fried chicory give the finished beer a more pronounced aftertaste and fullness (Langner et al., 2014).

Conclusions

The use of dried and fried roots makes it possible to produce new varieties of lager and dark beer of improved quality.

1. An innovative method of preparing wort for light beer involves adding crushed dried chicory, an enzyme preparation of inulinase with an activity of 14 units/g to the mash and an inulinase pause at a temperature of 55–56 °C for 20–30 minutes.
2. The selected technological modes allow to increase the content of reducing substances in wort by 1.6%, to increase the visible and actual degree of beer fermentation by 3.9%, and the content of alcohol and carbon dioxide in the finished beer by 3.2% and 10%, respectively.
3. According to physical, chemical and sensory properties, light beer with a content of dried chicory of 4% of the malt was found to be the best. The resulting lager beer was characterized by increased foam resistance and higher foam height, and the addition of chicory bitter substances did not impart excessive and extraneous bitterness to it.
4. An improved method of brewing dark beer involves the production of an aqueous extract of fried chicory and it's mixing with the cooled hopped wort. The optimal conditions for the extraction of water-soluble substances of fried chicory are a temperature of 85–90 °C, a hydraulic module of 1:6, and a time of 90 minutes.
5. According to physical, chemical and sensory properties, dark beer with a content of fried chicory of 3% of the malt was found to be the best. The resulting dark beer was

characterized by a more intense and fresh aroma of rye bread, greater sweetness, and better fullness of flavor and aftertaste.

6. Partial replacement of malt with chicory can reduce the use of bitter hops for light beer by 20% (from 14.8 to 12 g/dal) and for dark beer by 10% (from 10.3 to 9.3 g/dal), thus reducing the cost of lager beer.

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Improving the quality of apple juice by using hydrodynamically activated polymer flocculants in the coagulation process

Volodymyr Pogrebnyak[1], Iryna Perkun[1], Andriy Pogrebnyak[2], Tetiana Nikolaienko-Kamyshova[3], Marharyta Kucher[2], Oleksandr Sabirov[2], Natalia Nebaba[4]

1 – Ivano-Frankivsk National Technical University of Oil and Gas, Ivano-Frankivsk, Ukraine
2 – University of Customs and Finance, Dnipro, Ukraine
3 – Dnipro State Medical University, Dnipro, Ukraine
4 – Oles Honchar Dnipro National University, Dnipro, Ukraine

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Corresponding author:

Volodymyr Pogrebnyak
E-mail: VGPogrebnyak@gmail.com

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Abstract

Introduction. Improving the quality of apple juice by clarifying and purifying it using hydrodynamically activated polymer flocculants has been carried out.

Materials and methods. Apple juices from the following four varieties of apples: Jonathan, Snow Calville, Glory to the Winners, Wealthy. Polyethylene oxide and hydrolyzed polyacrylamide were chosen as the polymeric flocculants. A flocculator was used to activate the flocculant through turbulence in a circular tube that was rolled into a spiral coil without a frame. Evaluation of apple juice transparency was carried out via optical density measurements.

Results and discussions. It was found that treating a mixture of juice and polymeric flocculants polyethylene oxide and polyacrylamide during flow in a circular tube results in increased clarification rate of apple juice and reduced flocculant consumption only in turbulent mode. Experiments on apple juice clarification using activated flocculants demonstrated the high efficacy of the triple injection method.

The optimum concentration of polyethylene oxide was 1.5 and 1.5 mg/l (2 and 3 portions), and bentonite 340 mg/l (1 portion). On the basis of the established effect of turbulence on the flocculation process by varying the molecular concentration characteristics of polyethylene oxide solutions injected into the juice and the methods of their injection, rational parameters of apple juice clarification were obtained. The sensory analysis of apple juices made from different varieties of apples allowed to juice properties (appearance, color, consistency, smell, and taste) and proved the high quality of the products. The determined sensory assessments of the quality of apple juices processed using activated polyethylene oxide on a 5-point scale, taking into account the weighting coefficients of indicators (appearance, color, consistency, smell, and taste) for juices from apples of Glory to the Winners, Jonathan, Wealthy, and Snow Calville varieties were as follows: 4.97, 4.95, 4.93 and 4.93, respectively.

Conclusions. An innovative technological solution for the use of polymeric flocculants has been proposed, consisting in the processing of juice products using hydrodynamically activated polyethylene oxide and hydrolyzed polyacrylamide that has significantly increased the speed and degree of apple juice clarification, as well as improved their quality.

Introduction

A steady upward trend in the production and consumption of fruit and berry juices and other beverages is observed globally. To enable mass production of apple juice, it is necessary to address quality, stability, and safety of the final product. The resolution of this problem is primarily linked to how the apple juice clarification process affects its consumer properties. Fruit and berry juice clarification techniques commonly involve physical methods such as straining, settling, and separation, as well as biochemical methods that utilize enzymes. Treatment with enzyme preparations provides clarification of juices, particularly apple juice, which has a persistent colloidal system. Purified enzyme preparations authorized by health authorities for use in juice production are used for juice clarification: pectofoetidine, amylorisin, and glucavamorin. These methods are based on the process of adsorption of colloidal particles by the surface of clarifying agents: gelatin or a combination of tannin and gelatin, as well as the addition of solutions of colloids with opposite charge to the juice (Diblan et al., 2021; Heshmati et al., 2020; Polidori et al., 2018; Ricci et al., 2021). If physical and biochemical methods for enhancing the quality of apple juice through the clarification process have been adequately investigated (Abdullah et al., 2023; Urošević et al., 2017), further research is still required for physical and chemical methods.

The process of separating the colloidal system of apple juice into sediment and clear juice is called clarification when using the flocculation method. Juices immediately after receiving them can be clarified using organic and inorganic substances such as bentonite, flocculants like gelatin, polyvinylpyrrolidone, polyacrylamide, or polyethylene oxide (Kawaguchi and Hasegawa, 2014). Methods for clarifying apple juice in practice have both advantages and disadvantages. Advantages include the effective removal of colloidal compounds present in apple juice and low reagent costs. However, these methods have limitations on their use and can be costly. These findings have been supported by studies conducted by Aluko et al. (2023), Sachko et al. (2020) and Wongmaneepratip et al. (2023). All of these factors are necessary to find more efficient clarification agents that can meet technological and economical standards, while also satisfying production requirements for enhancing the transparency, stability, and safety of juices. They can also aid in intensifying the clarification process. Polymeric flocculants are extensively used for the purification of drinking water (Way and McLellan, 2012), the concentration of cell suspensions in biotechnology (Marbelia et al., 2016; Nones et al., 2015), and the treatment of wine materials and wines (Dordoni et al., 2015; Ghanem et al., 2017; Ren et al., 2020; Romanini et al., 2020).

Polymeric flocculants are effective reagents that can purify liquids from heavy metals. The ability of polymeric flocculants to precipitate heavy metals from the liquid can be used for deep purification of apple juice from heavy metals, i.e. to improve its safety (Pogrebnyak et al., 2022).

The main characteristics of flocculants that significantly affect the intensity of flocculation are their molecular weight, the flexibility of the polymer chain, the quality of the solvent and their concentration in the solution. As the molecular weight of the flocculant increases, its flocculating effect generally increases, too (Kawaguchi and Hasegawa, 2014; Pogrebnyak et al., 2022). This allows for a reduction in the value of optimal concentration of the flocculant needed to clarify the liquid. The increase in effect is due to large macromolecules being able to bind more particles in a floccule by using polymeric bridges between the particles. Calculations demonstrate that a doubling in macromolecule size leads to a significant increase in flocculation intensity, potentially by one or two orders of magnitude. This suggests that the flocculating effect of similarly-weighted macromolecules is reliant on the size of the macromolecular surface area, or its conformation, which is

influenced by chain flexibility. Chain flexibility can be altered by temperature, solvents, and the influence of a hydrodynamic field on a macromolecular coil (Latinwo et al., 2014).

The impact of a longitudinal hydrodynamic field on the flocculating behavior of macromolecules is simplified to the fundamental principle. The degree of elongation (or folding) of a flexible macromolecule can be characterized by the β parameter, which is equal to the ratio of the distance between the ends of the macromolecule h to its contour length l. From the standpoint of thermodynamics and physical kinetics, parameter β is more fundamental than the Flory chain flexibility parameter f: the fact is, that upon reaching a certain critical value of β_{cr}, the theory of dissipative structures and Prigogine's bifurcation come into play. Furthermore, the means by which β_{cr} is attained is insignificant as even a solitary macromolecule experiences diminished stability due to the presence of rotational isomers, which cause it to align (Pogrebnyak A. et al., 2022, 2020).

The foregoing allows us to state that under the influence of a tensile hydrodynamic field, it is possible to enhance the ability of macromolecules to flocculate without altering the molecular weight of polymer flocculant. This enhancement results in increased flocculation intensity and reducing the value of optimal concentration. The foregoing was decisive in order to propose innovative a method and device for hydrodynamic influence on the flocculating ability of macromolecules (Pat. 57600 Ukraine (2011). Clarification method for food liquids with polymeric flocculants; Pat. 51689 Ukraine, (2010). Adevice for clarifying liquid food products using flocculants).

Therefore, the consumable properties of apple juice during the clarification process can be improved by utilizing flexible-chain water-soluble polymers, which demonstrate an increase in flocculation action when exposed to a tensile hydrodynamic field (Pogrebnyak A. et al., 2022).

The aim of the present study – on the basis of experimental studies to establish rational parameters of flocculation process of clarification of apple juice, which provide a significant increase in the speed and degree of juice clarification, as well as an increase in its quality.

Materials and methods

Materials

The experiment conducted in this study examines the freshly obtained apple juices from four different apple varieties grown in ecologically safe regions of Ukraine (Kherson region, v. Stanislav): Jonathan (winter variety), Snow Calville (early winter variety), Glory to the Winners (autumn variety), and Wealthy (winter variety). The apples used for this study were harvested at their peak maturity. Apples were stored in a chamber at a temperature of 18°C for up to 5 days. Juice clarified with bentonite at a concentration of 340 mg/L was used as control juice samples. Juices clarified with flocculants without hydrodynamic influence were also used as control juice samples. Polymeric flocculants chosen include polyethylene oxide with molecular weights of $3 \cdot 10^6$, $4 \cdot 10^6$, and $6 \cdot 10^6$ (POLYOX WSR), hydrolyzed polyacrylamide (HPA) with a molecular weight of $4.5 \cdot 10^6$ and 5% degree of hydrolysis (Stokopol), and polyacrylamide with a molecular weight of 10^7 and 14% degree of hydrolysis (Praestol). The concentration of the flocculant in apple juice was chosen such that the inequality was fulfilled $[\eta]_0 \cdot C > 0.8$ (where $[\eta]_0$ is the intrinsic viscosity; C is a concentration of the flocculant).

Expererimental research methods

Definition of transparency and color. The apple juice's transparency was assessed using the photocolorimetric method at λ_{max}= 540 nm. Optical density was used to determine this. Before the optical density measurement, the juice was diluted with a water ratio of 1:10. The color was measured using a blue light filter (λ_{max}= 410 nm.) via optical density evaluation. The process of flocculation in apple juice by polymers was studied in cylindrical glass settling tanks, where clarified apple juice goes after hydrodynamic activation of the polymer in the flocculator.

The effect of the hydrodynamic field on the flocculating ability of macromolecules was estimated as

$$F = \left(\frac{n_{c0} - n_{c\dot{\varepsilon}}}{n_{c0}} \right) \cdot 100\ \%, \qquad (1)$$

where n_{c0} and $n_{c\varepsilon}$ – optical densities of apple juice with flocculant without exposure to hydrodynamic field and after hydrodynamic activation, respectively ($n_{c0} = n_{c\varepsilon}$ as the longitudinal velocity gradient ε tends to zero).

Flocculation with polymers in apple juice (without hydrodynamic influence) was studied in measuring glass cylinders with a volume of 200 ml. The cylinder was filled with apple juice up to the mark of 200 ml, then the required amount of the flocculant solution was injected, the cylinder was closed, and it was overturned five times with an interval of about 2 s. After the fifth tipping, the stopwatch was turned on and the time was measured for which the boundary separating the clarified apple juice and the precipitating flocculi reached the mark of 140 ml. By the time of the front movement and the distance between the marks, equal to l=51·10^{-3} m, the average clarification velocity of apple juice was found. The clarification rate of apple juice was also determined by the change in the optical density of the juice over time (with an interval of 10 min).

Experimental bench for clarification of apple juice. The experiments were conducted using a basic yet highly efficient flocculator that activated flocculants through turbulence within a circular tube, depicted in Figure 1.

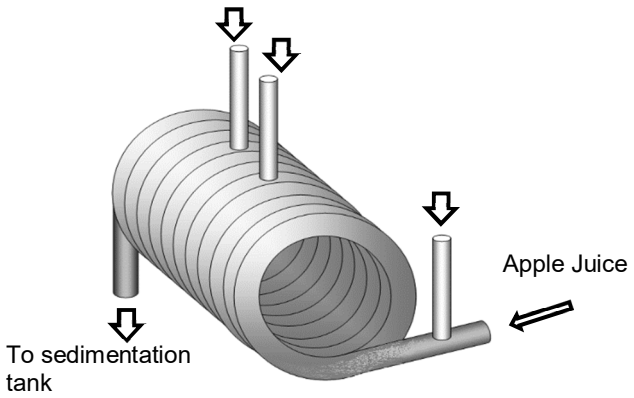


Figure 1. Tube flocculator with turbulence activation of flocculant in apple juice:
1 – inlet for bentonite (1st portion);
2 and 3 – inlets for polyethylene oxide (2nd and 3rd portions).

The essence of its operation is as follows. The clarification process involves pumping a precisely measured amount of flocculant together with apple juice, which is clarified, through a smooth pipe that is coiled in a frameless spiral configuration with one or more layers of winding prior to entering the sedimentation tank. By varying the flow rate and the length (number of turns) of the flocculator pipe, the processing time of the apple juice can be modified. The study determined the number of turns and diameter of the spiral, along with flow velocity of apple juice (based on Reynolds number) via empirical means. The assessment of the tubular flocculator (with consistent channel diameter) showed inferior efficiency compared to the flocculator assembled on coaxial cylinders (Pogrebnyak et al., 2022). However, its cost and maintenance expenses are significantly lower. In order to achieve the required temperatures for the juice, utilized thermostabilization system (Pashchenko et al., 2021; Pogrebnyak et al., 2021). The temperature stabilization was maintained at the specified level with precision up to 0.1 °C.

Evaluation of the quality of clarified juice

A comprehensive evaluation of the quality of apple juice that underwent clarification with hydrodynamically activated flocculants was conducted using the principle of qualimetry. Comprehensive evaluation of juice quality included several stages: determining the quality indicators, by which the quality of products was evaluated; ranking the quality indicators (appearance, color, consistency, smell, taste); evaluating the juice quality by selected indicators; calculating the indexes of improvement of properties; evaluating the juice quality by selected indicators, taking into account the weighting coefficients. In a comprehensive assessment of the quality of juices clarified by turbulence-activated polyethylene oxide, the content of vitamins was not taken into account, because their change in the composition of the evaluated samples in comparison with control samples is insignificant (Pogrebnyak et al., 2022).

Statistical analysis

The statistical data were processed using the small sample method. The values are reported as the mean ± standard deviation of quadruplicate or quintuplicate samples.

Results and discussion

The data presented in Table 1 provide evidence for enhancing the apple juice clarification process using polyethylene oxide. By processing the mixture of juice and flocculant – polyethylene oxide in a circular tube, there is a notable increase in the speed l/t (where $l = 51 \cdot 10^{-3}$ m, t is the settling time of flocculi on a given section of l) resulting in improvement in apple juice clarification and a decrease in the amount of polyethylene oxide consumption, exclusively in turbulent mode. There is a limiting value of Reynolds number – 7000, beyond which the effectiveness of the proposed method for increasing polyethylene oxide flocculation ability decreases. Experiments utilizing polyethylene oxide of molecular weight $4 \cdot 10^6$ were conducted with concentrations of 0.0002% and 0.0004% in apple juice.

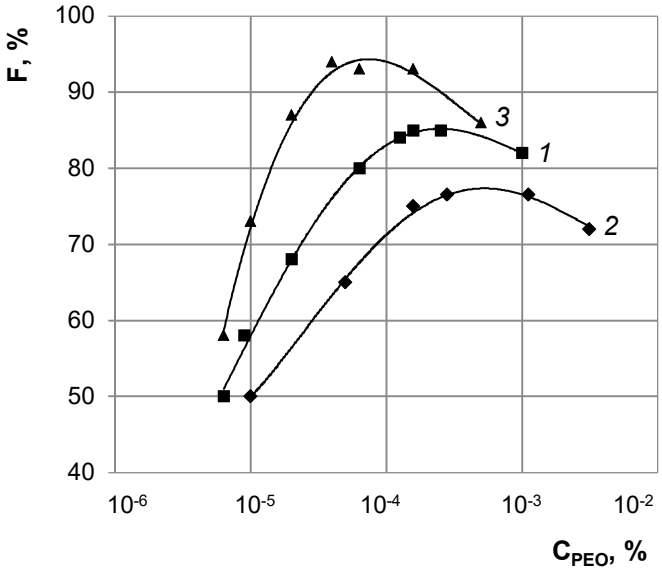
Table 1

Effect of flocculant- polyethylene oxide (PEO) concentration and Reynolds number on clarification rate of juice from Jonathan apples

PEO concentration in juice-PEO mixture, mg/L	Juice-PEO Re=0; $l \cdot 10^{-4}/t \cdot 60$ s	Juice-PEO in flow with Re=2000; $l \cdot 10^{-4}$ m/$t \cdot 60$ s	Juice-PEO in flow with Re=4000; $l \cdot 10^{-4}$ m/$t \cdot 60$ s	Juice-PEO in flow with Re=7000; $l \cdot 10^{-4}$ m/$t \cdot$ 60 s	Juice-PEO in flow with Re=10000; $l \cdot 10^{-4}/t \cdot 60$ s
2	10	10	35	50	20
4	20	20	60	70	50

The effect of polyethylene oxide concentration with molecular weights $3 \cdot 10^6$ and $6 \cdot 10^6$ on apple juice flocculation at 20 °C is illustrated in Figure 2. It is apparent that the dependence of the flocculation effect on concentration reaches a maximum at C_{PEO} = 20 mg/L and 10 mg/L concentrations, respectively, conforming to the polyethylene oxide molecular weights analyzed. Afterward, the effect magnitude decreases. Increasing the concentration of flocculant in apple juice leads to a higher viscosity of the juice- polyethylene oxide system. This is caused by emerging intermolecular interactions of polyethylene oxide molecules (Almásy et al., 2022; Dimitrova et al., (2002); Yang et al., 2005). Because of this, it hinders the settling of the formed flocculates, ultimately reducing the clarification rate, or the flocculation effect.

The data presented in Figure 2 (curve 3) and Tables 1 and 2 show that the effect of turbulent hydrodynamic field on the juice- polyethylene oxide and juice- polyacrylamide systems increases the flocculation effect. This is reflected in the optimum flocculant concentration, which decreases and the magnitude of the effect itself, which increases. If Reynolds is increased above $7 \cdot 10^3$, the clarification rate of apple juice begins to decrease.



M_{PEO}: 1 and 3 – $6 \cdot 10^6$, 2 – $3 \cdot 10^6$; 1 and 2 – Re < 2000; 3 – Re = 7000

Figure 2. The relationship between the efficiency of polyethylene oxide (PEO) flocculation and its concentration in apple juice from Jonathan variety apples

Table 2

Optimal concentrations of polyethylene oxide (PEO) and hydrolyzed polyacrylamide (HPA) for the flocculant-apple juice system with and without hydrodynamic treatment (HT) for f apple juice from Jonathan variety

M_{PEO}	C_{opt}, mg/L					
	Without HT	With HT, Re = 7000	M_{GPAA}	Hydrolysis rate, %	Without HT	With HT, Re = 7000
$6 \cdot 10^6$	10	4.5	10^7	14	0,5	0.35
$4 \cdot 10^6$	15	6.5				
$3 \cdot 10^6$	20	10	$4.5 \cdot 10^6$	5	1	0.75

Analysis of the experimental data reveals the expressions that demonstrate the correlation between molecular weight and optimal flocculant concentration. These expressions are obtained for cases where there is no hydrodynamic field impact on the apple juice- polyethylene oxide system (1) and with turbulent hydrodynamic field impact (2) at a Reynolds number of 7000.

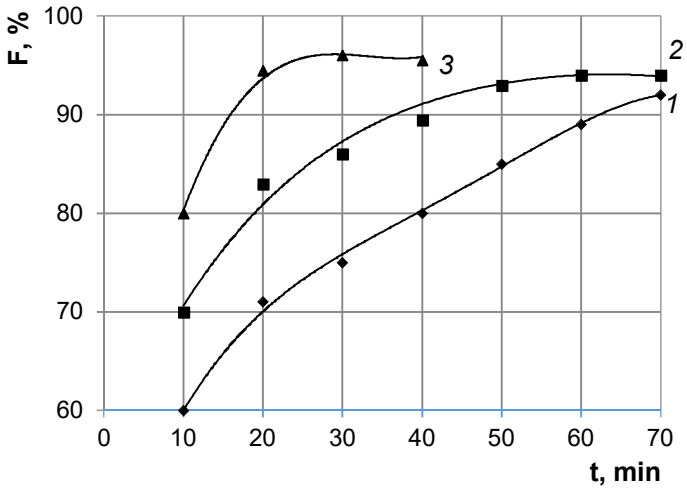
$$\frac{C_{opt} M_{PEO}}{100\%} = 60 \quad (1); \qquad \frac{C_{opt} M_{PEO}}{100\%} = 26. \quad (2)$$

Consequently, the experiments support the predicted phenomenon described (Pat. 57600, Ukraine, 2011). Clarification method for food liquids with polymeric flocculants), in which macromolecules gain increased flocculation ability when exposed to a turbulent hydrodynamic field. This increase is due to the macromolecular tangles' ability to alter their conformational state under tensile flow conditions at subcritical regimes, potentially resulting in complete unfolding (Latinwo et al., 2014).

This finding is further supported by studies from Pogrebnyak et al. (2019) and Pogrebnyak et al. (1991). The distribution of the strain factor, as obtained in the study conducted by Pogrebnyak et al. (2019) under conditions of tensile flow, indicates that macromolecules could transition to a highly unfolded state. The study of polyethylene oxide solutions showed that the ratio of the measured birefringence Δn to the maximum possible Δn_∞ when exposed to a tensile hydrodynamic field on macromolecules under model conditions of near-wall turbulence reaches 0.35-0.46, which corresponds to ~60-70% of the uncoiling degree of polymer chain.

To ensure a presentable appearance, apple juice should possess a maximum degree of clarification. However, the duration of the clarification process also plays a significant role in shaping the consumer properties. This is because a swift reduction in clarification time can halt many enzymatic reactions and oxidation processes (Alongi et al., 2019; Nehmé et al., 2019; Tong et al., 2022). Excluding or significantly reducing such reactions results in higher preservation of the valuable properties of apple juice, including organoleptic parameters, physical and chemical composition. Consequently, our study investigated the time-dependent change in transparency of the apple juice- polyethylene oxide system at the optimal flocculant concentration.

Figure 3 displays the results depicting the progression of flocculation effect in the apple juice- polyethylene oxide system over time at 20 °C, while Figures 4 and 5 illustrate the efficiency of clarifying apple juice based on the flocculant concentration at varying modes of turbulent impact on the juice- polyethylene oxide system. These figures pertain to apple juice extracted from the Jonathan apple variety.



$M_{PEO} = 6 \cdot 10^6$; 1 – without HT; 2 – with HT; 3 – with HT + 340 mg/L bentonite

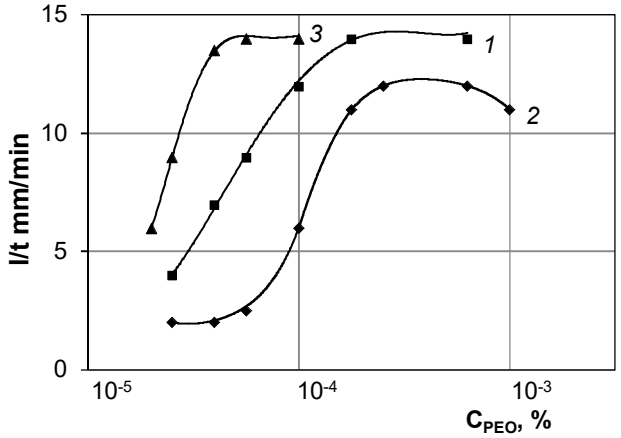
Figure 3. Variation of clarification degree of apple juice- polyethylene oxide (PEO) system at optimum flocculant concentration over time

The process of flocculation in apple juice using hydrodynamically activated flocculants was studied. Cylindrical glass sedimentation tanks were used, and clarified apple juice was delivered after polyethylene oxide was hydrodynamically activated in the flocculator. The transparency of the apple juice was measured at 10-minute intervals.

Figures 3, 4, and 5 demonstrate that using a turbulent hydrodynamic field to process the juice- polyethylene oxide system enhances the clarification rate of apple juice and reduces polyethylene oxide consumption, but only in turbulent mode. Table 1 and Figure 5 indicate that the flocculation efficiency of polyethylene oxide macromolecules decreases when the Reynolds number exceeds 7000, which sets a limit. The slight hydrodynamic impact unfolds macromolecular tangles, increasing macromolecule-particle contacts, leading to larger floccules. Conversely, intense hydrodynamic action worsens flocculation by destroying flocculi. Hydrodynamic activation of polyethylene oxide via turbulent flow allows for a reduced optimal concentration of 4 mg/L or lower, leading to apple juice with up to 85% transparency. The settling time for the juice was less than one hour.

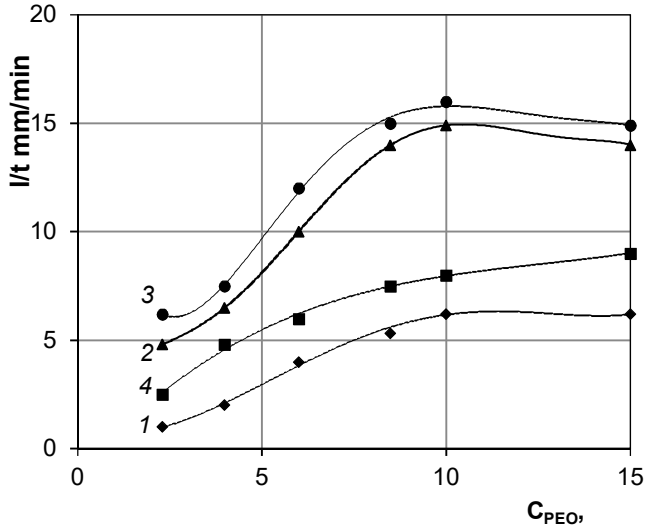
Flocculants Praestol and Stokopol were found to have a higher flocculating effect on apple juice compared to other flocculants, resulting in greater transparency. Transparency measured up to 93% with Praestol and 86% with Shtokopol. In addition, it was observed that the hydrodynamic activation of polyacrylamide was much less compared to polyethylene oxide, likely due to homonymous charges on polyacrylamide chains, which led to an increase in macromolecular clubs without the influence of a hydrodynamic field Qin et al., 2024).

Experiments utilizing activated flocculants to clarify apple juice demonstrated the triple injection method's efficiency, depicted in Figure 1. The best polyethylene oxide concentration was (1.5+1.5) mg/L (with bentonite concentration at 340 mg/L), leading to apple juice transparency of 95% (curve 3, Figure 2) at Reynolds $7 \cdot 10^3$ under turbulent flow from Jonathan apples. A ~5-minute interval elapsed between reagent portions (injection). The utilization of polyethylene oxide flocculant and bentonite in the combined clarification process of apple juice in turbulent flow resulted in a noteworthy reduction of sediment exposure time (3-5 times), leading to decreased production costs.



M_{PEO}: 1 – $6 \cdot 10^6$ (without HT); 2 – $3 \cdot 10^6$ (without HT); 3 – $6 \cdot 10^6$ (with HT)

Figure 4. The relationship between the clarification rate of apple juice from Jonathan variety apples and polyethylene oxide (PEO) concentration



$M_{PEO} = 4 \cdot 10^6$; Re: 1 – 0; 2 – $4 \cdot 10^3$; 3 – $7 \cdot 10^3$; 4 – 10^4

Figure 5. Impact of polyethylene oxide (PEO) concentration on the clarity of apple juice from Jonathan apples treated hydrodynamically at varying modes

The results in Figure 6 reveal the impact of polyacrylamide's molecular weight, concentration, and degree of hydrolysis on apple juice flocculation at 20 °C.

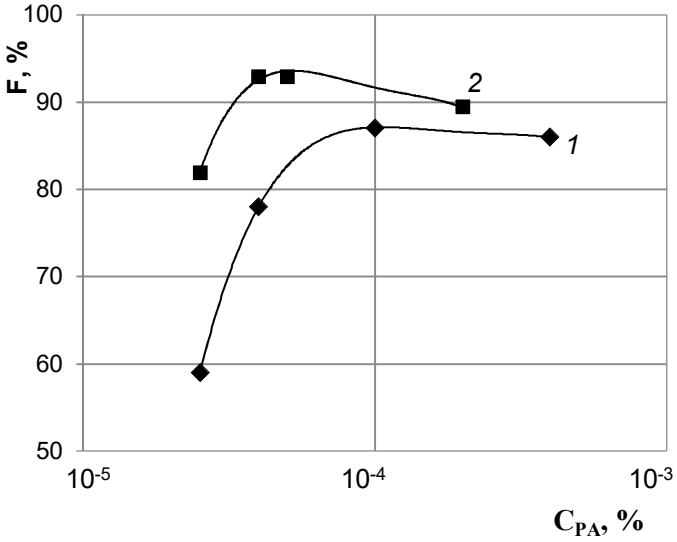


Figure 6. Dependence of flocculation efficiency of Praestol and Stokopol on concentrations of polyacrylamide in Jonathan apple juice
1 – Stokopol; 2 – Praestol

The substantial enhancement in the efficiency of clarifying apple juice via the combined utilization of bentonite and activated polyethylene oxide with turbulence is due to the rapid coagulation of positively-charged colloidal particles immediately following the introduction of bentonite. The particles then amalgamate into primary flocs directly after the initial dose of polyethylene oxide, which are then further enlarged into secondary flocs by the flocculant's macromolecules introduced with the second dose. The secondary flocs are larger than the primary flocs, enabling them to settle much more quickly (Kawaguchi and Hasegawa, 2014).

The last step of juice clarification involves filtering out aggregatively stabilized fine colloidal substances and residual turbidity. Obtaining a denser sludge will decrease the stress on filters and extend their service time. Our experiments showed a clear correlation between the effectiveness of polymer flocculation and sludge density. The density of the sludge was determined by measuring its volume in settling beakers intended for apple juice. A rise in the molecular weight of flocculant while under hydrodynamic influence in the apple juice-polyethylene oxide system (at $Re=7\cdot10^3$) results in a reduction of the optimal polyethylene oxide concentration in the juice, and an increase in sludge density. This outcome gives rise to the expected possibility of extending the life span of devices used for filtering apple juice.

To gain a better understanding of the potential application of hydrodynamically activated flocculants in apple juice production, a dual approach of technological and commodity science was used. The technological aspect encompasses not only the acceleration of the juice clarification process, but also an increase in juice clarification efficiency. This fosters another aspect of the study – commodity science – which centers on enhancing consumer properties and promoting juice stability.

The results of the sensory and qualimetric analysis of apple juice, clarified using activated flocculants, as well as toxicological studies, demonstrate that the use of flocculants leads to an enhancement in the transparency and color of apple juice (Table 3).

The sensory analysis of apple juices made from different varieties of apples allowed to evaluate consumer properties (appearance, color, consistency, smell, and taste) and proved the high quality of the products. The determined organoleptic assessments of the quality of apple juices processed using activated polyethylene oxide on a 5-point scale, taking into account the weighting coefficients of indicators (appearance, color, consistency, smell, taste) for juices from apples of Glory to the Winners, Jonathan, Wealthy and Snow Calville varieties were as follows: 4.97, 4.95, 4.93 and 4.93, respectively.

Table 3

Transparency and color measurements of apple juice (from Welsey apples) with varying concentrations of polyethylene oxide (PEO) molecular weight $3 \cdot 10^6$

Indicator	Control $C_{PEO}=0$, mg/L	C_{PEO}, mg/L			
		4	6	8	15
Transparency, %	41.1	75.2	85.0	86.8	84.6
Color, optical density units	0.611	0.278	0.125	0.071	0.125

The evaluation of apple juices treated with activated polyethylene oxide and Praestol for toxicological indicators (Pogrebnyak A. et al., 2022) suggests that such juices meet the standard requirements. A thorough commodity assessment of apple juice clarified with hydrodynamically activated flocculants under rational flocculation parameters in a turbulent flow indicates at least a 10% increase in the quality indicator value compared to juice treated with optimal concentration bentonite.

New regularities in the process of clarifying apple juice using hydrodynamic-activated flocculants, specifically polyethylene oxide and hydrolyzed polyacrylamide, have been identified. With this understanding, it is now possible to scientifically control the process to optimize the consumer properties of the clarified juice.

Conclusions

1. A phenomenon has been experimentally confirmed, which consists in an increase in the flocculating ability of polyethylene oxide and hydrolyzed polyacrylamide macromolecules under conditions of turbulent flow of apple juice. The nature of this phenomenon is present in the strong deforming effect of turbulence on the macromolecular coils of the flocculant under the action of longitudinal velocity gradients, and this, in turn, leads to an increase in the flocculating efficiency due to an increase in the degree of unfolding (elongation) of the macromolecular chains of the flocculant.
2. Based on the established impact of turbulence on the flocculation process when considering variations in molecular-concentration characteristics of polyethylene oxide solutions that are injected into juice along with the methods of injection, reasonable parameters (Reynolds number, flocculant concentration and its molecular weight, input methods) for apple juice clarification have been derived. By adhering to these parameters, the clarification process can be accelerated by over fivefold and the value

of the optimal concentration of flocculant can be decreased by 700 times or more in comparison to bentonite clarification methods.

3. On the basis of conducted research the possibility of using hydrodynamically activated polymeric flocculants for clarification of apple juices is shown. This method involves the treatment of juice products with hydrodynamically activated flocculants such as polyethylene oxide, Praestol and Shtokopol. This method significantly increases the speed and degree of clarification of apple juices, while improving their quality. The promise of flocculants lies in their ability to improve their properties under the influence of turbulence and at the same time ensure high quality of juices.

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Effects of the addition of xanthan gum and rice flour to maize starch on quality of gluten-free biscuit

Marko Jukić[1], Daliborka Koceva Komlenić[1], Gjore Nakov[2], Matea Begić[1], Sandi Keresturi[1], Jasmina Lukinac[1]

1 – Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Croatia
2 – College of Sliven, Technical University of Sofia, Sliven, Bulgaria

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Corresponding author:

Marko Jukić
E-mail:
marko.jukic@ptfos.hr

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Abstract

Introduction. The aim of this research was to study the interaction of xanthan gum and a mixture of rice flour and corn starch, its effects on the quality of gluten-free cookies, as well as optimization of the gluten-free cookie recipe to produce high-quality products.

Materials and methods. The effects of addition of rice flour to maize starch in ratio 6:4, 8:2 and 10:0 and the xanthan gum in amounts of 0, 2 and 4% were analysed. Texture parameters (breaking force), dimensions (spread factor), colour (browning index), water activity and total sensory score were determined as response variables.

Results and discussion. An increase in xanthan concentration led to an increase in hardness, browning index and water activity while reducing the spread factor of the gluten-free biscuits. Increased amounts of maize starch in the gluten-free mixture contributed in particular to a lower hardness and a lower browning index of the gluten-free biscuits, while at the same time the spreading of the biscuits increased.

An increase in the addition of xanthan gum led to an increase in hardness, browning index and water activity as well as a reduction in the spread factor of gluten-free biscuits. Meanwhile, increasing the amount of maize starch in the gluten-free mixture in combination with rice flour contributed significantly to a reduction in the hardness and browning index of gluten-free biscuits, while at the same time increasing the spread of the biscuits. The curvilinear effects of the addition of xanthan gum and maize starch on the sensory score showed that the optimal amounts were between the minimum and maximum addition values in the experiment.

Conclusions. The optimal conditions to obtain gluten-free biscuits of high quality with total sensory score 7.7 were as follows: ratio of rice flour to maize starch 88.9:11.1 in combination with an addition of 1.1% xanthan gum.

Introduction

It is believed that wheat grain can be an allergen and, according to statistics, about 1% of the world population is gluten sensitive (Wang et al., 2017). The rising prevalence of gluten-related disorders, including coeliac disease and non-celiac gluten sensitivity, has led to an increasing demand for gluten-free products worldwide (Ivanov et al., 2021; Stabnikova et al 2021). To produce gluten-free bakery products, partial replacement of wheat flour with gluten-free cereal flours, such as rise (Sciarini et al., 2010), soy (Taghdir et al., 2017), and sorghum (Marston et al., 2016) flours has been proposed. Among gluten-free bakery products, gluten-free biscuits are a popular choice due to their versatility and palatability (Di Cariano et al., 2018).

However, unlike traditional wheat flour biscuits, gluten-free biscuits require careful consideration of alternative ingredients to achieve the desired texture, flavour, and appearance. Rice flour, maize starch, and xanthan gum are often used in gluten-free baking recipes due to their functional properties (Di Cariano et al., 2018). Wheat biscuits made from short dough with high fat and sugar content usually rely on the gelatinisation of starch rather than the development of gluten. Therefore, the use of gluten-free flours offers the potential to produce gluten-free biscuits with customised texture and spread (Mancebo et al., 2015a; Xu et al., 2020).

Rice flour, a main ingredient in many gluten-free recipes, serves as a corn base due to its unique properties. Rice flour has a neutral flavour and light texture, making it an ideal substitute for traditional wheat flour in gluten-free baking (Mancebo et al., 2015b). Its widespread use is due to its versatility and its ability to mimic the texture and structure of gluten-containing products, ensuring a satisfying sensory experience for people with gluten-related disorders (Benkadri et al., 2018).

Maize starch, another key component in gluten-free formulations, plays a crucial role in improving the structural integrity and mouthfeel of baked goods. Maize starch acts as a thickening agent and adds a smooth and desirable texture to various foods. In gluten-free baking, maize starch contributes to the viscosity of the dough, facilitating proper handling and shaping of the dough while improving the overall palatability of the final product (Pérez-Carrillo et al., 2019).

Xanthan gum, a microbial high molecular weight exopolysaccharide synthesised by *Xanthomonas campestris* strains, serves as a multifunctional additive in gluten-free baking. Xanthan gum is known for its unique thickening and stabilising properties. It increases the viscosity and elasticity of dough, improving their handling and contributing to the desired texture of baked goods. In addition, xanthan gum helps to prevent ingredient separation and syneresis, resulting in products with improved storage stability and longer freshness (Benkadri et al., 2020).

The combined use of rice flour, maize starch and xanthan gum in gluten-free formulations enables the production of products that are very similar to their gluten-containing counterparts in terms of texture, flavour and appearance. By carefully adjusting the ratio of these ingredients, it is possible to achieve optimal results by balancing factors such as texture, structural integrity, and sensory properties to meet consumer expectations.

Overall, the use of rice flour, maize starch and xanthan gum represents a fundamental approach to gluten-free formulation allowing the development of high-quality, palatable products. Through continuous research and innovation, the gluten-free industry is able to offer a wide range of products that meet the nutritional needs and culinary preferences of consumers worldwide. Therefore, recipe optimisation is crucial for the production of gluten-free biscuits that meet consumer expectations and preferences (Lisovska et al., 2020).

The aim of this research was to study the interaction of xanthan gum and a mixture of rice flour and corn starch, its effects on the quality of gluten-free cookies, as well as optimization of the gluten-free cookie recipe to produce high-quality products.

Materials and methods

Materials

The following raw materials were used in this study: rice flour with 6.5% protein content (Galleria Internazionale Ltd., Zagreb, Croatia); maize starch (Dr. August Oetker KG, Bielefeld, Germany); xanthan gum powder (Doves Farm Foods Ltd., Berkshire, UK); shortening (Zvijezda plus Ltd., Zagreb, Croatia); granulated sugar (sucrose); table salt (NaCl), and sodium bicarbonate ($NaHCO_3$).

Methods

Design of experiment

The experimental design was based on the Response Surface Methodology and was created using the statistical software Statistica (ver. 14.0.0.15, TIBCO Software Inc., Palo Alto, USA) in a three-level full factorial design (3^2). The independent variables included the ratio of rice flour to maize starch in the GLUTEN-FREE flour blend and the amount of xanthan gum added. The experimental design included nine experiments with three replicates of the central point (Table 1). The actual values were set at 60:0, 80:20 and 100:0 for the rice flour to maize starch ratio and at 0, 2 and 4% of the xanthan gum addition.

Production of gluten-free biscuits

The biscuits were prepared according to the experimental design (Table 1).

Table 1

Design of experiment

N	Ratio: Rice flour : Maize starch	Xanthan gum,%
1	60	0
2	60	2
3	60	4
4	80	0
5	80	2
6	80	4
7	100	0
8	100	2
9	100	4

After weighing all ingredients (expressed in baker's percentages, where the flour mixture consisted of rice flour and maize starch), shortening, 40%; sucrose, 42%; salt, 1.25%, and sodium bicarbonate, 1.1%, were mixed for 3 minutes at low speed using an electronic

mixer (Gorenje MMC800W, Velenje, Slovenia). After the addition of water, 24%, mixing was continued at low speed for another minute, followed by another minute at medium speed.

The gluten-free flour mixture (100%) was then added and mixed at low speed for 2 minutes. The resulting dough was then rounded and refrigerated at 8 °C for 30 minutes (covered with plastic wrap). The dough was then rolled out to a thickness of 7 mm and pieces of dough with a diameter of 60 mm were cut out using a cylindrical mould. Baking took place in a convection oven (Wiesheu Minimat Zibo, Wiesheu GmbH, Großbottwar, Germany) at 205 °C for 12 minutes. The biscuits were baked in double batches. After a cooling time of one hour, the gluten-free biscuits were analysed.

Physical analysis

The dimensions of the gluten-free biscuits were assessed according to the International Method 10-50.05 protocol (Cereals and Grains Association, 2010). First, the total width of six biscuits stacked side by side was measured, then each biscuit was rotated 90° and measured again. The average width was calculated and divided by six to determine W, cm. Six biscuits were then stacked vertically and their combined height was measured. After the biscuits were randomly rearranged, the height was measured again. The average height of the six biscuits was divided by six to obtain T, cm. The spread factor was then calculated as W/T multiplied by ten. Measurements were taken on six sample biscuits from each batch.

The texture of the gluten-free biscuits was assessed using the TA.XTplus Texture Analyser (Stable Microsystems Ltd., Surrey, UK) by the three-point bend test. The knife blade moved towards the biscuit, which was positioned between two lower supports 30 mm apart. The test was performed at a blade speed of 1 mm/s until the breaking point was reached. The peak force (N) was recorded, giving an indication of the softness/hardness of the biscuit. The evaluation was carried out on three biscuits from each batch.

The water activity (aw) in the ground samples of the gluten-free biscuits was determined using the Hygropalm AW1 device (Rotronic AG, Bassersdorf, Switzerland).

Color evaluation

The colour of the surface of the gluten-free biscuits was assessed using the CR-400 Chromameter (Konica Minolta, Japan). The CIELab colour model was used, in which the L^* value (ranging from 0 to 100) represents the lightness or luminance, the b^* value (ranging from -128 to 127) represents the blue-yellow axis and the a^* value (ranging from -128 to 127) represents the green-red axis within the colour space. The CIELab values were used to calculate the browning index (BI) according to the following equations (Dadalı et al., 2007):

$$BI = \frac{\left(100\left(x - 0.31\right)\right)}{0.17}$$

where:

$$x = \frac{\left(a + 1.75L\right)}{\left(5.645L + a - 3.012b\right)}$$

Sensory analysis

The sensory analysis of gluten-free biscuits was carried out by a panel of fifteen trained persons (students and employees of the Faculty of Food Technology in Osijek, Croatia). Before starting the analysis, all panel members gave informed consent in accordance with the European Union guidelines for ethics and food-related research (Alfonsi et al., 2012). The sensory panel consisted of nine women and six men, with a median age of 24 years. Inclusion criteria for panel members included the absence of health conditions that could affect sensory evaluation (such as anosmia or colour blindness) and a typical preference for eating similar types of biscuits. The sensory evaluations were carried out in a dedicated tasting room equipped with individual test booths. Participants were given a brief introduction to familiarise them with the study and the samples to be evaluated. All gluten-free biscuit samples were presented to the tasters simultaneously and they were instructed to rinse their mouths with water between tastings. Sensory properties were rated using a 9-point hedonic scale, with scores ranging from 1 to 9 as follows: extremely dislike (1), very much dislike (2), moderately dislike (3), slightly dislike (4), neither dislike nor like (5), slightly like (6), moderately like (7), very much like (8), and extremely like (9). The sensory properties assessed were the external appearance, texture, flavour and taste. To optimise the recipe, the total sensory score was used as one of the response variables, which was determined by calculating the average of the ratings of the above-mentioned sensory properties.

Modelling the data and optimising the gluten-free biscuit recipe

The optimisation of the gluten-free biscuit recipe was carried out using Response Surface Methodology, with texture parameters (breaking force), dimensions (spread factor), colour (browning index), water activity and total sensory score as response variables. Regression analysis was used to determine the relationships between the independent input variables and each dependent output variable defined by the second-degree polynomial response surface model.

The analysis of variance (ANOVA) determined the statistical significance of each regression coefficient and the mathematical models were evaluated using the coefficient of determination (R^2). On the basis of the mathematical models obtained, response surfaces were created, which represent 3D diagrams that visually illustrate how changes in the ratio of rice flour to maize starch and the proportion of xanthan gum affect various quality parameters of gluten-free biscuits.

In the final step, the optimum ratio of rice flour to maize starch and the optimum proportion of xanthan gum were determined using the desirability function. The optimisation procedure involved converting all monitored responses into individual desirability functions with values between 0 and 1, with the overall desirability function determined as their geometric mean. The responses were maximised (browning index and total sensory score), minimised (water activity) or set to a target value (peak force at 50 N and spread factor at 54.3). The optimisation of the gluten-free biscuit formulation was performed using the statistical software Statistica (ver. 14.0.0.15, TIBCO Software Inc., Palo Alto, USA).

Results and discussion

In the field of gluten-free baking, achieving the desired texture, appearance and overall quality of products is a major challenge, especially due to the absence of gluten, an important

structural component in conventional baked goods. Therefore, researchers and food technologists are constantly exploring different ingredients and recipes to develop high-quality gluten-free products that meet consumer expectations. Rice flour and maize starch are commonly used ingredients in gluten-free baking, while xanthan gum is an important additive that mimics the functional properties of gluten. In order to optimise the recipes of gluten-free biscuits, it is important to understand how these ingredients interact and influence the final product. In this context, the present study investigated the influence of different ratios of rice flour to maize starch as well as different levels of xanthan gum on the quality of gluten-free biscuits. According to the experimental design (Table 2), baking tests were carried out and the quality of the gluten-free biscuit samples was examined in order to optimise the recipe with regard to the ratio of rice flour to maize starch and the proportion of xanthan gum. The ratio of rice flour to maize starch in the gluten-free mix was 60:40, 80:20 and 100:0, while the proportion of xanthan gum was 0%, 2% and 4%. The total amount of rice flour and maize starch mixture was constant in all samples. The actual samples of baked gluten-free biscuits are shown in Figure 1.

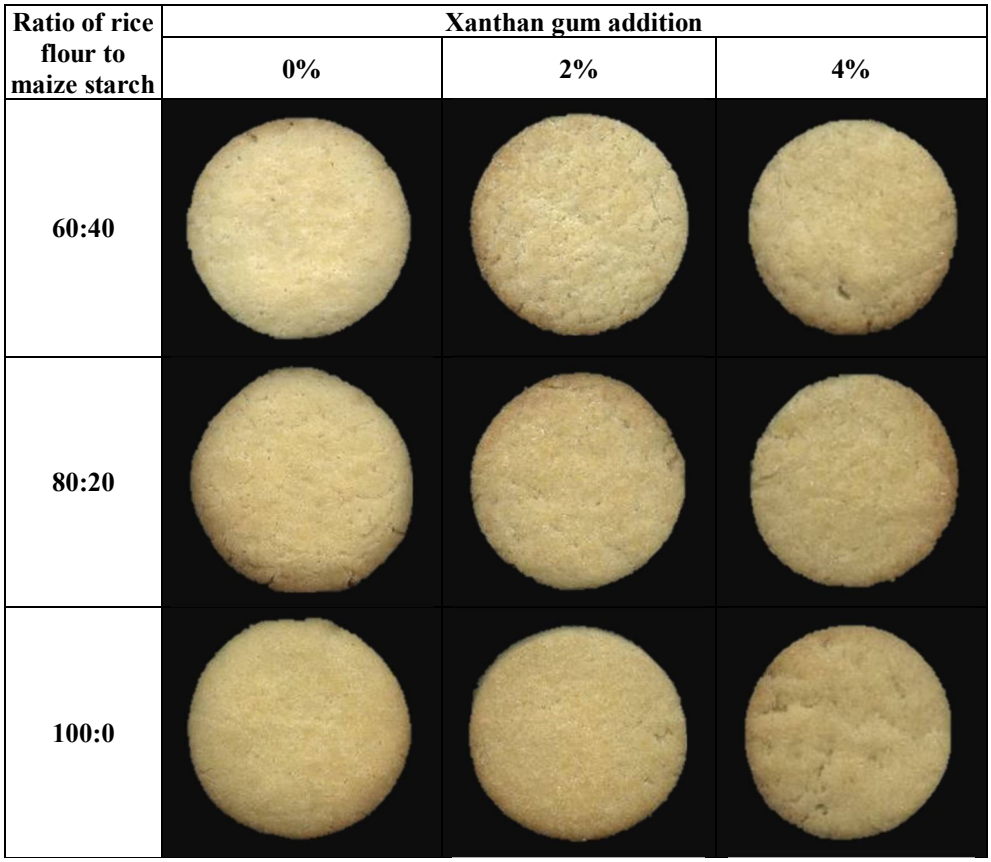

Figure 1. Produced gluten-free biscuits with different ratios of rice flour to maize starch and different proportions of xanthan gum

The results of testing the influence of different formulations on the peak force required to break gluten-free biscuits during the 3-point band test are shown in Figure 2 and in Tables 2 and 3.

Table 2

Quality parameters of gluten-free biscuits

Run	Rice flour : Maize starch ratio	Xanthan gum,%	Dependent variables				
			Peak force (N)	Spread factor	Browning index	Water activity	Total sensory score
1	60:40	0	15.7	60.9	38.6	0.407	6.0
2	60:40	2	37.6	54.0	39.0	0.452	6.9
3	60:40	4	47.2	53.2	43.7	0.505	6.8
4	80:20	0	17.0	57.8	43.6	0.424	7.2
5	80:20	2	56.5	53.1	44.2	0.465	7.5
6	80:20	4	59.4	52.4	44.6	0.482	7.3
7	100:0	0	28.1	55.5	45.3	0.445	6.6
8	100:0	2	62.6	53.0	47.2	0.477	7.5
9	100:0	4	67.8	52.3	47.3	0.515	7.2
10	80:20	2	55.2	53.6	43.5	0.483	7.3
11	80:20	2	58.0	52.9	45.0	0.471	7.8
12	80:20	2	55.1	52.5	44.4	0.484	7.8

Table 3

Analysis of variance (ANOVA) and regression model of the influence of the different xanthan gum content and the ratio of rice flour to maize starch on biscuit hardness

Source of variation	*SS*	*DF*	*MS*	*F*	*p*
X_1 – Rice flour : Maize starch ratio	560.7	1	560.7	40.1	0.001*
X_1^2	14.2	1	14.2	1.0	0.352
X_2 – Xanthan gum content	2148.6	1	2148.6	153.7	< 0.001*
X_2^2	537.8	1	537.8	38.5	0.001*
$X_1 \cdot X_2$	17.0	1	17.0		
Error	83.9	6	14.0		
Total	3496.8	11			
Model: $Y = -45.602 + 1.305 \cdot X_1 + 19.538 \cdot X_2 - 0.006 \cdot X_1^2 - 3.550 \cdot X_2^2 + 0.052 \cdot X_1 \cdot X_2$					
$R^2 = 0.976$					

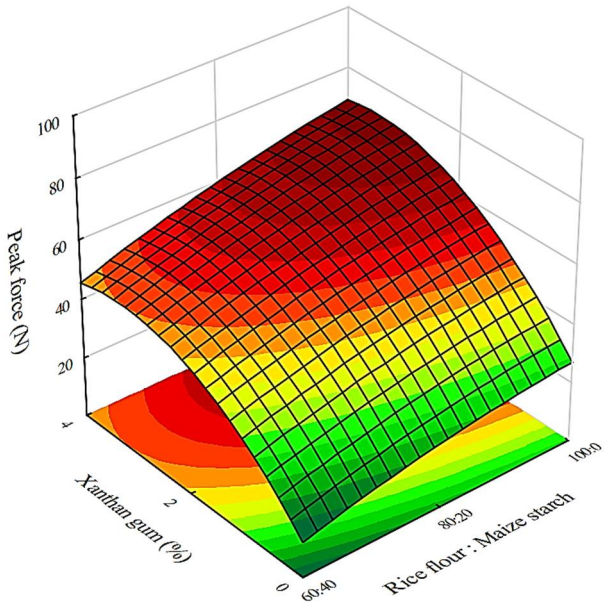

Figure 2. Response surface for the effect of different xanthan gum content and the ratio of rice flour to maize starch on biscuit hardness

Of the formulations tested, the biscuits without maize starch (100% rice flour) and 4% xanthan gum had the highest hardness (67.8 N). The main effect of the ratio of rice flour to maize starch in the flour blend was statistically significant ($F = 40.1$, $p = 0.001$). The results showed a clear trend of decreasing hardness with increasing maize starch content. This observation can be attributed to the ability of maize starch to interfere with the formation of a strong protein network, resulting in lower biscuit hardness. Fitzgerald et al. (2003) suggested that rice flour may lead to the development of a gel matrix of denatured proteins when heated, which may provide specific mechanical reinforcement and help to stabilise the overall structure. The increasing hardness of gluten-free biscuits with increasing proportion of rice flour in the mixture with maize starch can be attributed to the fact that the protein content in the flour mixture increases with the higher addition of rice flour. The quadratic effect of the ratio of rice flour to maize starch was not statistically significant ($F = 1.0$, $p = 0.352$), suggesting that the relationship between maize starch content and peak force may have been linear rather than curvilinear in this context.

The main effect of xanthan gum content was also highly significant ($F = 153.7$, $p < 0.001$), indicating its significant influence on the increase in hardness of the biscuits. When the amount of xanthan gum was increased from 0% to 4%, there was a consistent increase in biscuit hardness at each ratio of rice flour and maize starch. Xanthan gum is known for its binding properties and its ability to improve the texture of gluten-free products. The increase in hardness with higher xanthan gum concentrations supports its role in improving the structural integrity of gluten-free biscuits (Benkadri et al., 2020). The quadratic effect of xanthan gum content was also statistically significant ($F = 38.5$, $p = 0.001$), indicating a curvilinear relationship between xanthan gum content and peak force. The interaction between the ratio of rice flour to maize starch in the flour mixture and xanthan gum content

was not statistically significant ($F = 1.2$, $p = 0.312$), indicating that the combined effect of these factors on peak force was not significantly different from what would have been expected based on their individual effects. The regression model provided coefficients for each predictor variable and their interactions. The model had a high coefficient of determination ($R^2 = 0.976$), suggesting that the model explained 97.6% of the variance in peak force, indicating a very good fit.

Several other studies have also investigated the effect of xanthan gum on the quality of gluten-free biscuits (Devisetti et al., 2015; Gül et al., 2018; Shahzad et al., 2021). Their studies showed that the addition of xanthan gum significantly changed the texture characteristics of gluten-free biscuits, particularly by increasing their hardness. Gül et al. (2018) attributed this effect to the branched structure of xanthan gum, along with its interactions with other ingredients in the biscuits. However, it is worth noting that Benkadri et al. (2018) found an opposite effect of xanthan gum on biscuit hardness. This discrepancy could be due to differences in the methodology of texture analysis. While our study employed a 3-point bend test, Benkadri et al. (2018) used the Volodkevich bite upper jaw probe method.

The results presented in Table 2 and Figure 3 show that the spread factor of gluten-free biscuits tends to decrease with the addition of xanthan gum and increases with the increase of maize starch content in the gluten-free flour mixture. The highest biscuit spread factor (60.9) was observed in a sample without added xanthan gum and with a 60:40 rice flour to maize starch ratio, and the lowest spread factor (52.3) was observed in the sample with 4% xanthan gum without maize starch in the biscuit recipe. This decrease in spread factor could be due to the higher viscosity caused by the combination of rice flour and xanthan gum, which inhibits the spread of the biscuit dough during baking.

The analysis of variance (ANOVA) and the regression model revealed significant effects of both the ratio of rice flour to maize starch and the xanthan gum content on the spread factor of gluten-free biscuits. The linear effect of maize starch ratio was found to be statistically significant ($F = 24.4$, $p = 0.003$), indicating that changes in the ratio of rice flour to maize starch significantly affected the spread factor of the biscuits. In addition, the quadratic effect of rice flour to maize starch ratio was not statistically significant ($F = 1.4$, $p = 0.279$), indicating that the relationship between maize starch ratio and spread factor was mostly linear (Table 4).

Table 4

Analysis of variance (ANOVA) and regression model of the influence of the different xanthan gum content and the ratio of rice flour to maize starch on biscuit spread

Source of variation	*SS*	*DF*	*MS*	*F*	*p*
X_1 – Rice flour : Maize starch ratio	8.6	1	8.6	24.4	0.003*
X_1^2	0.5	1	0.5	1.4	0.279
X_2 – Xanthan gum content	44.9	1	44.9	127.4	< 0.001*
X_2^2	10.9	1	10.9	30.8	0.001*
$X_1 \cdot X_2$	5.0	1	5.0	14.3	0.009*
Error	2.1	6	0.4		
Total	75.1	11			
Model: $Y = 73.985 - 0.289 \cdot X_1 - 5.630 \cdot X_2 + 0.001 \cdot X_1^2 + 0.504 \cdot X_2^2 + 0.028 \cdot X_1 \cdot X_2$					
$R^2 = 0.972$					

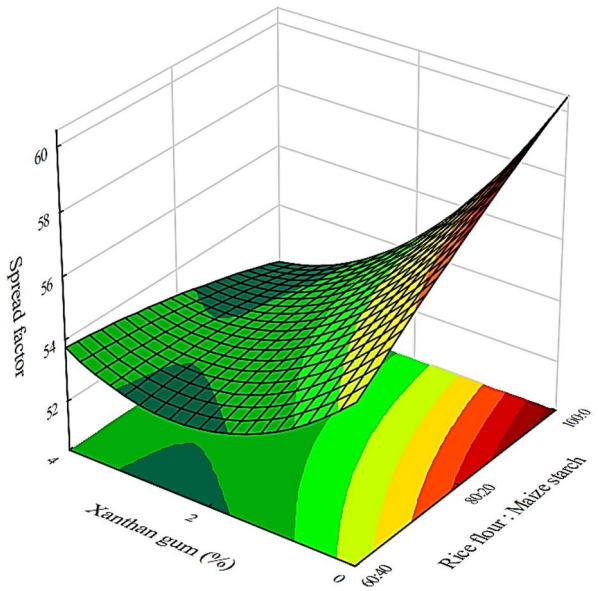

Figure 3. Response surface for the effect of different xanthan gum content and the ratio of rice flour to maize starch on biscuit spread

On the other hand, the linear effect of xanthan gum content was highly significant (F = 127.4, $p < 0.001$), indicating that variations in xanthan gum content significantly affected the spread factor of gluten-free biscuits. In addition, the quadratic effect of xanthan gum content was also statistically significant ($F = 30.8$, $p = 0.001$), highlighting the non-linear relationship between xanthan gum content and spread factor. The interaction effect between the ratio of rice flour to maize starch and xanthan gum content was significant (F = 14.3, p = 0.009), indicating that the combined effect of these factors on the spread factor differs from their individual effects. As in the case of gluten-free biscuit hardness, the resulting regression model showed a high degree of agreement ($R^2 = 0.972$).

Comparing these results with previous studies, they are consistent with the studies by Shahzad et al. (2021), Gül et al. (2018), Kaur et al. (2015) and Devisetti et al. (2015), who also reported a significant decreasing effect of xanthan gum content on the spread factor of gluten-free biscuits. Xanthan gum, which is known for its thickening and stabilising properties, could contribute to the lower spread factor by increasing the dough viscosity. However, in contrast to the influence of xanthan gum, the addition of maize starch causes an increase in the spread factor of biscuits, which, similar to the effect on biscuit hardness, can be explained by the general decrease in the amount of protein in the gluten-free mixture. This weakening of the biscuit structure leads to increased spread. This conclusion is also supported by Schroder et al. (2003), who found that low protein and high starch ingredients in gluten-free mixes contribute to weak structural stability. These results underline the importance of both the rice flour to maize starch ratio and the xanthan gum content in determining the spread factor of gluten-free biscuits, which has implications for optimising gluten-free biscuit formulations to achieve the desired texture properties.

Table 5

Analysis of variance (ANOVA) and regression model of the influence of the different xanthan gum content and the ratio of rice flour to maize starch on biscuit browning index

Source of variation	*SS*	*DF*	*MS*	*F*	*p*
X_1 – Rice flour : Maize starch ratio	57.0	1	57.0	50.0	< 0.001*
X_1^2	1.6	1	1.6	1.4	0.281
X_2 – Xanthan gum content	10.9	1	10.9	9.6	0.021*
X_2^2	0.1	1	0.1	0.1	0.743
$X_1 \cdot X_2$	2.4	1	2.4	2.1	0.197
Error	6.8	6	1.1		
Total	78.8	11			
Model: $Y = 15.183 + 0.503 \cdot X_1 + 2.000 \cdot X_2 - 0.002 \cdot X_1^2 + 0.056 \cdot X_2^2 - 0.019 \cdot X_1 \cdot X_2$					
$R^2 = 0.913$					

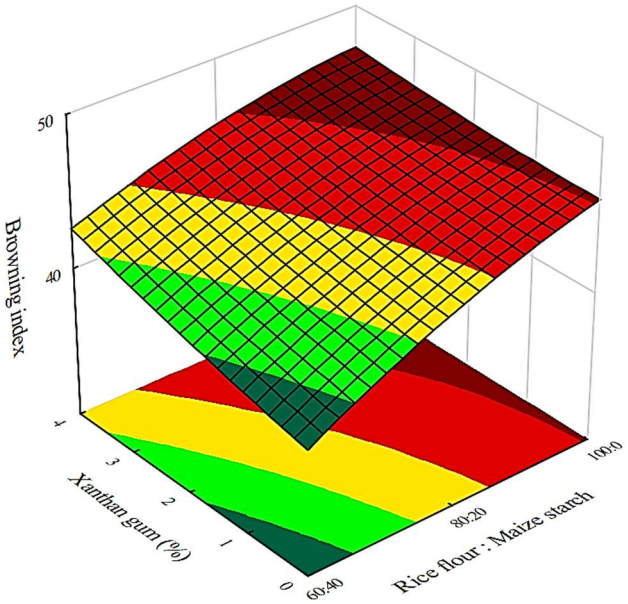

Figure 4. Response surface for the effect of different xanthan gum content and the ratio of rice flour to maize starch on browning index of biscuits

It is generally known that gluten-free bakery products often have a less pronounced colour than their wheat flour counterparts. This applies in particular to products that contain certain amounts of different starch preparations in their composition. The reason for this is that different starches are often used in the production of gluten-free products, including gluten-free biscuits, due to their techno-functional properties. However, large amounts of starch preparations in the recipe can lead to a pale colour of the product, which is less accepted by consumers. Their use reduces the total protein content in the flour blend, which is involved in the Maillard reactions, known to be one of the most important mechanisms in the formation of product colour (Mancebo et al., 2015b). Therefore, in our study we also

considered the browning index of biscuits as an important parameter in the evaluation of the quality of gluten-free biscuits.

The results obtained showed that increasing the maize starch content tends to reduce the browning index of gluten-free biscuits. For example, with a xanthan gum content of 0%, the browning index increases from 38.6 to 45.3 when the maize starch content in gluten-free blend decreases from 40% to 0% (Table 2 and Figure 4). Conversely, the effect of the xanthan gum content on the browning index appears to be less pronounced compared to maize starch. Although the browning index increases slightly with a higher xanthan gum content, the effect is not as significant as that of maize starch. The influence of xanthan gum on the increase in the browning index of gluten-free biscuits is probably only due to the fact that the samples with higher levels of xanthan gum also had a higher moisture content and higher water activity, which could have influenced the slightly darker appearance of the biscuits. The availability of water molecules can play an important role in facilitating Maillard reactions. Higher water activity provides a more favourable environment for these reactions as it increases the mobility of reactants and promotes their interaction. Consequently, biscuits with higher water activity are more prone to extensive Maillard browning, resulting in darker and more intensely coloured crusts and surfaces (Lund et. al., 2017).

According to the ANOVA results (Table 5), both the ratio of rice flour to maize starch (F = 50.0, p < 0.001) and the xanthan gum content (F = 9.6, p = 0.026) significantly influenced the browning index of gluten-free biscuits, with only a linear relationship with the browning index. However, the interaction between the ratio of rice flour to maize starch and xanthan gum showed no significant effect on the browning index. The model had a high coefficient of determination (R^2 = 0.913).

A comparison of these results with previous studies showed similar trends regarding the influence of maize starch and xanthan gum content on the browning index of gluten-free biscuits. The study by Mancebo et al. (2015) also reported significant effects of the influence of increased maize starch addition on the decrease in brightness of gluten-free biscuits made from rice flour and maize starch, confirming the consistency of the current results. Similar results were obtained in the gluten-free bread samples, where the replacement of rice flour with starch also resulted in a lighter crust colour compared to the gluten-free bread with rice flour in the control (Mancebo et al., 2015; Miñarro et al., 2010). The darker colour of gluten-free biscuits with added xanthan gum was also observed in the study by Shahzad et al. (2021).

Table 6

Analysis of variance (ANOVA) and regression model of the influence of the different xanthan gum content and the ratio of rice flour to maize starch on biscuit water activity

Source of variation	*SS*	*DF*	*MS*	*F*	*p*
X_1 – Rice flour : Maize starch ratio	0.0009	1	0.0009	5.6	0.055
X_1^2	0.0000	1	0.0000	0.1	0.815
X_2 – Xanthan gum content	0.0085	1	0.0085	54.1	< 0.001*
X_2^2	0.0002	1	0.0002	1.6	0.257
$X_1 \cdot X_2$	0.0002	1	0.0002	1.2	0.307
Error	0.0009	6	0.0002		
Total	0.0108	11			
Model: $Y = 0.377 + 0.0002 \cdot X_1 + 0.043 \cdot X_2 + 0.000005 \cdot X_1^2 - 0.002 \cdot X_2^2 - 0.0002 \cdot X_1 \cdot X_2$					
R^2 = 0.913					

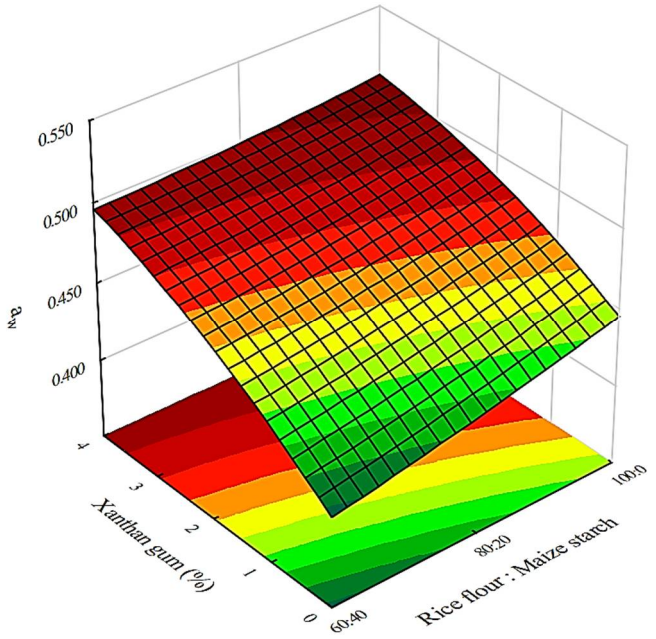

Figure 5. Response surface for the effect of different xanthan gum content and the ratio of rice flour to maize starch on biscuit water activity (a_w)

Water activity (aw) is an important parameter in gluten-free biscuits as it influences various aspects of their quality, including texture, shelf life and microbial stability (Červenka et al., 2006). Therefore, maintaining an optimal water activity level is crucial for the desired quality of gluten-free biscuits. As previously mentioned, water activity also influences Maillard browning reactions, which contribute to the development of colour, flavour and aroma in baked goods (Lund et al., 2017). Therefore, understanding and controlling water activity are important aspects in the formulation of gluten-free biscuits to achieve optimal texture, shelf life and sensory quality.

The water activity of the gluten-free biscuits, as shown in Table 2 and Figure 5, varied in the different formulations, which were characterised by different contents of maize starch and xanthan gum. Increasing the xanthan gum content in particular led to a significant increase in water activity, as can be seen from the rising values when the xanthan gum content was increased from 0% to 4%. The influence of maize starch content on water activity was not statically significant, with marginal differences observed between the different maize starch contents. These results indicate that xanthan gum plays a greater role than maize starch in determining the water activity of gluten-free biscuits.

A further investigation using the regression model shown in Table 6 confirmed these observations. The model showed a significant positive coefficient for xanthan gum content (F = 54.1, p < 0.001), indicating its strong influence on water activity. In contrast, the coefficient for maize starch was relatively small and not statistically significant (F = 5.6, p = 0.055), supporting the assumption that variations in maize starch content have minimal effects on water activity. In addition, the lack of significance of the interaction term indicates that the combined effect of maize starch and xanthan gum on water activity was not

significantly different from their individual effects. The coefficient of determination was the same as in the model for the browning index (R^2 = 0.913).

These results emphasise the importance of xanthan gum in influencing the water activity of gluten-free biscuits, which is probably due to its hygroscopic properties and its ability to interact with water molecules. Comparing these results with the existing literature, our findings are in agreement with the studies of Gül et al. (2018), Benkadri et al. (2018), Benkadri et al., 2020) and Shahzad et al. (2021), who also reported a significant influence of xanthan gum content on the increased water activity in gluten-free biscuits.

Table 7

Analysis of variance (ANOVA) and regression model of the influence of the different xanthan gum content and the ratio of rice flour to maize starch on total sensory score

Source of variation	*SS*	*DF*	*MS*	*F*	*p*
X_1 – Rice flour : Maize starch ratio	0.427	1	0.427	7.8	0.032*
X_1^2	0.667	1	0.667	12.1	0.013*
X_2 – Xanthan gum content	0.375	1	0.375	6.8	0.040*
X_2^2	0.540	1	0.540	9.8	0.020*
$X_1 \cdot X_2$	0.010	1	0.010	0.2	0.685
Error	0.330	6	0.055		
Total	2.949	11			
Model: $Y = -2.333 + 0.216 \cdot X_1 + 0.675 \cdot X_2 - 0.001 \cdot X_1^2 - 0.113 \cdot X_2^2 - 0.001 \cdot X_1 \cdot X_2$					
$R^2 = 0.888$					

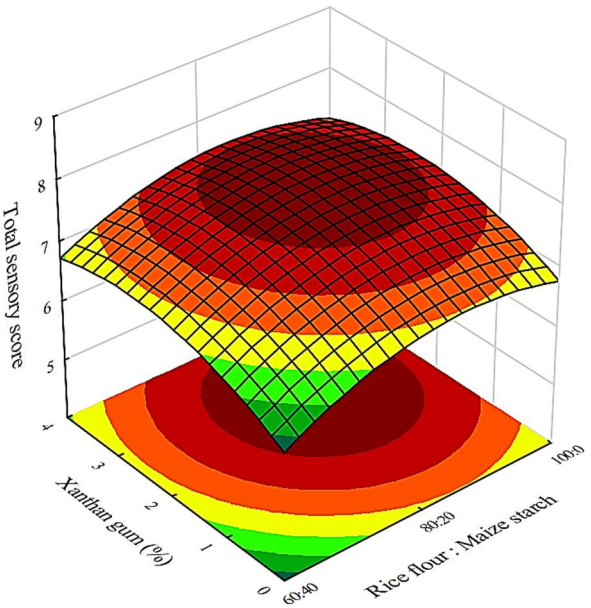

Figure 6. Response surface for the effect of different xanthan gum content and the ratio of rice flour to maize starch on total sensory score

The total sensory score of the gluten-free biscuits, as shown in Table 2 and Figure 6, showed variability between the different recipes, characterised by different ratios of rice flour to maize starch and xanthan gum content. These variations indicate that both maize starch and xanthan gum content play an important role in influencing the overall sensory perception of gluten-free biscuits. The lowest sensory rating (6.0) was found for the samples with no added xanthan gum and the 60:40 ratio of rice flour and maize starch in the flour blend. The samples with an addition of 2% xanthan gum, regardless of the ratio of rice flour to maize starch, received the highest total sensory scores (6.9–7.5).

A further investigation using the regression model shown in Table 7 provided information on the individual and combined effects of the ratio of rice flour to maize starch and the xanthan gum content on the total sensory score. The model showed significant linear and quadratic effects for both maize starch and xanthan gum content, indicating that they influence the total sensory score of gluten-free biscuits. In addition, the F values for the quadratic effects ($F = 12.1$, $p = 0.013$ and $F = 9.8$, $p = 0.020$) were higher than for the linear effects ($F = 7.8$, $p = 0.032$ and $F = 6.8$, $p = 0.040$), suggesting that the optimal amount of maize starch and xanthan gum lies between the minimum and maximum addition levels. In contrast to the linear and quadratic effects observed for the ratio of rice flour to maize starch and xanthan gum content, the interaction term was not statistically significant, indicating that the combined effect on sensory score does not deviate significantly from the individual effects.

Comparing these results with the existing literature, our findings are consistent with a previous study by Mancebo et al (2015a), which also reported that a high addition of maize starch reduces the sensory acceptability of gluten-free biscuits. Benkadri et al. (2018) concluded that the addition of xanthan gum in an amount of 0.5% to 1.5% did not significantly affect sensory acceptability. Similar to our results, Gül et al. (2018) found that the addition of xanthan gum improved the sensory quality of gluten-free biscuits, but this improvement was only noticeable up to an addition level of 3%, after which the values decreased slightly. Shahzad et al. (2021) found that the addition of xanthan gum in the recipe for the production of biscuits led to a decrease in sensory ratings, but in their study, they added xanthan gum at a level of 5%, as opposed to the maximum of 4% in our study.

The final step in this study was the optimisation of the gluten-free biscuit recipe. From the results presented above, it can be seen that the quality of gluten-free biscuits was influenced by several factors and not by a single main factor. Each independent variable played a significant role in shaping the characteristics of the gluten-freE biscuits. All effects resulting from the response surface plots were taken into account during the optimisation process, as the ideal solution requires a balance between the different responses. In this study, the responses were maximised (browning index and total sensory score), minimised (water activity) or fixed at a target value (peak force at 50 N and spread factor at 54.3).

The optimisation process resulted in the following optimal conditions to achieve the desired response values: the ratio of rice flour to maize starch 88.9:11.1 and an addition of 1.1% xanthan gum. The predicted response values based on these conditions were: peak force 47.7, spread factor 54.3, browning index 44.7, water activity 0.458 and total sensory score 7.4. A desirability score of 0.72 was calculated for the gluten-free biscuits using the optimal recipe (Figure 7). After identifying the best solution, the gluten-free biscuits were produced according to the optimised recipe. These biscuits were analysed for all five responses to validate the predictive capability of the models and to compare the theoretical predictions with the experimental results. The experiments carried out under optimal conditions showed a high degree of agreement between the predicted and experimental values for all responses (Table 8). The total sensory score obtained (7.7) was even higher than the score predicted by

the model (7.4), placing the biscuit samples between a score of 7 (moderately liked) and 8 (very much liked) on the hedonic scale used, indicating a high preference for the gluten-free biscuits produced.

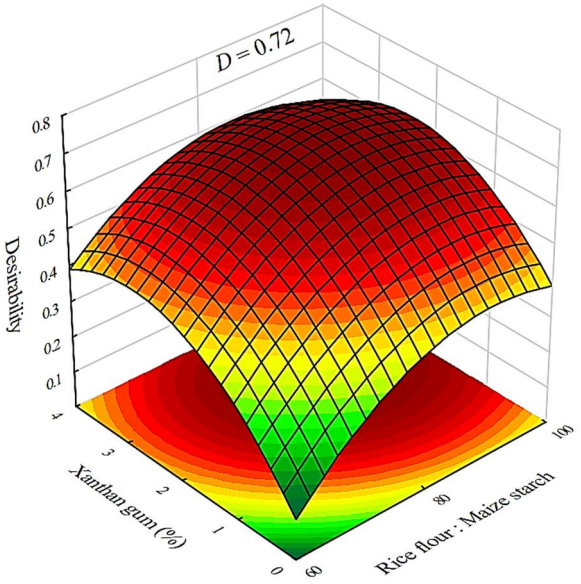

Figure 7. Response surface for the effect of different xanthan gum content and the ratio of rice flour to maize starch on desirability of gluten-free biscuits

Table 8

Optimal formulation of gluten-free biscuits with predicted and experimental values of response variables

Parameter	Predicted	Experimental
Peak force (N)	47.7	49.1
Spread factor	54.3	53.4
Browning index	44.7	44.1
Water activity	0.458	0.449
Total sensory score	7.4	7.7
Optimal rice flour to maize starch ratio – 88.9:11.1		
Optimal xanthan gum content – 1.1%		

Conclusions

1. This study emphasised the significant influence of the ratio of rice flour to maize starch in the flour blend and the xanthan gum content on the quality characteristics of gluten-free biscuits. The observed effects underlined how important it is to optimise the recipe of gluten-free biscuits in order to achieve the desired sensory properties and consumer acceptance.

2. An increase in the addition of xanthan gum led to an increase in hardness, browning index and water activity as well as a reduction in the spread factor of gluten-free biscuits.
3. Increasing the proportion of maize starch in the gluten-free mixture in combination with rice flour contributed significantly to a reduction in the hardness and browning index of gluten-free biscuits, while at the same time increasing the spread of the biscuits. However, the effect of maize starch on water activity was inconclusive.
4. The curvilinear effects of the addition of xanthan gum and maize starch on the sensory score showed that the optimal amounts were between the minimum and maximum addition amounts in the experiment.
5. Through the optimisation process, the optimal conditions were determined to achieve the desired response values: a ratio of rice flour to maize starch of 88.9:11.1 in combination with an addition of 1.1% xanthan gum.

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Integrated technology of the surfactants and phytohormones biosynthesis by *Nocardia vaccinii* IMV B-7405 for their use in crop production

Tetyana Pirog[1,2], Daria Piatetska[1], Natalia Leonova[2], Tetyana Shevchuk[2]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, Kyiv, Ukraine

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Corresponding author:

Tetyana Pirog
E-mail: tapirog @nuft.edu.ua

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Abstract

Introduction. The strain *Nocardia vaccinii* IMV B-7405, which produces phytohormones and surfactants, is a promising agent for the development of integrated technologies to obtain complex preparation to be used in crop production.

Materials and methods. Cultivation of bacteria was carried out in a liquid medium with 2% refined or waste oil and 100–300 mg/l of tryptophan (the precursor of the phytohormone auxin biosynthesis) was added to the medium. The concentration of auxins was determined by the method of high-performance liquid chromatography, surfactants – by weight method. Antimicrobial activity of surfactant was analyzed by the indicator of minimum inhibitory concentration (MIC). Greenhouse experiments were carried out using tomato plants; the number of fruits and their weight were analyzed. Determination of the protective effect of surfactants against bacterial diseases of tomatoes was carried out by the method of separated leaves.

Results and discussion. It was established that in the presence of tryptophan in the culture medium of *N. vaccinii* IMV B-7405 the concentration of auxins was one to two orders of magnitude higher than without the biosynthesis precursor. The increase in auxins synthesis correlated with tryptophan transaminase activity – the key enzyme of auxins biosynthesis. Surfactants synthesized in the presence of tryptophan were characterized by higher antimicrobial activity against phytopathogenic bacteria: MICs were 2–4 times lower compared to those established for preparations formed without a precursor. Treatment of tomato leaves with solutions of surfactants synthesized by *N. vaccinii* IMV B-7405 in the presence of tryptophan contributed to the protection of leaves from phytopathogens damage. The exometabolites of *N. vaccinii* IMV B-7405 increased the productivity of tomatoes: the total weight increased by 82–91%, and the average fruit weight by 12–18%.

Conclusions. The complex preparation based on the exometabolites (the surfactants and phytohormones) of *N. vaccinii* IMV B-7405 can be used to control the number of phytopathogens and increased the productivity of tomatoes.

Introduction

At the present stage, the classical monobiotechnologies, the main postulate of which is "one producer – one carbon substrate – one target product", are being replaced by the so-called "integrated biotechnologies" (one producer – one or several carbon substrates – several target products) (Hori et al., 2011; Krishnan et al., 2019; Nitschke et al. 2011; Que et al., 2014; Zeng et al., 2013). The effectiveness of such technologies is obvious. First, there is the implementation of one technological process instead of several. Secondly, the scope of application of the target product, which contains a complex of metabolites with different properties, is much wider compared to mono bioformulations (Pirog et al., 2019a).

The available individual data on the surface-active substances (surfactants) biosynthesis as concomitant metabolites of phytohormones of auxin`s nature were summarized in the review (Pirog et al., 2019a). Note that the concentration of phytohormones synthesized by surfactant producers was low and didn`t exceed 5 mg/l. At the same time, microbial surfactants due to a complex of unique properties (the ability to reduce surface and interfacial tension, emulsify various substrates, destroy biofilms, and have antimicrobial and anti-adhesive activity) are multifunctional formulations and can be used in food technologies, pharmaceutical industry, agriculture, medicine and environmental technologies (Fenibo et al., 2019; Pirog et al., 2019a).

The ability of the surfactant producer *Nocardia vaccinii* IMV B-7405 to synthesize stimulating phytohormones such as auxins, cytokinins, and gibberellins was shown in the studies of Pirog et al. (2016). Since surfactants of *N. vaccinii* IMV B-7405 strain have a wide range of anti-adhesive and antimicrobial activity (Pirog et al., 2023), including phytopathogenic bacteria, the multifunctional formulation containing surfactant and phytohormones is promising for use in crop production. At the same time, the concentration of phytohormones synthesized by *N. vaccinii* IMV B-7405 was low (70-100 μg/l), which significantly reduced the effectiveness of using such formulation to stimulate plant growth.

In modern biotechnologies, one of the approaches to increasing the concentration of the target product can be the introduction of biosynthesis precursors. Thus, most researchers (Liu et al., 2019; McClerklin et al., 2018) determine the ability of microorganisms to synthesize auxins with the addition of tryptophan to the culture medium, which is a precursor to the synthesis of indole-3-acetic acid (IAA).

It was shown that the surfactant producer *N. vaccinii* IMV B-7405 synthesizes auxins under growth media conditions with various substrates without a precursor (Pirog et al., 2016), and, therefore, there are potential opportunities for increasing their synthesis.

However, it noted that both surfactants and phytohormones are secondary metabolites that are usually synthesized in the form of a complex of similar compounds, the composition and ratio of which may vary depending on the cultivation conditions (Pirog et al., 2019b). Therefore, there is no guarantee that surfactants synthesized in the presence of tryptophan in the culture medium will be characterized by high antimicrobial activity.

Therefore, the aim of this research was: (a) to define the possibility of auxin`s synthesis intensification due to introducing a precursor into the culture medium of *N. vaccinii* IMV B-7405; (b) to determine the antimicrobial activity of surfactants synthesized in tryptophan presence in the medium against phytopathogenic bacteria; (c) to check the effectiveness of a complex microbial bioformulations in vegetation conditions to stimulate the growth and increase the yield of tomatoes.

Materials and methods

Research objects

The main object of research was a strain of oil-oxidizing bacteria, *Nocardia vaccinii* IMV B-7405 from the Collection of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (Pirog et al., 2019, 2023). By chemical nature, extracellular surfactants of *N. vaccinii* IMV B-7405 are a complex of glyco-, amino- and neutral lipids. Glycolipids are represented by trehalosomycolates.

This research used phytopathogenic bacteria from the Ukrainian Collection of Microorganisms (UCM): *Pseudomonas syringae* UCM B-1027, *Agrobacterium tumefaciens* UCM B-1000, *Xanthomonas vesicatoria* UCM B-1106, *Pectobacterium carotovorum* UCM B-1075. Also, strains from the Collection of the Department of Phytopathogenic Bacteria of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine: *Clavibacter michiganensis* subsp. *michiganensis* IMV B-10_2 and *Pseudomonas syringae* pv. IMV B-9167 were used.

Cultivation conditions

The strain *N. vaccinii* IMV B-7405 grown in a medium containing (g/l): $NaNO_3$ – 0.5–1.25; $MgSO_4 \cdot 7H_2O$ – 0.1; $CaCl_2 \cdot 2H_2O$ – 0.1; KH_2PO_4 – 0.1; $FeSO_4 \cdot 7H_2O$ –0.001; yeast autolysate – 0.5% (v/v). The source of carbon and energy is refined sunflower oil and oil after frying potato or meat, in a concentration of 2% (v/v).

Tryptophan was introduced into the medium as the 1% solution in the amount of 100, 200 or 300 mg/l at the beginning of the process or the end of the exponential phase of strain growth (at 48 h of cultivation).

Cultures in the exponential growth phase, grown on appropriate liquid media containing 0.5–1% (v/v) of the substrate used as inoculum. The amount of inoculum (10^4–10^5 cells/ml) was 5–10% of the volume of the nutrient medium. Cultivation of bacteria was carried out in flasks with a volume of 750 ml with 100 ml of medium on a shaker (320 rpm) at 28–30 ºC for 120 hours.

Release of extracellular auxins

Preparation of auxin extracts. Extracellular phytohormones auxins were isolated by triple extraction with organic solvents from the culture broth after surfactant extraction (Negretsky, 1988). Ethyl acetate, pH 3.0, was used as an organic solvent. The obtained extracts were evaporated under a vacuum at 40-45 °C. The dry sediment was dissolved in 80% ethanol and transferred to microtubes. The obtained extracts were stored at a temperature of – 24°C.

Determination of the qualitative and quantitative composition of auxins. Preliminary purification and concentration of extracts containing phytohormones (accumulative thin-layer chromatography) were carried out on plates with silica gel brand "Silufol UV_{254}" (Chemapol, Czech Republic) in a mixture of solvents, which were introduced sequentially: chloroform, 12.5% aqueous ammonia, and ethyl acetate: acetic acid (20:1).

The qualitative and quantitative composition of auxins was analysed by high-performance liquid chromatography (HPLC), using an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) and an Agilent G1956B mass spectrometry (mass spectrometry – MS) detector. HPLC/MS analysis of auxin extracts was performed at the Center for Collective Use of Scientific Equipment at the Institute of Microbiology and Virology.

For comparison, standard synthetic phytohormones *Sigma* (Germany) and *Acros Organic* (Belgium) were used:

IAA – Indole-3-acetic acid, Indole-3-acetic acid (IOK);

ICal – Indole-3-carboxaldehyde, Indole-3-carboxaldehyde;

IC – Indole-3-carbinol, Indole-3-carbinol;

ICA – Indole-3-carboxylic acid, Indole-3-carboxylic acid;

IAA-hydr. – Indole-3-acetic acid hydrazide, Indole-3-acetic acid hydrazide;

IBut – Indole-3-butyric acid, Indole-3-butyric acid;

Methanol (A) and a 1% solution of acetic acid in water (B) were used as the mobile phase. The separation was carried out on a Zorbax SB-C18 chromatographic column (2.1 mm × 150 mm, 3 μm) (Agilent Technologies, USA), the flow rate through the column was 0.25 ml/min, the temperature of the thermostat was 30 °C, and the injection volume was 2 μl. Elution was carried out in the gradient mode: 0 min – A (30%): B (70%); 25 min – A (30%): B (70%); 35 min – A (100%): B (0%); 35 min - A (100 %): B (0 %).

Detection of compounds was carried out using a diode-matrix detector with signal registration at 254 and 280 nm and fixation of absorption spectra in the range of 191–700 nm. An Agilent G1956B mass spectrometric detector (Agilent Technologies, USA) was used to determine the molecular masses of the studied compounds. Ionization was carried out in the ESI and APCI modes with the fixation of positive ions in the SCAN mode in the range of 100–1200 m/z. Calibration was performed using standard auxin solutions. The amount of phytohormones was estimated in μg per 1 g of dry biomass of the producer.

Enzymatic analyses

Preparation of cell-free extracts. To obtain cell-free extracts, the culture liquid after cultivation of *N. vaccinii* IMV B-7405 in a liquid mineral medium with used oil after frying potatoes was centrifuged (4000 g, 15 min, 4 °C). The cell sediment was washed twice from the residues of the medium with 0.05 M K-phosphate buffer (pH 7.0), centrifuging (4000 g, 15 min, 4 °C). Washed cells were suspended in 0.05 M K-phosphate buffer (pH 7.0) and destroyed by ultrasound (22 kHz) 3 times for 60 seconds at 4 °C on the UZDN-1 apparatus. The disintegrated biomass was centrifuged (12,000 g, 30 min, 4 °C), the sediment was discarded, and the supernatant was used as a cell-free extract.

Tryptophan transaminase activity. The activity of tryptophan transaminase (EC 2.6.1.27, other names: L-phenylalanine-2-oxoglutarate aminotransferase; tryptophan aminotransferase; 5-hydroxytryptophan-ketoglutaric transaminase; hydroxytryptophan aminotransferase; tryptophan aminotransferase; L-tryptophan transaminase) was determined by the formation of indole-pyruvate from L- tryptophan and 2-oxoglutarate, which was analysed spectrophotometric at 330 nm (Collier and Kohlhaw, 1972).

Determination of the concentration of surface-active substances and preparation of surfactant solutions

The amount of extracellular surfactants was determined by the weight method after their extraction from the supernatant of the culture liquid.

Surfactants were obtained from a supernatant via extraction with a chloroform–methanol mixture at a ratio of 2:1 (Folch mixture). Cultural liquid obtained after cultivation of *N. vaccinii* IMV B-7405 in the medium containing used oil after frying meat or potato as a carbon source with 300 mg/L tryptophan added at the beginning of growth was used.

Grown cells were centrifuged (5000 g) for 45 min, and the supernatant was further treated. For this purpose, 50 mL of supernatant was placed in a 200 mL cylindrical separating funnel, 50 mL of the Folch mixture was added, and the funnel was closed with a stopper and vortexed (lipids were extracted) for 5 min. The mixture obtained after the extraction procedure was left in a separating funnel for phase separation; after that, the lower fraction was poured out (organic extract 1), and the water phase was re-extracted as described above. After the phase separation, the lower fraction was poured out and organic extract 2 was obtained. In the third stage, 50 mL of the Folch mixture was added to the water phase, extraction was performed, and organic extract 3 was obtained.

In the studies, solutions of surface-active substances with different concentrations were used as preparations. For this, the dry residue was diluted by sterile tap water to the original volume. All the preparations were sterilized at 112°C for 30 min.

Determination of the surfactant antimicrobial activity

The antimicrobial activity of surface-active substances was determined according to the indicator minimum inhibitory concentration (MIC). Determination of MIC was carried out by the method of two-fold serial dilutions in meat-peptone broth (MPB). Under sterile conditions, 1 ml of the medium was introduced into 10 test tubes, 1 ml of a surfactant solution of a certain concentration was added to the first, after which it was mixed, 1 ml was taken and transferred to the next test tube. Similarly, dilution was carried out for the next nine test tubes. 1 ml was taken from the last test tube. Thus, the final volume in each tube was 1 ml (MPB and surfactant solution), and the concentration of surfactant in each subsequent tube was reduced by 2 times. As a control, 1 ml of MPB without the addition of a surfactant solution was used. Next, 0.1 ml of test culture suspension (10^5–10^6 CFU/ml) was added to each test tube and mixed. The test tubes were incubated for 24 hours at 28-30 °C.

The results were assessed visually by the turbidity of the medium: (+) – test tubes in which the turbidity of the medium was observed (growth of the test culture), (−) – there was no turbidity (no growth). The minimum inhibitory concentration of the surfactant solution was determined as the concentration of surfactant in the last test tube where growth was absent.

Determination of the exometabolites effect on the growth and yield of tomatoes

The experiment was conducted in greenhouses from April to September. Tomatoes plant variety Salad (*Solanum lycopersicum* L.) was used as a test crop. The crop was harvested in the period from July to September. Before planting in the soil, the root system of tomato seedlings was kept for two hours in the supernatant or culture liquid of *N. vaccinii* IMV B-7405 (dilution 1:200 and 1:400). Seedlings kept for two hours in tap water were used as a control. There were three plants in each variant. During the experiment, the amount of tomato fruits and their weight were analysed.

Determination of the protective effect of surface-active substances of *N. vaccinii* IMV B-7405 against bacterial diseases of tomatoes

The determination was carried out by the method of detached leaves. *X. vesicatoria* IMV B-9098 and *C. michiganensis* subsp. *michiganensis* IMV B-102 were used as test cultures of phytopathogens, which cause necrosis on the plant leaves.

2 months after setting up the vegetative experiment, the plants in the flowering phase were treated with a surfactant solution with a concentration of 20 μg/ ml. A day later, leaves were collected from the surfactant-treated and untreated plants. The leaves were placed in Petri dishes on sterile cotton swabs moistened with 5 ml of sterile tap water. After that, the leaves were sprayed with a suspension of phytopathogenic bacteria (10^7-10^8 CFU/ml), and grown on MPA for 48 hours. Leaves treated with sterile tap water were used as a control. Closed Petri dishes were incubated at room temperature under natural light for 7 days and the course of the disease was observed. The degree of development of tomato bacterial disease was estimated as a percentage ratio of the area of the leaf affected by the disease to the area of the entire leaf.

Statistical processing

All experiments were repeated three times; the number of determined parameters was from 3 to 5. The experimental data were statistically processed according to Lakin (Pirog et al., 2016). Differences between mean parameters were considered significant at $p < 0.05$.

Results and discussion

Effect of precursors on auxins biosynthesis by *N. vaccinii* IMV B-7405

Previous studies have shown that the synthesis of auxin metabolites depended on the nature of the carbon source in the culture medium of *N. vaccinii* IMV B-7405 (Pirog et al., 2016).

The data given in Table 1 testify those regardless of the time of tryptophan introduction into the culture medium of strain *N. vaccinii* IMV B-7405 with both refined and spent oil, a significant increase in auxin synthesis was observed compared to indicators on the medium without this precursor.

Among the synthesized auxins, IAA prevailed, whose precursor is tryptophan. Note that the highest concentration of auxins was achieved when 300 mg/l of tryptophan was added to the medium with both substrates.

It is known (Pidgorsky et al., 2010) that most of the precursors are involved in the processes of the biosynthesis of secondary metabolites at the end of the exponential or the beginning of the stationary phases of growth. This was observed under the conditions of growing *N. vaccinii* IMV B-7405 on refined oil: the introduction of tryptophan at the end of the exponential phase of growth was accompanied by an increase in the concentration of synthesized auxins by 1.8-41.3 times.

At the same time, other regularities were observed during the cultivation of *N. vaccinii* IMV B-7405 in a medium with spent oil: for most variants, the highest concentration of auxins was observed when tryptophan was added at the beginning of the cultivation process. We assume that such results may be due to the quality of the waste oil used as a substrate, in particular, the presence of components in its composition that can somehow affect the biosynthesis of phytohormones. Our further research will be devoted to clarifying these issues.

The data given in Table 1, testify that the concentration of synthesized auxins increased with an increase in the concentration of the precursor in the culture medium of *N. vaccinii* IMV B-7405. A further increase in tryptophan may be accompanied by an intensification of auxin synthesis. However, at this stage, to create an effective microbial

preparation with growth-stimulating properties, this is not necessary, since at the achieved concentration of auxins (3000−5000 μg/l, see Table 1), the culture liquid of *N. vaccinii* IMV B-7405 to process seeds or the root system of plant seedlings must be diluted at least 200 times.

Table 1

Synthesis of auxins under growth conditions of *N. vaccinii* IMV B-7405 in an environment with oil-containing substrates and tryptophan

Oil as a substrate	Trp, mg/l	The moment of introduction of tryptophan (growth phase)	Concentration of auxins, μg/l				
			IAA	ICA	ICal	IAA-hydr.	Total amount
Refined oil	Without tryptophan		64.9	6.3	2.1	3.6	76.9
	100	Lag phase	29.0	11.6	17.7	–	58.3
		The end of the exponential	40.6	14.4	84.9	–	139.9
	200	Lag phase	348.6	71.7	11.3	–	431.6
		The end of the exponential	854.0	501.5	–	–	1355.5
	300	Lag phase	331.4	90.0	–	–	421.4
		End of the exponential	1986,7	1157.1	–	–	3143.7
Waste oil	0	Lag phase	4.5	1.8	–	6.9	13.2
	100	Lag phase	1258.9	472.6	–	–	1731.5
		The end of the exponential	874.3	292.1	–	–	1185.7
	200	Lag phase	2331.2	470.6	–	–	2801.8
		The end of the exponential	2166.4	725.8	12.7	–	2910.8
	300	Lag phase	4666.7	1139.2	–	–	5806.0
		The end of the exponential	1538.8	719.73	–	–	2258.6

Note. Oil after frying potatoes was used as a substrate. IAA – indole-3-acetic acid; ICA – indole-3-carboxylic acid; ICal – indole-3-carboxaldehyde; IAA-hydr. – indole-3-acetic acid hydrazide. "–" – not found. When determining the concentration of auxins, the error did not exceed 5%.

Activity of tryptophan transaminase under different conditions of cultivation of *N. vaccinii* IMV B-7405

To confirm that exogenous tryptophan is involved in the biosynthesis of auxins, the activity of tryptophan transaminase was analysed – one of the key enzymes in the synthesis of indole-3-acetic acid, which catalyses the reaction of the formation of indole-3-pyruvic acid from tryptophan and 2-oxoglutarate. As evidenced by the data shown in Figure 1, in the presence of tryptophan in the culture medium of the *N. vaccinii* IMV B-7405, a 2.4-5.7-fold increase in the activity of this enzyme was observed compared to the cultivation of this strain without a precursor.

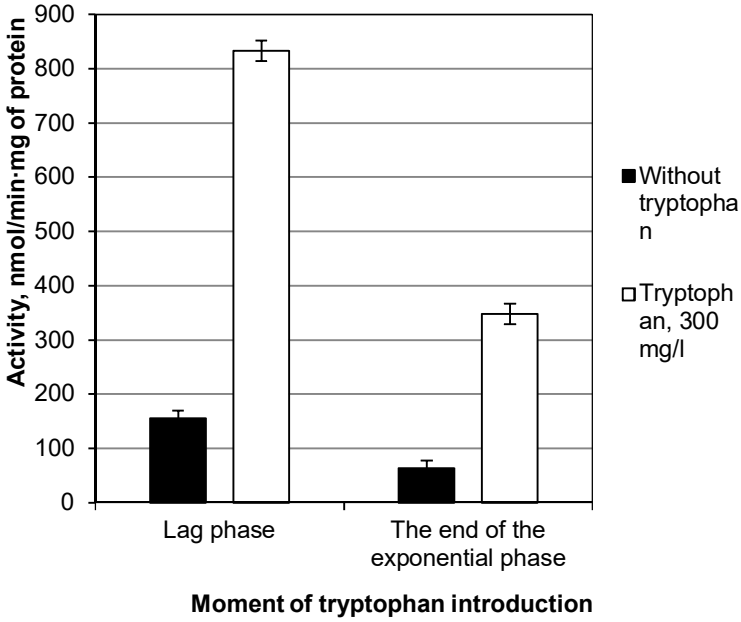

Figure 1. The influence of tryptophan on the activity of tryptophan transaminase in *N. vaccinii* IMV B-7405 cells grown on refined oil

Antimicrobial activity of surfactant *N. vaccinii* IMV B-7405 against phytopathogenic bacteria

The data given in Table 2 showed that surfactants synthesized in the presence of tryptophan were characterized by high antimicrobial activity against phytopathogenic bacteria: the MICs of such surfactants were 2-4 times lower compared to the indicators established for surfactants formed in an environment without a precursor.

Table 2

Antimicrobial activity of surfactants synthesized by *N. vaccinii* IMV B-7405 in the presence of tryptophan

Growth substrate	Tryptophan, mg/l	Minimum inhibitory concentrations (μg/ml) relative to phytopathogenic bacteria					
		UCM B-1000	UCM B-1027	UCM B-1106	UCM B-1075	IMV B-10_2	IMV B-9167
Refined oil	0	5.6	5.6	22.5	5.6	90	90
	300	1.4	1.4	11.3	1.4	22.5	45
Waste oil	0	90	22.5	2.8	22.5	2.8	90
	300	45	5.6	0.7	1.4	1.4	22.5

Note. When determining the minimum inhibitory concentrations, the error did not exceed 5%.

Influence of surfactants on tomato bacterial pathogens

The following experiments showed that pretreatment of tomato leaves with solutions of surfactants synthesized by *N. vaccinii* IMV B-7405 in the presence of tryptophan contributed to the protection of leaves from damage by phytopathogens (Figures 2 and 3). Thus, within 7 days, no symptoms of the disease were detected on the treated leaf infected with phytopathogens (Figure 2a, Figure 3a). The treatment proved to be effective protection against *C. michiganensis* subsp. *michiganensis* IMV B-102 and *X. vesicatoria* IMV B-9098 strains.

At the same time, the degree of damage by phytopathogens to untreated leaves with solutions of surface-active substances ranged from 8 to 50% (Figure 2b, Figure 3b). Such results testify to the high efficiency of the antimicrobial effect of surface-active substances against bacterial diseases of tomatoes.

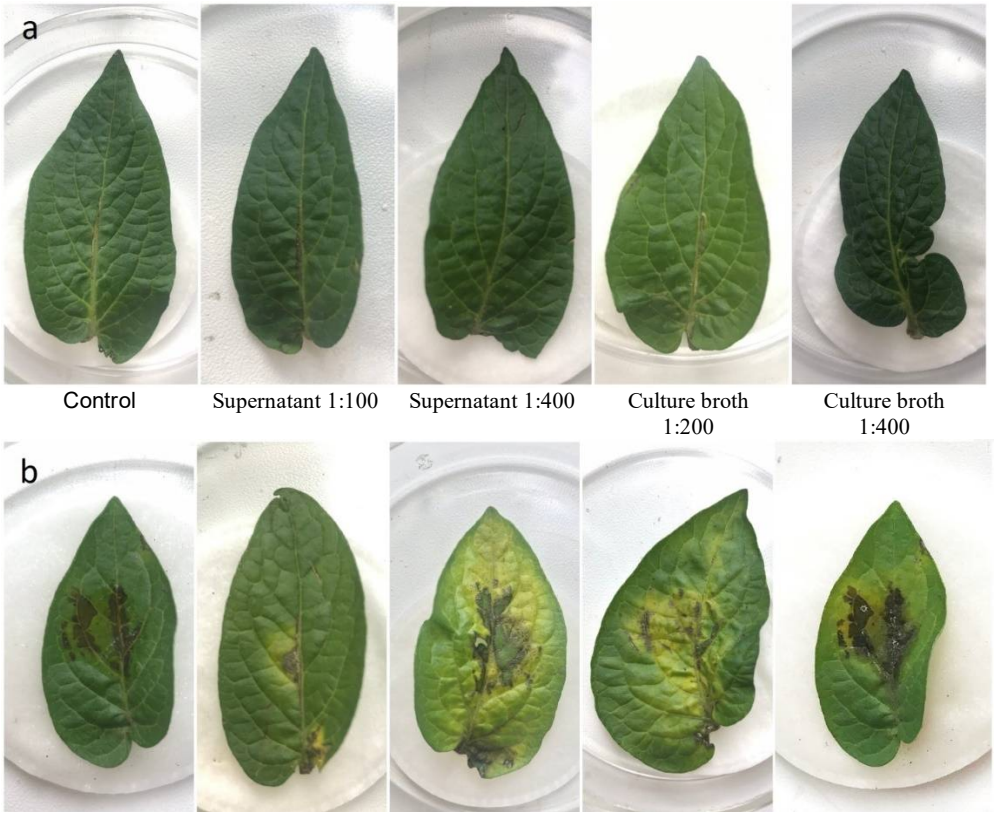

Figure 2. Inoculation of tomato leaves with strain *Xanthomonas vesicatoria* IMV B-9098 pre-treated with surfactant (a) and without pre-treatment (b)

Figures 2 and 3: leaves were taken from tomatoes, the root system of seedlings of which before planting in the soil was treated with water (control), diluted 200 and 400 times with the supernatant and culture liquid after growing *N. vaccinii* IMV B-7405 on oil spent after frying potatoes according to the presence of tryptophan (300 mg/l)

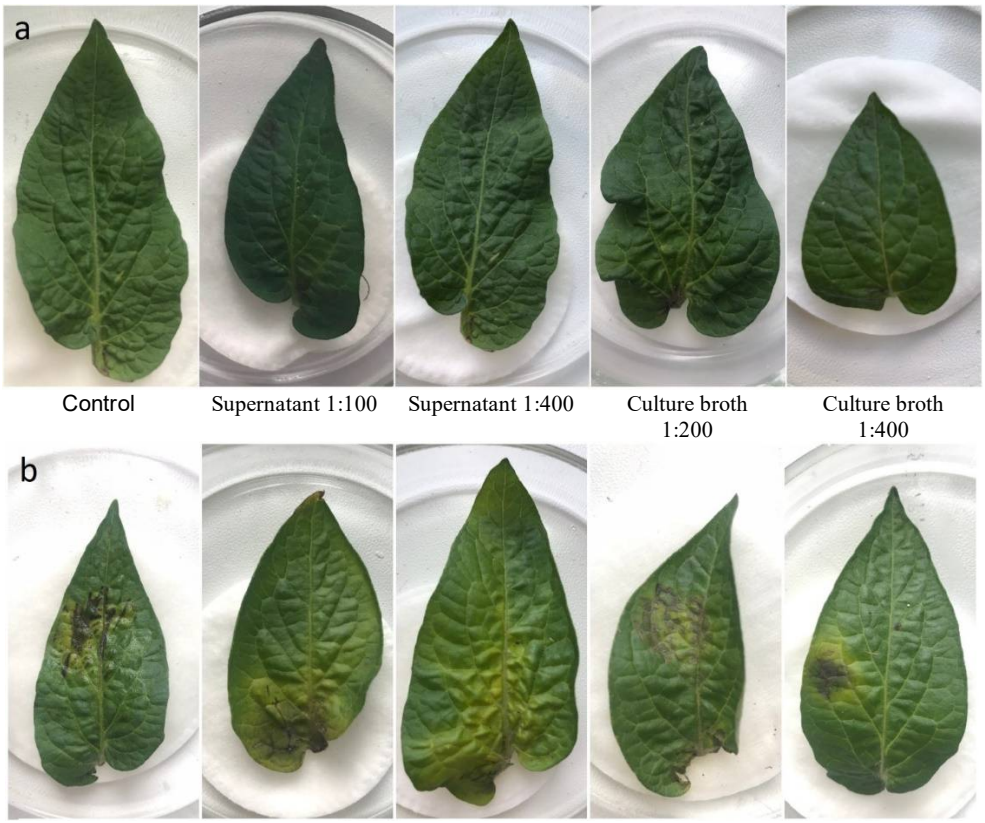

Figure 3. The effect of surfactants pre-treatment of tomato leaves (a) and its absence (b) on development of *Clavibacter michiganensis* subsp. *michiganensis* IMV B-102

Effects of *N. vaccinii* IMV B-7405 exometabolites on the yield of tomatoes

Data on the yield of tomatoes are given in Table 3. These data prove that for all variants of plant treatment with exometabolites of *N. vaccinii* IMV B-7405, an increase in yield indicators was observed. The greatest increase in both the total weight of tomatoes and the average weight of the fruit (82-91 and 12-18%, respectively) was observed in the case of using culture liquid and supernatant in a dilution of 1:400. In the case of a decrease in the degree of dilution of the culture liquid and supernatant, the yield indicators decreased slightly. The obtained data can be explained by the fact that the optimal concentration of phytohormones in the working solution was achieved at a dilution of 1:400.

It should be noted that the yield indicators of tomatoes after treatment with supernatant and culture liquid were almost the same, but the use of culture liquid in crop production is more expedient from an economic point of view, as it makes it possible to exclude the biomass separation stage from the technological process.

Table 3

Effects of *N. vaccinii* strain IMV B-7405 exometabolites on the yield of tomatoes

Variant of treatment of the tomato root system	Total weight, % of control	Average fruit weight, % of control
Culture liquid supernatant (1:200)	147	106
Culture liquid supernatant (1:400)	**191**	**118**
Culture liquid (1:200)	128	103
Culture fluid (1:400)	**182**	**112**

Note. Control (100%) – treatment of the seedling root system with water; control variants were not treated with surfactant solutions. To obtain the culture fluid (supernatant), *N. vaccinii* IMV B-7405 was grown on oil used after frying potatoes in the presence of 300 mg/l tryptophan. Dilutions were chosen based on the effective action of phytohormones.

In this research, the choice of substrates for growing *N. vaccinii* IMV B-7405 was determined by the following reasons. First, under the conditions of growth on refined oil, the strain of *N. vaccinii* IMV B-7405 synthesized the highest amount of auxins (770.4 μg/ l) compared to that on other substrates (Pirog et al., 2016). Secondly, a complex microbial preparation should be characterized by high antimicrobial activity against phytopathogenic bacteria, and earlier (Pirog et al., 2023) it was established that such properties are inherent in surface-active substances synthesized in the process of cultivating *N. vaccinii* IMV B-7405 on refined and spent after frying potato oil. Thirdly, used oil is a toxic waste, the emissions of which are not regulated in Ukraine, and its use as a substrate will make it possible to simultaneously dispose of hazardous waste and reduce the cost of the target product for crop production.

The first reports about the ability of South African producers to synthesize phytohormones appeared in 2008 (Buensanteai et al., 2008). (The ability of surfactant producers' *R. erythropolis* IMV As-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *N. vaccinii* IMV B-7405 to synthesize phytohormones auxin nature was shown in study (Pirog et al., 2016). Later, there was a report on the formation of indolyl-3-acetic acid by bacteria isolated from soils contaminated with hydrocarbons and heavy metals (Pacwa-Płociniczak et al., 2016). In 2018-2019, several works (Jayakumar et al., 2019; Sabaté et al., 2018; Wu et al., 2018) were published, in which the ability of producers of surface-active lipopeptides and rhamnolipids to synthesize auxins was established. However, in those works, the authors did not try to improve the synthesis of IAA. Thus, our work is the first to report the intensification of auxin synthesis by surfactant producers in the presence of tryptophan.

Note that the literature contains data on the tryptophan effect on the IAA microorganisms' synthesis that don't synthesize surfactants (Dasri et al., 2014; Gopalakrishnan et al., 2015; Kumari et al., 2018; Lebrazi et al., 2020; Nutaratat et al., 2017). Kumari et al. (2018) found that the IAA concentration synthesized by *Bacillus subtilis* DR2 (associated with *Eragrostis cynosuroides*) increased almost 1.2 times (up to 168.1 μg/ml) in the presence of 1.2 g/l tryptophan in a medium with tryptone. In 2020, Lebrazi et al. (2020) showed that the rhizosphere strain *Rhizobium* sp. formed 116.42 μg/ml IAA when 2 g/l tryptophan was added to the medium, which is 1.3 times more than without the precursor. The synthesis of IAA by the unidentified strain DPY-05 increased almost 27 times (up to 67.18 μg/ml) in the presence of 0.5 g/l tryptophan (Dasri et al., 2014). *Enterobacter* sp. DMKU-RP206 strain, isolated from the surface of rice leaves, synthesized on a medium with

lactose and 11 g/l tryptophan up to 5.56 g/l IAA, which is 13.4 times more than without tryptophan (Nutaratat et al., 2017).

It is worth noting that the described producers (Dasri et al., 2014; Kumari et al., 2018; Lebrazi et al., 2020; Nutaratat et al., 2017) are in a certain interaction with plants – as endophytes, phyllosphere or as associated microorganisms. This leads to the formation of higher phytohormones concentrations by these strains to ensure a beneficial interaction with plants. At the same time, the *N. vaccinii* IMV B-7405 studied by us belongs to free-living soil bacteria, for which the synthesis of compounds that stimulate plant growth is not characteristic at all.

However, there are reports about the ability to synthesize phytohormones by free-living bacteria that don't directly participate in the vital activity of plants (McClerklin et al., 2018; Myo et al., 2019). Thus, Myo et al. (2019) established that the neomycin producer *Streptomyces fradiae* NKZ-259 synthesized 4.876 mg/l of IAA on a medium without tryptophan, and in its presence (2 g/l) the level of synthesis increased 20 times (up to 82.363 mg/l).

Data (Dasri et al., 2014; Nutaratat et al., 2017; Kumari et al., 2018; Myo et al., 2019; Lebrazi et al., 2020) indicate that the introduction of precursors is effective in increasing the synthesis of phytohormones. However, in these works, high concentrations of tryptophan (2-11 g/l) were introduced into rich nutrient media with tryptone (Kumari et al., 2018), mannitol (Lebrazi et al., 2020), peptone (Dasri et al., 2014), lactose (Nutaratat et al., 2017), starch (Myo et al., 2019), and the degree of intensification was not exceeded 20 times. Our studies have shown the possibility of intensifying the synthesis of IAA more than 400 times on the medium with spent oil (if only 0.3 g/l of tryptophan, see Table 2). There is currently no such information in the literature.

Data (Gopalakrishnan et al., 2015) show that the auxins synthesis intensification in the presence of tryptophan was caused by the fact that in microorganisms this amino acid is a precursor to the biosynthesis of IAA. The conversion of tryptophan to IAA can be carried out in three ways: through indole-3-pyruvic acid and indole-3-acetaldehyde, tryptamine or indole-3-acetamide.

The results obtained (Figure 1) allow us to assume that the biosynthesis of IAA in *N. vaccinii* IMV B-7405 occurs through the formation of indole-3-pyruvate.

It is known that the criterion of the antimicrobial effect of bioformulations is the MIC – the lowest concentration that causes complete inhibition of the test culture growth visible to the naked eye (Lotfabad et al., 2013). Determination of the MIC makes it possible to simultaneously compare the effectiveness of different antimicrobial formulations.

The choice of test cultures to determine the antimicrobial activity of *N. vaccinii* IMV B-7405 surfactant was determined by the fact that the bacteria *A. tumefaciens*, *P. syringae*, *X. vesicatoria*, *P. carotovorum*, *C. michiganensis*, *P. syringae* pv. *tomato* are known phytopathogens that affect tomato plant cultures (Mansfield et al., 2012). Among these cultures, three cause diseases of tomatoes specifically on the territory of Ukraine (*X. vesicatoria*, *C. michiganensis*, *P. syringae* pv. *tomato*), and three are polyphagous (*A. tumefaciens*, *P. syringae*, *P. carotovorum*), that is except for tomatoes also affect other plants.

The results of our research showed that the addition of tryptophan to the culture medium of *N. vaccinii* IMV B-7405 was accompanied by the synthesis of surfactants, the antimicrobial activity of which not only didn't decrease but also higher than that of surface-active substances obtained without precursor of auxin synthesis. Thus, in the presence of tryptophan, both an increase in the synthesis of auxins (see Tables 1, 2) and an increase in the antimicrobial activity of surfactants synthesized in a complex with phytohormones were observed.

Currently, lipopeptides are the most studied surfactants with antimicrobial action. However there are few works in the literature that determined the MIC of lipopeptides against phytopathogenic bacteria (Abdallah et al., 2018; Bais et al., 2004; Chopra et al., 2020; Etchegaray et al., 2008; Fan et al., 2017; Luo et al., 2015; Mansfield et al., 2012; Phae et al., 1990; Zeriouh et al., 2011). Thus, the MIC of *B. subtilis* 6051 surfactin against *P. syringae* pv. *tomato* DC3000 was 25 μg/ml (Bais et al., 2004). The *B. subtilis* 9407 surfactant complex, the main component of which is surfactin A C13-C16, had an antimicrobial effect on *Acidovorax citrulli* MH21, *P. syringae* pv. *tomato* DC3000, *Khanthomonas campestris* pv. *campestris* Xcc 8004, *Pectobacterium carotovorum* subsp. *carotovorum* Ecc 09, *Pectobacterium atrosepticum* SCRI1043 (zones of growth inhibition 10–18 mm) (Fan et al., 2017). Iturin, synthesized by *B. subtilis* NB22, showed antimicrobial activity against *Xanthomonas oryzae* and *Pseudomonas lachrymans* (MIC 3.13−12.5 μg/ml) (Phae et al., 1990). Iturin, formed by *B. subtilis* OG inhibited the growth of phytopathogenic bacteria *Xanthomonas axonopodis* pv. *citri* and X. *campestris* pv. *campestris* (MIC 10-50 μmol/l) (Etchegaray et al., 2008).

In 2020, Medeot et al. reported that *B. amyloliquefaciens* MEP218 synthesizes fengycin with antibacterial activity against *Xanthomonas axonopodis* pv. *vesicatoria* activity, which distinguishes it from other fengycins, which are mainly antifungal.

In addition to lipopeptides, rhamnolipids and sophorolipids show antibacterial activity. Thus, it was established that the MIC for *Erwinia carotovora* pv. *carotovora*, *R. solanacearum* and *X. campestris* pv. *vesicatoria*, rhamnolipids of *P. aeruginosa* B5, were >50 μg/ml (Kim et al., 2000). Leite et al. (2016) established that under the influence of 15 μl of supernatant (rhamnolipid concentration 0.57 g/l), obtained after cultivation of *P. aeruginosa* P1R16 in olive oil, the growth retardation zone of *R. solanacearum* 1226 was 22 mm. Finally, Chopra et al. (2020) showed that the MIC for *X. campestris* dirhamnolipid synthesized by *P. aeruginosa* RTE4 on glucose (2%) was 5 mg/ml.

Schofield et al. (2013) reported that sophorolipid derivatives (with different degrees of lactonization and acetylation, as well as different acyl chain lengths) and combinations of sophorolipid derivatives showed antibacterial activity against *Acidovorax carotovorum*, *Erwinia amylovora*, *Pseudomonas cichorii*, *P. syringae*, *P. carotovorum, R. solanacearum* and *X. campestris*. Minimum inhibitory concentrations ranged from 2.5 to 10 mg/ml for individual sophorolipid derivatives and from 0.009 to 10 mg/ml for combinations of these surfactants.

The MIC of *N. vaccinii* IMV B-7405 about most of the phytopathogenic bacteria studied by us was in the range of 1.41-22.5 μg/ml. Such indicators are lower than in the above-mentioned articles (Kim et al., 2000; Bais et al. 2004; Schofield et al., 2013; Fan et al., 2017; Chopra et al., 2020), which indicates the high antimicrobial activity of surface-active substances *N. vaccinii* IMV B-7405 against phytopathogenic bacteria. The advantage of the *N. vaccinii* IMV B-7405 strain compared to those described in these works is the ability to simultaneously synthesize surfactants with high antimicrobial activity and phytohormones.

It should be noted that in recent years, reports on the antimicrobial activity of surfactants synthesized in a complex with phytohormones began to appear in the literature (Chen et al., 2021; Chlebek et al., 2020). Thus, the endophyte *Pseudomonas fluorescens* BRZ63 isolated from rapeseed roots produced rhamnolipids, IAA (59.62 μg/ml), siderophores, and salicylic acid (Chlebek et al., 2020). Under the influence of *Pseudomonas fluorescens* BRZ63 metabolites, the degree of growth inhibition of the phytopathogenic fungi *Rhizoctonia solani* W70, *Colletotrichum dematium* K, *Sclerotinia sclerotiorum* K2291 and *Fusarium avenaceum* ranged from 37 to 62%.

Bacillus atrophaeus B44 synthesizes lipopeptides and gibberellins at the same time

(Chen et al., 2021). Lipopeptides of strain B44 showed antimicrobial activity against *R. solani* (growth inhibition zone 16 mm). In contrast to the *N. vaccinii* IMV B-7405 which synthesizes phytohormones and surfactants and is characterized by high antibacterial activity against phytopathogens the surfactants described in (Chen et al., 2021; Chlebek et al., 2020) have only an antifungal effect.

To confirm the possibility of using surfactant *N. vaccinii* IMV B-7405 to control the number of phytopathogens during infection in vivo, *X. vesicatoria* IMV B-9098 and *C. michiganensis* subsp. *michiganensis* IMV B-102 were chosen as test cultures. During their interaction with plants, these pathogens affect, first, the leaves, in connection with which it is possible to conduct a visual assessment of the intensity of the course of the disease.

The results of our research showed the absence of infection for 7 days on a leaf previously treated with surfactant (see Figures 2 and 3). So it was assumed that the treatment of tomato plants with the *N. vaccinii* IMV B-7405 strain will help protect them from damage by phytopathogens.

In the literature (Bolivar-Anillo et al., 2021; Ghadamgahi et al., 2022; Tomar et al., 2014) there is similar information about the use of similar model systems to study the ability of surfactant and phytohormone producers to reduce damage by phytopathogens to the leaves of crops. However, in these works, only the antifungal activity of surfactants was researched. At the same time, in the last two decades, the protection of vegetable crops from bacterial diseases is one of the urgent problems due to their high prevalence and harmfulness. Bacterial diseases cause great economic losses to agriculture, affecting almost all cultivated plant species.

It was established that the pre-treatment of potato leaves with surfactant-containing supernatant of *P. aeruginosa* 1 or a suspension of surfactant-producing cells made it possible to reduce by almost 100% damage to leaves by the phytopathogenic fungus *Phytophtora infestans* (Tomar et al., 2014). Preliminary inoculation of bean leaves with a suspension of *B. subtilis* (lipopeptide producers and phytohormones) led only to the appearance of weak symptoms of *Botrytis cinerea* B05.10 (Bolivar-Anillo et al., 2021). Treatment of potato and strawberry leaves with a *P. aeruginosa* FG106 suspension (producer of rhamnolipids and auxins) made it possible to reduce the infection zone of *B. cinerea*, *P. infestans* and *Phytophthora colocasiae* from 1.6-2.1 cm^2 in control variants (treatment with phosphate-salt buffer) up to 0.1-0.2 cm^2 (Ghadamgahi et al., 2022).

At the last stage, the effect of exometabolites of *N. vaccinii* IMV B-7405 on the yield of tomatoes was studied. For the plants treatment the culture liquid and supernatant after cultivation of the strain on waste oil in the presence of 300 g/l tryptophan was used, since under such conditions the maximum level of auxins was achieved (see Table 2) and high antimicrobial activity of surfactants against tomato bacterial pathogens.

It is known that phytohormones have a positive effect on the tomatoes growth and development (Ahirwar et al., 2015; Almaghrabi et al., 2013; Babu et al., 2015). It was shown that the pre-sowing treatment of tomatoes with PGPR (plant growth-promoting rhizobacteria) strains *P. putida*, *P. fluorescens*, *Serratia marcescens*, *Bacillus amyloliquefaciens*, *B. subtilis* and *B. cereus* increased stem weight, plant height and their yield (Almaghrabi et al., 2013). Thus, after treatment with the *S. marcescens*, the number of fruits increased by 6 pcs/plant, and their total weight increased by 180.3 g. Babu et al. (2015) found that after seed treatment with five unidentified PGPR strains capable of IAA synthesis, the weight of tomatoes exceeded the control by 51.3–116.0%. In addition, the authors noted an earlier formation of flowers on plants treated with bacteria. Note that the same effect was observed during the study of the *N. vaccinii* IMV B-7405 exometabolites influence on tomatoes. Ahirwar et al. (2015) showed that the treatment of seeds with a culture liquid after growing the

Pseudomonas fluorescence SS5 strain isolated from the rhizosphere of tomatoes, capable of IAA synthesis, was accompanied by an increase in the number of fruits by 57% and the total weight of tomatoes by 28% compared to the control.

Conclusion

Therefore, in the presence of low concentrations of tryptophan in the medium with both refined and spent sunflower oil, the surfactant producer *N. vaccinii* IMV B-7405 synthesizes one to two orders higher amounts of auxins than without the precursor of phytohormone biosynthesis. The activity of tryptophan transaminase confirms that tryptophan is involved in the metabolism of *N. vaccinii* IMV B-7405 strain through the indole-3-pyruvate pathway of auxin biosynthesis. In addition, surfactants synthesized in a medium with tryptophan were characterized by higher antimicrobial activity of surfactants against phytopathogenic bacteria compared to those obtained during cultivation without the precursor of auxin synthesis. The ability of surfactant *N. vaccinii* IMV B-7405 to biocontrol the number of phytopathogenic bacteria was manifested not only in *in vitro* studies but also in vivo when conducting research with detached leaves (detached leaf assay). Treatment of seeds of tomato plants with a cultural liquid contributed to the increase in yield and increase in fruit weight. Thus, in the future, the bioformulations based on the exometabolites of *N. vaccinii* IMV B-7405 can be used to control the number of phytopathogens due to the ability to synthesize surfactants and to stimulate plant growth due to the formation of auxins.

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Influence of starch products on the vitality and activity of lactic acid bacteria in yogurt

Olga Ivashchenko[1], Myroslav Khonkiv[1], Viktor Stabnikov[1], Galyna Polishchuk[1], Andrii Marynin[1], Magdalena Buniowska-Olejnik[2]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – Institute of Food Technology and Nutrition, University of Rzeszow, Poland

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Corresponding author:

Galyna Polishchuk
E-mail: milknuft@i.ua

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Abstract

Introduction. Products of enzymatic hydrolysis of corn starch are promising multifunctional ingredients in yogurt, but their use requires studying their influence on the vitality and activity of lactic acid bacteria, as well on some physico-chemical characteristics of the product.

Materials and methods. Yoghurt with a fat content of 1% with maltodextrin, glucose, and glucose-fructose syrup in an amount of 9% in terms of dry matter were studied. The number of lactic acid bacteria cells was determined by plating on MRS solid nutrient medium, water activity was measured using a HygroLab 2 device, syneresis of clots was estimated by centrifugation, and active acidity – by the potentiometric method.

Results and discussion. The influence of starch product with different dextrose equivalents addition on the viability and activity of lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* during fermentation and storage of yogurt has been studied. An increase of dextrose equivalent and monosaccharides content in starch products reduce the fermentation time of milk due to the increase of lactic acid bacteria activity. A slight decrease in water activity in the presence of glucose-fructose syrup in yogurt in an amount of 9% had virtually no effect on the milk fermentation process. The number of lactic acid bacteria increased during the first seven days of yogurt storage added with glucose-fructose syrup. On the 14th day of storage, the concentration of cells of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* became almost the same in all yogurts due to almost complete consumption of carbon sources. When the storage of yogurt was extended to 28 days, the most stable content of lactic acid bacteria was found in yogurt added with maltodextrin due to its prebiotic properties. The increases of active acidity and syneresis in all yogurts were greatest in the first 8–14 days. Presence of dextrins in yogurt stabilizes its physical and chemical properties during storage.

Conclusions. Adding starch-containing products to yogurt allows to activate the activity of lactic acid bacteria and improve their survival during yogurt storage.

Introduction

Yogurt is one of the most popular fermented beverage characterized by exceptional taste, thick consistency and containing at least 10^7 CFU/ml of lactic acid bacteria beneficial to human health (Gómez-Gallego et al., 2018, Hadjimbei et al., 2013). Dessert yoghurts additionally contain food additives or fillers including sweeteners, of which sucrose, honey and fructose are most often used (Kang et al., 2019; Martínez et al., 2024; Prokisch et al., 2022).

Carbohydrates are consumed by lactic acid bacteria during the fermentation of milk mixtures (Ayivi et al., 2020; Yeboah et al., 2023;), therefore, when using sweeteners of a carbohydrate nature, it is relevant to study their effect on activity and vital functions of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* present in starter cultures.

There is limited information about the specific effects of carbohydrates of sweeteners on the activity of lactic acid bacteria in yogurt. Thus, Popa and co-authors (2011) did not find a significant difference between the effects of various sweeteners (honey, sucrose, glucose-fructose syrup, and inulin) on the activity and viability of lactic acid bacteria and bifidobacteria in bio-yogurt.

The highest activity of *Lactobacillus acidophilus* was observed in the presence of honey with different oligosaccharide contents (Popa et al., 2011). Addition of fructose and oligofructose in an amount of 2% in fermented milk drinks during their treatment with probiotic starter cultures of *Lactobacillus*, in particular *L. brevis* B1, improved their physicochemical, rheological, sensory and microbiological properties (Danylenko et. al., 2022; Zielinska et al., 2021).

The presence of oligofructose and fructose ensured a higher amount of lactic acid bacteria in fermented milk drinks for 35 days during storage compared to control where no saccharides were added. Oligofructose turned out to be the most effective stimulator of bacterial growth. During storage, pH values decreased in all samples, which was most obvious between days 7 and 21 (Zielińska et al., 2021).

Along with traditional sweeteners, products of enzymatic hydrolysis of starch are widely used in dessert products (Eke-Ejiofor, 2015; Nikolić et al., 2023).

The degree of starch hydrolysis is expressed by the dextrose equivalent value, which indicates the number of dextrose molecules released as a result of starch hydrolysis. The dextrose equivalent value can range from 0 for starch to 100 for glucose (Hidayat et al., 2015). Dextrins in starch products with medium and low dextrose equivalent effectively bind water, thicken milk drinks and ice cream mixtures, reduce the perception of sweetness, serve as a source of solids, and also imitate fat (Bass et al., 2017; Rayhani et al., 2008). Dextrins with the same dextrose equivalent may differ in physicochemical characteristics, in particular viscosity, sweetness, solubility, and bioavailability (Sun et al., 2020), which requires studying the functional and technological properties of each type of starch product in food.

Research of the dextrin use in yogurt are limited. Thus, it was shown that the addition of wheat dextrin in an amount of 15 g had a positive effect on the fermentation process, as well as viscosity, acidity, syneresis and overall acceptability of the product (Peerkhan et al., 2021). But the work did not study the peculiarities of the fermentation process and the microbiological characteristics of yogurt with dextrins.

It is known that monosaccharides (glucose and fructose) formed during the hydrolysis of starch affect fermentation processes (McGregor et al., 1987; Parker et al.,

2010), act as sweeteners, and reduce water activity, which can affect shelf life of food products (Jia et al., 2017). However, they do not exhibit structuring ability.

Despite their low cost, availability on the market and technological advantages, starch products are still used quite limitedly in dairy industry (Polischuk et al., 2019; Zargaraan et al., 2016). At the same time, glucose-galactose syrup (Mosquera-Martínez et al., 2023) and chemically modified starches (Cui et al., 2014; Schmidt et al., 2007) are more widely used in yogurt production. Meanwhile, functional waxy corn starches (CLARIA® Elite, Plus, Essential Starch), obtained from Tate & Lyle, (USA) in quantities of 1.88 and 2.35 (by nominal phase volume) demonstrated a consistent texture accompanied by the fullest taste.

The phase volume was calculated based on the effective starch concentration (c) in the continuous phase and the effective swelling volume after treatment (q). It has been found that yoghurts containing starch with a low effective volume of starch swelling (30 ml/g) and a phase volume slightly above the dense packing point (1.41) have the most preferable sensory properties (Wong et al., 2020). The combined effect of lactic acid bacteria producing exopolysaccharides with a known structure and starch (0.75%) on the rheological and physical properties of yoghurts was also studied (Verni et al., 2019). It has been found that the addition of starch to yogurt increases the importance of the rheological and physical properties of all blended yogurts, which probably occurs through repulsion between proteins and polysaccharides that contributes to thermodynamic incompatibility. But the microbiological indicators of yogurt with exopolysaccharides and starch were also not studied by the authors.

Based on a set of physicochemical indicators of yogurt samples with starch products, the feasibility of using molasses with an average dextrose equivalent in its composition was proven (Ivashchenko and Polishchuk, 2023). Molasses added into yogurt served as a thickener, stabilizer and sweetener. At the same time, the influence of starch destruction products on microbiological properties and physico-chemical characteristics of yogurt has not been studied.

Thus, chemically modified starches in yogurt perform only the function of structuring and moisture binding, which affects the consistency of the fermented milk clot and prevents syneresis. At the same time, starch hydrolysis products, as multifunctional ingredients, can thicken and stabilize the structure (at medium and low dextrose equivalent), sweeten dairy products with varying intensity depending on the depth of starch hydrolysis, and be a source of solid substances. In turn, their influence on the activity and viability of lactic acid bacteria has been practically not studied.

Therefore, the aim of the presented research was to study the influence of starch products with different dextrose equivalents on the microbiological and physicochemical characteristics of yogurt.

Materials and methods

Materials

The following products of enzymatic hydrolysis of corn starch were selected for the study: dry maltodextrin MD-10 (dextrose equivalent 10); dry glucose syrup GS-42 (dextrose equivalent 40-42); glucose-fructose syrup GFS-42 (dextrose equivalent 98).

The carbohydrate composition of the hydrolysis products of corn starch is given in Table 1.

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Table 1

Carbohydrate composition of maltodextrin, dry glucose syrup and glucose-fructose syrup

Content	MD-10	GS-42	GFS-42
Dry matter (DM)%, not less	95	95	70
Glucose, % DM	1.0	16	54
Fructose, % DM	-	-	42
Maltose, % DM	3.4	15	2
Maltotrioses, % DM	5.0	16	1
Dextrins, % DM	90.6	53	1

Preparation of yogurt samples

Yogurt samples were prepared from normalized milk to obtain a finished product with a fat content of 1%. According to changes to the Standard of Identity for Yogurt developed by the Food and Drug Administration (FDA, 2023), 1% fat yogurt can be classified as low-fat (0.29 to 1.76% fat).

The control yogurt contained 4% skim milk powder and 5% sucrose. The total content of dry matter of milk powder and sucrose (9%) in the test samples was replaced with an equivalent dry matter content of starch products.

The specified composition of yogurt was previously justified based on the results of an analysis of quality indicators of the studied samples with varying contents of starch hydrolysis products (Ivashchenko and Polishchuk, 2023). The balance of dry matter content in the sample with liquid glucose-fructose syrup was maintained by adding an equivalent content of skimmed milk powder.

The abbreviations for the yoghurt samples are given below:

- Control (contains 5% sucrose and 4% skimmed milk powder);
- Sample 1 (contains 9% dry maltodextrin MD-10);
- Sample 2 (contains 9% dry glucose syrup GS-42);
- Sample 3 (contains 9% dry matter of GFS glucose-fructose syrup).

Yogurt samples were prepared in volumes of 200 ml. Starch products and other recipe ingredients were added to milk normalized for fat content before pasteurization with stirring using a Daihan HS-50 A laboratory mixer for 2 minutes at a speed of 200 rpm.

The samples were filtered and pasteurized at a temperature of 87±2ºC for 2-3 minutes. The pasteurized mixtures were cooled to a temperature of 41±1°C and inoculated with 10^7 CFU in 1 ml of pure cultures until titrated acidity values did not exceed pH 4.8. For fermentation of milk mixtures, a starter was used to produce yogurt containing lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Before fermentation, the dry lyophilized starter was activated in part of the thermally treated milk for 2 hours at a temperature of 41±1°C.

During fermentation, the dynamics of changes in the active acidity of yogurt samples was studied. After fermentation, yogurt samples were cooled to a temperature of 4±2 C and the water activity and concentration of lactic acid bacteria cells were determined. During storage in a refrigerator for up to 28 days at a temperature of 4±2 °C, the degree of syneresis, active acidity and concentration of lactic acid bacteria cells were determined in yogurt samples.

Methods

Active acidity was measured potentiometrically using a laboratory pH/MV/ISE/Temp ADWA AD1200 ATC meter.

Water activity (Aw) was determined using a HygroLab 2 device (Rotronic, Switzerland), with an accuracy of ±0.001 Aw units. The water activity indicator was expressed from 0.00 to 1.00 Aw (0-100% rh). The measurement was carried out at a temperature of 20 ºC. Before measurement, the device was calibrated against a special humidity standard (95% HR).

The number of lactic acid bacteria cells in yogurt was determined by serial tenfold dilution with sterile saline (0.85% NaCl) followed by plating on MRS (De Man–Rogosa–Sharpe) solid nutrient medium (CondaLab, Spain). To create anaerobic conditions, inoculated Petri dishes were filled with an additional layer of MRS agar, cooled to 45ºC. Colony-forming units (CFU) were counted after anaerobic incubation at 37°C for 48 hours.

Syneresis of milk-protein yogurt clots was determined by centrifugation. To do this, 25 ml of yogurt, after mixing in a calibrated test tube, was centrifuged using a laboratory centrifuge Sigma 2-6E (Germany) for 20 minutes at 1000 rpm and a temperature of 20ºC, and the volume of separated whey was measured, which was expressed by in ml per 100 g of product (Polischuk et al., 2020). The measurements were carried out during storage of yogurt samples after 0, 7, 14, 28 days.

Statistical analysis of the experiment results was carried out using Statistica 6.0, Microsoft Office Excel 2007 and Mathcad. Data were expressed as mean ± standard deviation to define three measurements.

Results and discussion

The content of lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* in freshly prepared yogurt samples is shown in Figure 1.

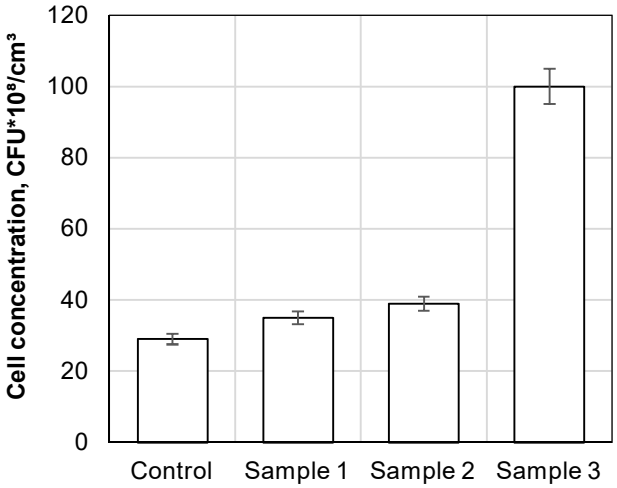

Figure 1. Concentration of lactic acid bacteria cells in fresh yogurt

According to Figure 1, the positive effect of all starch hydrolysis products on the activity of lactic acid bacteria is obvious. It should be noted the significant role of glucose-fructose syrup in activating the vital activity of lactic acid bacteria cells in the composition of yogurt starter due to its higher bioavailability for bacteria compared to other starch products.

By the level of additive influence on the lactic acid bacteria activity, they can be placed in the following sequence: GFS > GS-42 > MD-10. Thus, the content of bacterial cells in the sample with glucose-fructose syrup was 2.8 times higher than in yogurt with maltodextrin and 2.5 times higher than in yogurt with glucose syrup.

Fructose consists 42% of dry matter in glucose-fructose syrup, the positive effect of which on lactic acid bacteria is well known (Zielińska et al., 2021).

Mono-, di- and trisaccharides in the composition of GFS and CS-42 are bioavailable carbon sources and promote the active development of lactic acid bacteria during milk fermentation, in contrast to indigestible high-molecular prebiotics, such as fructooligosaccharides and galactooligosaccharides activating beneficial microbiota in the human intestine (Davani-Davari et al., 2019).

The prebiotic properties of maltodextrin were shown by Bisar et al. (2019). In this study, maltodextrin moderately activated lactic acid bacteria *S. thermophilus* and *L. delbrueckii* spp. *bulgaricus* during milk fermentation.

The effect of GS-42 on milk fermentation was close to that of the yogurt with MD-10, which is explained by the rather high content of higher sugars in them – 53% and 90.6%, respectively.

The concentration of cells of lactic acid microorganisms in yogurt samples (Figure 1) is correlated with data on changes in active acidity during fermentation of milk mixtures (Table 2).

Table 2

Changes in the active acidity of milk mixtures with starch products during fermentation*

Yogurt	Duration of fermentation, hours						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Control	6.57 ±0.21	6.10 ±0.14	5.60 ±0.19	5.02 ±0.15	4.72 ±0.19	4.68 ±0.15	4.55 ±0.16
Sample1	6.54 ±0,20	6.42 ±0.18	6.05 ±0.20	5.70 ±0.18	5.14 ±0.19	4.75 ±0.18	4.63 ±0.16
Sample 2	6.40 ±0.20	6.27 ±0.17	5.71 ±0.17	5.10 ±0.20	4.80 ±0.15	4.55 ±0.14	4.51 ±0.13
Sample 3	6.23 ±0.17	5.73 ±0.16	5.17 ±0.16	4.77 ±0.18	4.45 ±0.16	4.38 ±0.14	4.30 ±0.15

* pH values of 4.8 and below are highlighted with a dark background, which is a criterion for completing the milk fermentation process.

The results show that glucose-fructose syrup, due to its high content of monosaccharides, accelerates fermentation by about 1 hour; glucose syrup has virtually no effect on the activity of lactic acid bacteria, and maltodextrin even somewhat prolongs the achievement of proper acidity. This could be explained by the different ratio between monosaccharides, oligosaccharides and dextrins in the products of starch hydrolysis, which exhibit prebiotic properties differently and bind free water (Gänzle et al., 2012).

Changes of the concentration of lactic acid bacteria cells in yogurt samples with starch products of varying degrees of hydrolysis during storage up to 28 days is shown in Figure 2.

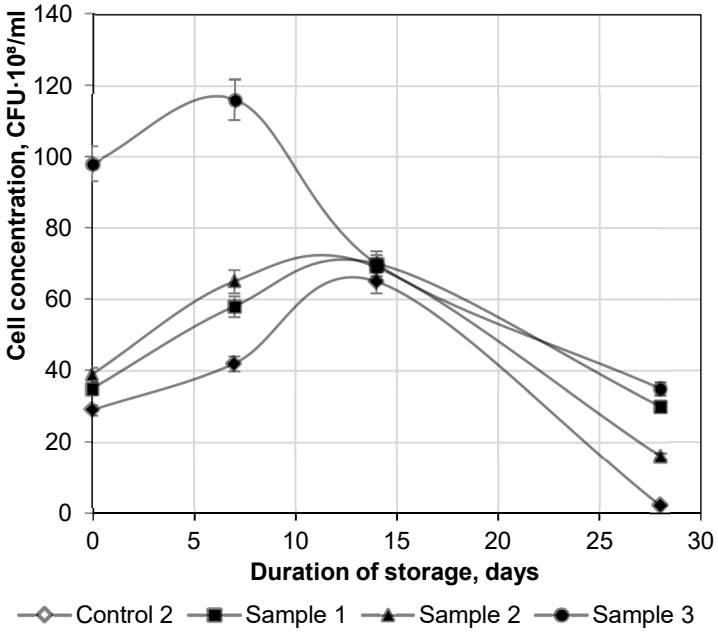

Figure 2. Concentration of lactic acid bacteria cells during storage of yogurt samples with starch products

During storage of yogurt, the increase in the concentration of microbial cells actively continued in sample 3 with glucose-fructose syrup in the first 7 days, after which, on the 14th day, their minor dieback was observed. Samples 1 and 2 showed a similar pattern of changes in the concentration of microbial cells, which maximally increased on the 14th day of storage as the limit for the suitability of yogurt for consumption.

In the control yogurt, the content of bacterial cells also increased on the 14th day, followed by their significant decrease on the 28th day of storage. It should be noted that the concentration of lactic acid bacteria on the 14th day of storage becomes almost the same for all yoghurts, which is due to the almost complete consumption of available carbon sources by the bacteria. Maltodextrin (sample 1) had the greatest positive effect on the viability of lactic acid bacteria during long-term storage of yogurt, which to some extent contributed to the stabilization of the quality of the product. The detected effect coincides with the data of Batawy et al. (2018).

Figure 3 shows the dynamics of active acidity of yogurt samples during storage for up to 28 days at a temperature of 4±1 °C.

According to Figure 3, in all samples during storage there was a decrease in active acidity, which is especially typical during the period from the beginning of storage from 2-4 days to 16-20 days. Sample 1 with maltodextrin had the most stable acidity indicators. At the same time, the acidity of sample 3 with glucose-fructose syrup demonstrated high activity of lactic acid bacteria, even when yogurt was stored at low positive temperatures, especially in the period from 2 to 16 days. The recommended active acidity value for yogurt is usually set at 4.5 and below (Weerathilake et al., 2014). But from January 1, 2024, in accordance to the decision of the Food and Drug Administration (FDA), the maximum pH value of yogurt is set to 4.6 and below.

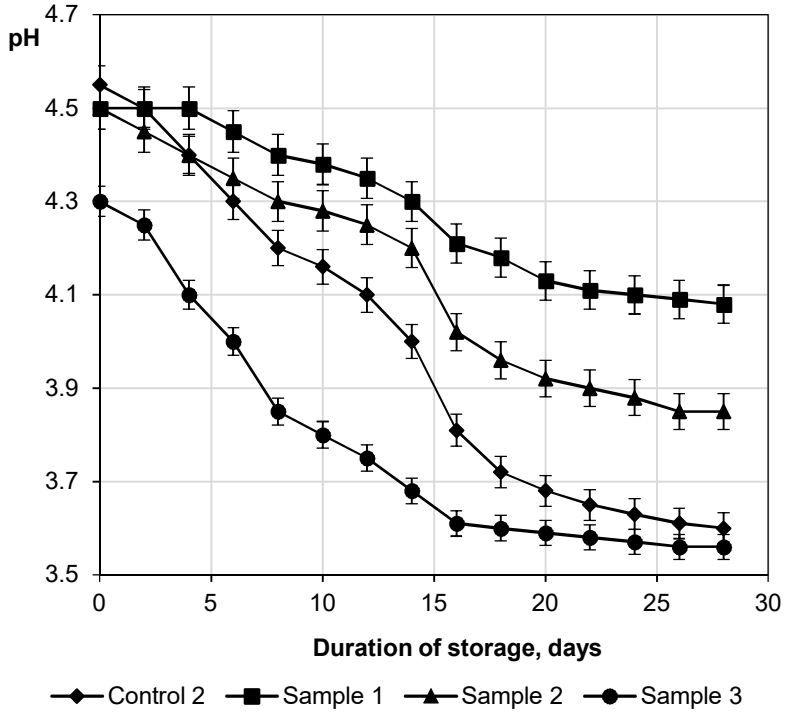

Figure 3. Changes in active acidity of yogurt samples with starch products during storage

As for the minimum pH value of yogurt, it is recommended to keep it at 4.0 to avoid the product having an overly sour taste (Deshwal et al., 2021). Following these requirements, the recommended pH range for yogurt is 4.0 to 4.6. According to the specified criterion, a sample of yogurt with GFS meets requirements for active acidity values only during the first 6 days of storage. The control yogurt meets requirements for active acidity within 14 days, and the sample with glucose syrup within 18 days. As for the sample with maltodextrin, its active acidity is quite stable throughout the entire storage period.

At the next stage of study, the degree of syneresis of yogurt with starch products during storage was studied. The results are shown in Table 3.

Table 3

Syneresis of yoghurt samples during storage

Yogurt	Time of storage, days							
	0	**4**	**8**	**12**	**16**	**20**	**24**	**28**
Control	2.0 ±0.11	3.5 ±0.18	5.3 ±0.20	5.8 ±0.21	6.4 ±0.19	6.6 ±0.22	6.8 ±0.23	6.9 ±0.24
Sample1	0.5 ±0.02	2.2 ±0.15	3.2 ±0.17	3.8 ±0.14	4.2 ±0.16	4.3 ±0.16	4.3 ±0.22	4.4 ±0.21
Sample 2	1.1 ±0.04	2.8 ±0.16	4.0 ±0.17	4.5 ±0.18	4.7 ±0.18	4.9 ±0.19	5.0 ±0.20	5.2 ±0.19
Sample 3	2.0 ±0.15	4.2 ±0.17	6.1 ±0.02	6.6 ±0.20	6.9 ±0.23	7.3 ±0.24	7.7 ±0.20	7.9 ±0.28

During storage, syneresis of all samples increased most in the first 8-12 days, after which this indicator somewhat stabilized. The control sample of yogurt without starch products and the sample of yogurt with glucose-fructose syrup retained moisture the worst.

Samples with maltodextrin and glucose syrup, containing moisture-binding higher sugars, demonstrated the least syneresis, which can be explained by the participation of high-molecular starch destruction products in additional cross-linking of the protein gel (Arab et al., 2023). Thus, the presence of high molecular weight residues of starch destruction improves the consumer properties of yogurt, including storage time.

The water activities in the yogurt samples are shown in Figure 4.

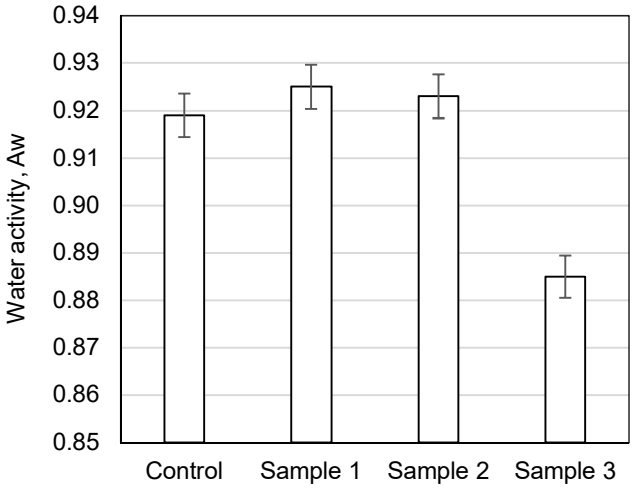

Figure 3. Water activity in the yogurt samples

Glucose-fructose syrup has the greatest influence on water activity in yogurt due to high concentration of glucose and fructose, 96% from total solids (Zuorro, 2021). As a result of a decrease in the content of monosaccharides in glucose syrup and maltodextrin, the activity of water in yogurt 1 and 2 increases slightly, but is almost no different from the activity of water in the control yogurt. So, starch products with low and medium dextrose equivalent affect water activity, similar to the combined effect of sugar and skimmed dry milk in the control yogurt. As for yogurt 3, a slight decrease in water activity to 0.855 did not significantly effect on the activity and vital activity of lactic acid bacteria during yogurt storage.

Thus, based on the results of the study, it can be concluded that the carbohydrate composition of starch products has a direct effect on the microbiological and physico-chemical parameters of yogurt, including throughout the entire shelf life. With an increase in the dextrose equivalent of starch products, their ability to accelerate the fermentation process of milk mixtures increases, which is accompanied by a more active accumulation of lactic acid bacteria cells in freshly made yogurt. At the same time, maltodextrin, due to its prebiotic properties, to some extent stabilizes the viability of lactic acid bacteria during long-term storage of yogurt.

The results of the study are of practical importance, since they allow to take into account the peculiarities of using starch products with different carbohydrate compositions.

Conclusions

1. The ability of starch products to influence water activity and the dynamics of milk fermentation by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* depends on their carbohydrate composition. By increasing the dextrose equivalent, the monosaccharides in starch syrup, bioavailable to bacteria, reduce the fermentation time of milk in the production of yogurt. The effectiveness of this influence increases in the following sequence: maltodextrin → glucose syrup → glucose-fructose syrup. A slight decrease in water activity when the glucose-fructose syrup content in yogurt is 9% has virtually no effect on the dynamics of the milk fermentation process.
2. During storage of yogurt, there is an active increase in the concentration of bacterial cells in the presence of glucose-fructose syrup in the first 7 days, and on the 14^{th} day their minor dieback was observed. On the 14^{th} day of storage, the concentration of microbial cells *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* becomes almost the same for all samples due to almost complete consumption of the available carbon sources. When yogurt storage is extended to 28 days, maltodextrin has the greatest effect on the viability of lactic acid bacteria due to its prebiotic properties.
3. Starch products with different dextrose equivalents can be used in yogurt, taking into account the specifics of their effect on the activity and viability of bacterial cells of the starter and the specified physico-chemical parameters of the finished product.
4. Further research will include studying the sensory characteristics and syneretic ability of milk-protein yogurt clots during storage

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Consumption and frequency of wine drinking in V4 countries

Katarína Bírová[1], Patrik Rovný[1], Jozef Palkovič[1], Martin Vondráček[2]

1 – Slovak University of Agriculture in Nitra, Slovak Republic
2 – Czech University of Life Sciences Prague, Czech Republic

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Corresponding author:

Katarína Bírová
E-mail: xbirovak@uniag.sk

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Abstract

Introduction. The aim of the article is to evaluate the consumption and frequency of drinking wine in the countries of the Visegrad Four.

Materials and methods. The data were obtained from the sources: Standard EU Alcohol Survey / Deep Seas, DATACube, Public Database VDB, Wine Consumption per Capita in Hungary, Wine Consumption per Capita in Poland. The following methods were used: The Kruskal Wallis test, Analysis of Variance Kruskal Wallis and Mann-Whitney test.

Results and discussion. Wine consumption in the individual countries of the Visegrad Four varies. Hungary has achieved the highest consumption in the last period, where the average consumption of wine in pure alcohol for the last decade (2010–2021) is 3.2 liters. The analysis shows that when evaluating all age categories in terms of frequency of wine drinking, the largest group (18.42%) are wine consumers who drink wine 2–5 days a year. The second largest group (16.58%) are wine consumers who drink wine 1–2 days a week. The third group are consumers (16.38%) with a frequency of drinking 2–3 days a month. From the point of view of the comparison of individual countries, it follows that in all four analyzed countries consumers consume wine occasionally but the most in Poland. From the point of view of regular consumption, the Czech Republic stands out. Wine consumers in Hungary and Slovakia have the same behaviour in contrast to the Czech Republic and Poland. On the other hand wine consumers in the Czech Republic and Poland have similar consumer behaviour. Among other comparisons of the states, there are significant differences in wine consumption in terms of the frequency of alcohol drinking.

Conclusion. Czech, Slovak and Hungarian winemakers who are starting to expand into the Polish market have a chance. Hungary has recorded the highest wine consumption in recent years, with an average consumption of 3.2 liters of pure alcohol per capita. Due to the globally decreasing area of vineyards, the demand as well as the prices of wine will increase.

Introduction

Alcoholic beverages are among the basic cultural features all over the world (World Population Review, n.d.). Therefore, many of the countries take alcohol consumption as a normal thing and accept the availability of alcoholic beverages on the market as well as its promotion (Rehm et al., 2003). The first study on alcohol consumption was made in 1965 (Mandelbaum, 1965). However, the consumption of alcohol has a deeper character because alcohol is an addictive substance and its consumption has a negative effect on human health (McClure et al., 2013; Probst et al., 2020; Rehm et al., 2003). A recent study pointed out that for people between the ages of 15 and 39 consuming excessive amounts of alcohol can have a negative effect on overall health. Although differences in the risks of ill health by age or gender have not been demonstrated (GBD 2020 Alcohol Collaborators, 2022). For example, a French study talks about the positive effects of red wine on the cardiovascular system (Brownlee, 2006; Das et al., 2011; Higgins and Llanos, 2015). However, alcohol does not only affect health aspects if we look at it from another point of view, an equally important economic, business, trade and social perspective appears (Gavurova & Tarhanicova, 2021; Ranaweera et al., 2018; Laramee et al., 2013; Smutka et al., 2015; Svatoš & Smutka, 2012; Vološin et al., 2011; Hamdan et al., 2023). Therefore, it is important to consider the responsibility in business (Jurásek et al., 2021; Mnerie et al., 2016; Oliinyk et al., 2023). Combining it with the investigation of current peculiarities of food and beverage manufacturing development (Al-Zu'bi & Albloush, 2022; Ditsiou et al., 2023; Fachrurrozie et al., 2023, Huszka et al., 2022; Jencova et al., 2022; Nurliza & Oktoriana, 2021; Yousuf et al., 2022; Yousuf et al., 2023; Zsarnóczai et al., 2021)

In Europe, drinking alcohol is strongly linked to culture and is therefore characterized by its high consumption and by preference a different type of alcohol (Glover, 2000). According to Iontchev (1998) and Popová et al. (2007), alcohol drinking cultures are divided into three patterns. The Mediterranean pattern dominated by wine and fruit brandy. The Central European pattern includes countries such as Slovakia, the Czech Republic and Germany, which are specific for drinking beer as well as fruit spirits. With a closer specification the consumption of fruit spirits has been increasing in Slovakia for the last decades, while in the Czech Republic, on the contrary, beer drinking still dominates. The Northern European pattern is characterized by drinking spirits, respectively vodka. Following the fall of the communist regime a wider range of alcohol came onto the market and the lack of regulation led to an increase in its consumption (Leon et al., 1997; Zatonski & Jha, 2000). Even after the collapse of the communist bloc the countries of the Vyšehrad Four are important representatives. policies (Čábelková et al., 2022; Strielkowski et al., 2022) such as infrastructure, energy, digitization and, last but not least, agriculture (Visegrad Group (V4) | Ministry of Investments, Regional Development and Informatization of the Slovak Republic, 2020).

Even the V4 countries have one common tradition, the tradition of drinking alcohol which is also mentioned by the WHO. In 2019 the Czech Republic ranked in the top ten countries with the highest consumption of pure alcohol, namely in third place with an average value of 13.29 liters. Before the Czech Republic there were countries like Romania and Georgia. Next, Poland took twelfth place with an average value of 11.63 liters. Hungary and Slovakia ranked 23rd and 25th with average values of 10.60 and 10.48 liters. The V4 countries are therefore in the first top 25 out of a total of 198 countries in the world monitored by the WHO (Indicator Details, n.d.-b).

If we compare wine with other drinks or foods it is wine that stands out in terms of cultural and social factors and traditions such as age, gender, education, perceived risk,

religion or ethnicity (Camillo, 2012; Hussain et al., 2007; Somogyi et al. al., 2011; Outreville and Desrochers, 2016; Pape et al., 2017). The association of wine in relation to food, the symbolism of wine or other characteristics such as the frequent association of wine with a gift (Deroover, et all., 2021). As we mentioned, wine has its characteristic position in human association, and therefore we can say that people satisfy psychological and sensory aspects with wine. These aspects evoke in consumers a desire for communit or the need to belong to a chosen company, mutual communication and the possibility of untying the personality (Bruwer and Li, 2007; Mnerie et al., 2016; Platania, 2016; Naglova et al., 2017; Olsen et al., 2007; Orth, 2005; Orth and Bourrain, 2005).

The wine industry in the V4 countries is strongly influenced by climatic conditions. The most suitable territory for the wine industry is Hungary which also has the highest consumption of wine in the entire V4. Hungary has more than 65,000 hectares of vineyards and annual consumption exceeds 240,000 liters. Next comes the Czech Republic which has approximately 16,000 hectares and their annual wine consumption exceeds 200,000 liters. Although Slovakia is a small country, it has more than 11,000 hectares of vineyards and an annual consumption of 85,000 liters of wine. The most inhospitable country for wine is Poland, even though it is large in area, it has only 700 hectares of fertile vineyards and annual consumption is 320,000 liters. However, when we compare it in terms of wine consumption per capita, we can determine the following order: Hungary, the Czech Republic, the Slovak Republic and Poland with the lowest wine consumption (Rogovska, 2018).

The aim of research is to evaluate the consumption and frequency of drinking wine in the countries of the Visegrad Four.

Materials and methods

The data were obtained from the sources (Standard EU Alcohol Survey/ Deep Seas, n.d.), (DATACube., n.d.), (Public Database VDB, n.d.), (Wine Consumption per Capita in Hungary, 2021), (Wine Consumption per Capita in Poland, 2021) and were processed in Excel and SAS. The pan-European alcohol survey was conducted from 24 April to 22 July 2020. The number of respondents for each country was: Slovakia (1505), the Czech Republic (1417), Hungary (1876) and Poland (1509), taking into account a sample that it consisted only of respondents who consume alcohol and specifically wine. The following data were processed on the basis of statistical characteristics such as age range, gender, countries of the Visegrad Four and specification of the frequency of wine drinking.

Coding:

- Age range:
  - 1 age group – 18–34 years
  - 2 age group – 35–44 years
  - 3 age group – 45–64 years

Only adults (economically active) between the ages of 18 and 64 were approached in the research. From the point of view of age categories the following intervals were used: 18-34 (young adults), 35-44 (middle-aged adults, 45–64 (old-aged adults). These categories are used in international research on alcohol consumption (Kilian, 2023).

- Frequency of drinking wine

Within the frequency of drinking alcohol in the first part we analyzed the frequency of drinking alcohol for each answer separately, and in the second part of the research, 3 basic sets were developed according to Table 1. The division was organized for the purpose of easier interpretation of the frequency of wine drinking.

Table 1

Frequency of drinking wine and its distribution

1	Every day	Regularly
2	5-6 days a week	
3	3-4 days a week	
4	1-2 days a week	
5	2-3 days out of the month	Occasionally
6	One day of the month	
7	6-11 days of the year	
8	2-5 days of the year	
9	Day in the last year	Almost never
10	I don't drink now but i used to drink	
11	I never drank in my life	

Statistical Methods

The Kruskal Wallis test is a nonparametric hypothesis test that compares three or more independent groups. Statisticians also refer to it as one-way ANOVA on ranks. This analysis extends the Mann Whitney U nonparametric test that can compare only two groups. If you analyze data, chances are you're familiar with one-way ANOVA that compares the means of at least three groups. The Kruskal Wallis test is the nonparametric version of it. Because it is nonparametric, the analysis makes fewer assumptions about your data than its parametric equivalent. Many analysts use the Kruskal Wallis test to determine whether the medians of at least three groups are unequal. However, it's important to note that it only assesses the medians in particular circumstances. Interpreting the analysis results can be thorny. At its core, the Kruskal Wallis test evaluates data ranks. The procedure ranks all the sample data from low to high. Then it averages the ranks for all groups. If the results are statistically significant, the average group ranks are not all equal. Consequently, the analysis indicates whether any groups have values that rank differently. For instance, one group might have values that tend to rank higher than the other groups. The Kruskal Wallis test doesn't involve medians or other distributional properties—just the ranks. In fact, by evaluating ranks, it rolls up both the location and shape parameters into a single evaluation of each group's average rank. Like one-way ANOVA, the Kruskal Wallis test is an "omnibus" test. Omnibus tests can tell you that not all your groups are equal, but it doesn't specify which pairs of groups are different.

Specifically, the Kruskal Wallis test evaluates the following hypotheses:

- Null: The average ranks are all the same.
- Alternative: At least one average rank is different.

Again, if the distributions have similar shapes, you can replace "average ranks" with "medians." Imagine you're studying five different diets and their impact on weight loss. The Kruskal Wallis test can confirm that at least two diets have different results. However, it won't tell you exactly which pairs of diets have statistically significant differences.

So, how do we solve this problem? Enter post hoc tests. Perform these analyses after (i.e., post) an omnibus analysis to identify specific pairs of groups with statistically significant differences. A standard option includes Dunn's multiple comparisons procedure. Other options include performing a series of pairwise Mann-Whitney U tests with a Bonferroni correction or the lesser-known but potent Conover-Iman method.

Analysis of variance Kruskal-Wallis

This is a method for comparing several independent random samples and can be used as a nonparametric alternative to the one way ANOVA.

The Kruskal-Wallis test statistic for k samples, each of size ni is:

$$T = \frac{1}{s^2}\left[\sum_{i=1}^{k}\frac{R_i}{n_i} - N\frac{(N+1)^2}{4}\right] \quad (1)$$

where N is the total number (all ni) and Ri is the sum of the ranks (from all samples pooled) for the ith sample and:

$$S^2 = \frac{1}{N-1}\left[\sum_{all} R_{ij}^2 - N\frac{(N+1)^2}{4}\right] \quad (2)$$

The null hypothesis of the test is that all k distribution functions are equal. The alternative hypothesis is that at least one of the populations tends to yield larger values than at least one of the other populations.

Assumptions:

- Random samples from populations
- Independence within each sample
- Mutual independence among samples
- Measurement scale is at least ordinal
- Either k population distribution functions are identical, or else some of the populations tend to yield larger values than other populations.

If the test is significant, you can make multiple comparisons between the samples. You may choose the level of significance for these comparisons (default is a = 0.05). All pairwise comparisons are made and the probability of each presumed "non-difference" is indicated (Hollander and Wolfe, 2013; Conover, 1999; Critchlow and Fligner, 1991;). Two alternative methods are used to make all possible pairwise comparisons between groups; these are Dwass-Steel-Critchlow-Fligner and Conover-Iman. In most situations, you should use the Dwass-Steel-Critchlow-Fligner result. By the Dwass-Steel-Critchlow-Fligner procedure, a contrast is considered significant if the following inequality is satisfied:

$$W_{ij} = \frac{\frac{n_i(n_i+n_j+1)}{2}}{\frac{n_i n_j}{24}\left[n_i+n_j+1-\frac{\sum_{b=1}^{g_{ij}}(t_b-1)t_b(t_b+1)}{(n_i+n_j)(n_i+n_j-1)}\right]} > q_{a,k}, for\ 1 \le i \le j \le k \quad (3)$$

Where q is a quantile from the normal range distribution for k groups, ni is size of the ith group, nj is the size of the jth group, tb is the number of ties at rank b and Wij is the sum of the ranks for the ith group where observations for both groups have been ranked together. The values either side of the greater than sign are displayed in parentheses in StatsDirect results. The Conover-Iman procedure is simply Fisher's least significant difference method performed on ranks. A contrast is considered significant if the following inequality is satisfied:

$$\left|\frac{R_j}{n_j} - \frac{R_i}{n_i}\right| > t_{1-a/2}\sqrt{S^2\frac{N-1-T}{N-k}}\sqrt{\frac{1}{n_i}+\frac{1}{n_j}} \quad (4)$$

Where T is a quantile from the Student T distribution on N-k degrees of freedom. The values either side of the greater than sign are displayed in parentheses in StatsDirect results. An alternative to Kruskal-Wallis is to perform a one way ANOVA on the ranks of the observations. StatsDirect also gives you an homogeneity of variance test option with Kruskal-Wallis; this is marked as "Equality of variance (squared ranks)". Please refer to homogeneity of variance for more details.

Technical validation

The test statistic is an extension of the Mann-Whitney test and is calculated as above. In the presence of tied ranks the test statistic is given in adjusted and unadjusted forms, (opinion varies concerning the handling of ties). The test statistic follows approximately a chi-square distribution with k-1 degrees of freedom; P values are derived from this. For small samples you may wish to refer to tables of the Kruskal-Wallis test statistic but the chi-square approximation is highly satisfactory in most cases (Conover, 1999).

Results and discussion

Wine consumption in the individual countries of the Visegrad Group varies (Figure 1).

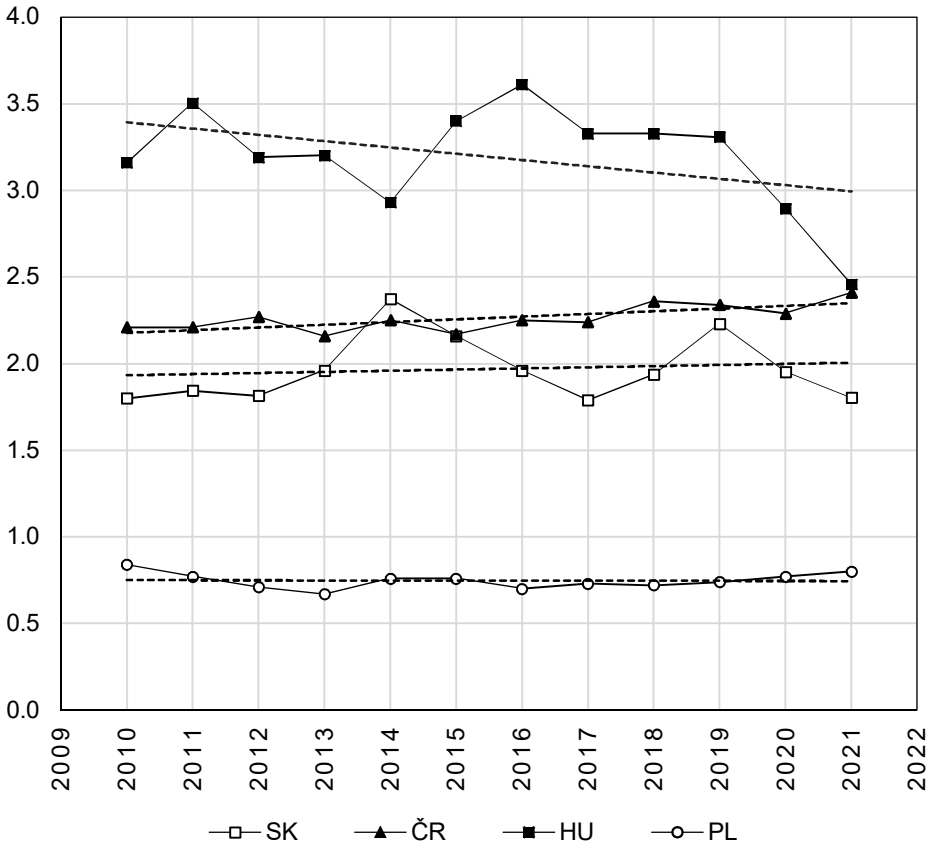

Figure 1. Consumption of wine from the Visegrad countries per capita in 100% alcohol

We are talking about the consumption of wine in 100% alcohol in liters per inhabitant. Hungary has achieved the highest consumption in the last period where the average consumption of wine in pure alcohol for the last decade (2010–2021) is 3.2 liters. The Czech Republic took second place with a value of 2.3 liters. Compared to Hungary it is almost one liter more which proves that Hungary is a wine powerhouse and the inhabitants like this alcoholic drink. The Slovak Republic is not too different from the Czech Republic where on average 2.0 liters are drunk in Slovakia. The most significant difference is represented by Poland where consumption during the monitored period reached only 0.7 liters. Poland is a country where wine is not grown much but it is not even widely consumed. The development trend of wine consumption in the V4 countries indicates a stable character. Overall, during the monitored period, countries such as Slovakia and the Czech Republic show a slow but increasing trend in wine consumption. Poland is more conservative in terms of consumption and the development (Hornowski et al., 2020) is neither decreasing nor increasing. On the contrary, from a long-term perspective, Hungary shows a downward trend in wine consumption, especially in 2020 and 2021, which represent the years affected by the pandemic. From the year-on-year point of view and sharper fluctuations in consumption, 2014 was the year for Slovakia and Hungary. The trends show opposite developments if something decreases in one country, it increases in the other until the last two years, 2020 and 2021, when both countries saw a significant decrease in consumption. Especially in Hungary, to the value of 2.5 liters, which represents a decrease compared to the average by 0.7 liters. In 2014, the consumption of wine in Slovakia increased and reached a value of 2.4 liters which represents the highest value of the monitored period. In Slovakia, the overall development of wine consumption is very fluctuating, but in the long term consumption is still increasing. As part of the comparison in the volume of consumption, the Czech Republic and Slovakia have the most similar wine consumption among the countries of the Visegrad Four.

Another part of the research deals with the frequency of drinking wine. Figure 2 shows the frequency of wine drinking for all V4 countries together by individual age groups (age groups 1, 2, 3). The analysis shows that when evaluating all age categories in terms of frequency of wine drinking, the largest group (18.42%) are wine consumers who drink wine 2–5 days a year. The second largest group (16.58%) are wine consumers who drink wine 1–2 days a week. The third group are consumers (16.38%) with a frequency of drinking 2–3 days a month. Figure 2 shows a graphic representation of the number of respondents of the age range depending on the individual answers to the frequency of drinking wine in the countries of the Visegrad Four. In age category 1, the most respondents (415) indicated that they consume wine 2 to 3 days a month. The second most numerous answer (362) was that respondents drink alcohol 1 to 2 days a month. The third most numerous group of answers (349) is the group that drinks wine only rarely, 2–3 days a year, and the fourth most numerous group of answers (279) is that they consume wine once a month. In age category 2, the most numerous group of respondents' answers (286) was one to two days a week. The second most numerous group of respondents (277) who drink wine again very rarely. The third significant answer (254) was respondents who consume wine 2 to 3 times a month. In age category 3, there were answers from respondents (536) which represents the largest number of respondents who indicated that they rarely drink alcohol, once a month. The second (398) and third (364) most numerous group of respondents' answers is that they consume wine 1–2 days a week and 2–3 days a month. From the analysis, we can conclude that people in age categories 1 and 3 consume wine more often, most often 2 to 3 times a month.

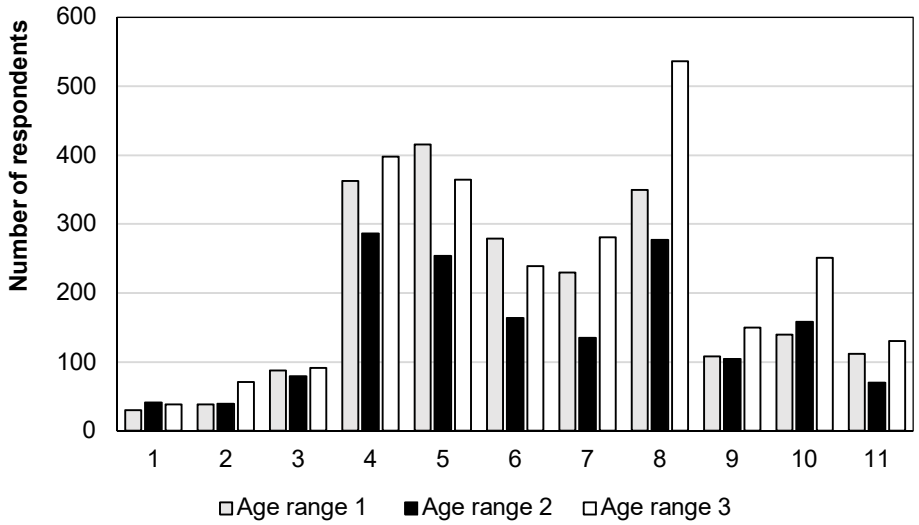

Figure 2. Frequency of drinking wine in Visegrad countries
Source: own processing

The situation is the same in terms of frequency of wine drinking and individual countries. In the Czech Republic there is the largest group of wine consumers (292) with a frequency of drinking 1–2 days a week. In Hungary (401) and Slovakia (312) the most numerous group of consumers is with the frequency of drinking wine 2–5 days a year. However, the analysis shows that in Hungary, the two most numerous answers are 5 (282) and 4 (267), which indicate that consumers drink wine 1 to 2 times a week and 2 to 3 times a month. In Poland, on the other hand there is the largest group (288) with a frequency of drinking 2–3 days a month.

Based on the addition of individual frequencies, summaries were processed. From the point of view of opportunity it follows that the largest group consists of occasional wine consumers of age category 1 (18–34 years). In the category of regular alcohol consumption, the largest age group is 2 (35–44 years). In general, if we take the individual categories of occasional wine drinking, the age categories are balanced (Figure 3).

Table 2

Frequency of drinking wine (how often they drink wine) in V4 countries

Age		Frequency of drinking Wine (how often they drink Wine) in V4 country											
Range		1	2	3	4	5	6	7	8	9	10	11	Total
CZ	F	29	49	78	292	261	142	142	180	71	102	71	1417
	%	0.46	0.78	1.24	4.63	4.16	2.25	2.25	2.85	1.62	1.62	1.13	22.47
HU	F	31	46	67	267	282	158	225	401	102	201	96	1876
	%	0.49	0.73	1.06	4.23	4.47	2.51	3.57	6.36	1.62	3.19	1.52	29.74
PL	F	30	23	62	265	288	214	133	269	93	99	33	1509
	%	0.48	0.36	0.98	4.2	4.57	3.39	2.11	4.27	1.47	1.57	0.52	23.93
SK	F	19	30	51	222	202	168	146	312	96	147	112	1505
	%	0.3	0.48	0.81	3.52	3.2	2.66	2.31	4.95	1.52	2.33	1.78	23.86
Total	F	109	148	258	1046	1033	682	646	1162	362	549	312	6307
	%	1.73	2.35	4.09	16.58	16.38	10.81	10.24	18.42	5.74	8.7	4.95	100

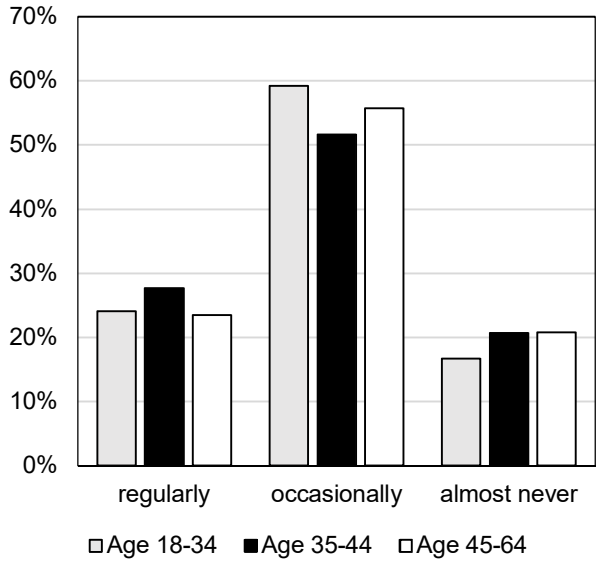

Figure 3. Summary of age range and frequency of wine drinking

From the point of view of comparing individual countries, it follows that in all four analyzed countries, consumers consume wine occasionally but the most in Poland. From the point of view of regular consumption, the Czech Republic stands out (Figure 4).

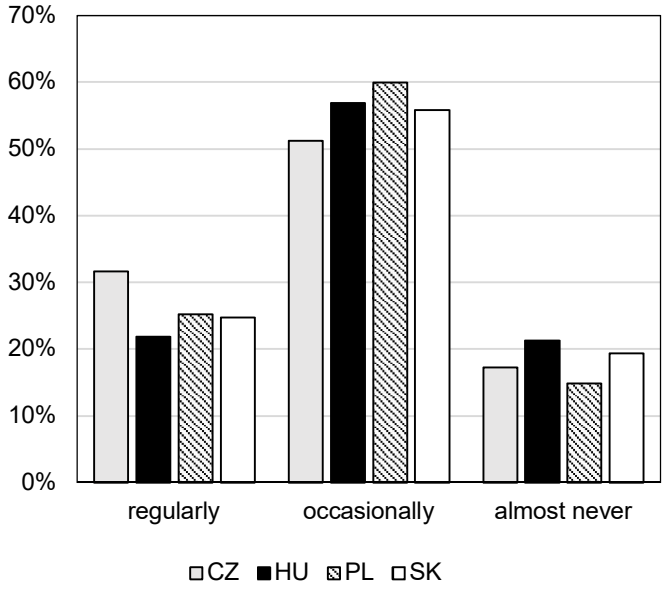

Figure 4. Summary of individual countries of Visegrad countries and frequency of drinking wine

Non-parametric tests were used to assess dependence between groups of respondents in individual countries. The tests show that when comparing the group of respondents between countries and individual age categories there is significant dependence (significant differences) between respondents in the Czech Republic and Hungary, between the Czech Republic and the Slovak Republic, between Hungary and Poland, and between Poland and Slovakia.

The results show that wine consumers in Hungary and Slovakia have the same behavior in contrast to the Czech Republic and Poland. On the other hand, wine consumers in the Czech Republic and Poland have similar consumer behavior. Among the other comparisons of the states there are significant differences in wine consumption in terms of the frequency of alcohol drinking (Table 3 and Figure 5). The Table also shows a comparison of consumer behavior in terms of age categories which shows that the situation is the same in almost all age groups which was described above, except for the group of respondents in the age category of 45–64 years where when comparing the states of the Czech Republic and Poland, behavior is different.

Table 3

Dependencies between age groups and individual states

Pairwise Two-Sided Multiple Comparison Analysis				
Dwass, Steel, Crichow-Fligner Method				
Variable: Wine				
	Pr>DSCF	Pr>DSCF	Pr>DSCF	Pr>DSCF
Stat	**Age range 1**	**Age range 2**	**Age range 3**	**All age range**
CZ vs. HU	0.0077	0.0017	<0.0001	<0.0001
CZ vs. PL	0.7557	0.7109	0.0014	0.0836
CZ vs. SK	0.0124	<0.0001	<0.0001	<0.0001
HU vs. PL	<0.0001	0.0316	0.026	<0.0001
HU vs. SK	0.9999	0.1396	0.9706	0.5722
PL vs. SK	<0.0001	<0.0001	0.0159	<0.0001

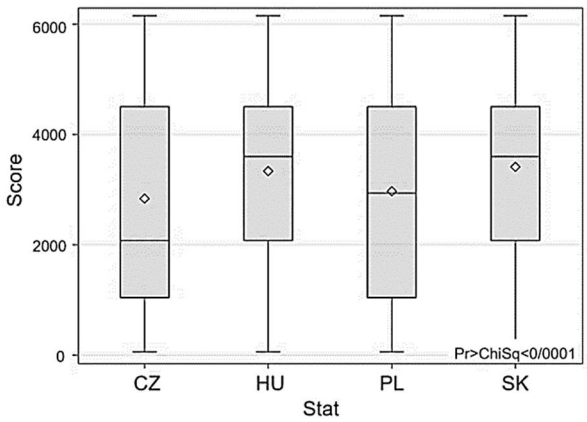

Figure 4. Dependencies between age groups and individual states

Furthermore, in the research we dealt with the comparison of the frequency of drinking wine in age categories depending on gender in the Visegrad Four countries as a whole and also in individual countries in percentage terms (Table 4). The analysis showed that there are no significant differences in the frequency of wine consumption between the sexes. The average difference between the sexes in the categories of frequency of drinking wine as a whole is 1.42%, while the biggest difference is represented by occasional wine consumers in the Czech Republic where the difference between the sexes is 9.71%, which means that women are more likely to be occasional wine consumers in the Czech Republic as men regardless of age category. From the point of view of the age categories and the individual countries of the Visegrad Four, the largest difference between the sexes is again recorded in the Czech Republic and Poland, namely in age category 2. In this case, again, a larger percentage of regular wine consumers are represented by women. Likewise, in the category of occasional wine consumers, the biggest gender difference is in age category 1 (difference 5.47%) and age category 2 (4.99%). Overall, the analysis shows that the frequency of drinking wine in the countries of the Visegrad Four is the same between the sexes, and it has not been proven that one gender consumes significantly more wine.

Table 4

Frequency of wine drinking by age and sex in %

Frequency	Age range	V4		SK		CZ		HU		PL	
		M	W	M	W	M	W	M	W	M	W
Regular	1	4.18	3.64	3.96	3.24	4.63	4.98	3.85	3.22	4.39	3.36
	2	6.96	9.47	7.23	7.13	7.29	12.94	6.50	7.26	6.94	11.33
	3	8.86	6.63	7.09	4.80	11.08	9.67	8.37	6.02	9.07	6.48
	Sum	24.97	24.53	18.28	15.18	23.00	27.60	18.72	16.49	20.40	21.17
Occasio	1	12.97	15.09	12.82	13.36	14.45	19.91	12.56	11.93	12.18	16.31
	2	11.86	14.04	10.37	11.15	14.31	15.93	10.79	12.24	12.32	17.31
	3	14.12	11.79	12.55	10.12	17.81	17.21	13.22	9.96	13.17	10.83
	Sum	56.24	55.54	27.15	25.29	32.82	42.53	27.42	25.21	28.19	34.12
Almost	1	16.96	21.88	14.73	18.81	17.81	25.46	17.29	17.12	17.99	27.40
	2	14.28	14.22	12.14	11.28	16.97	14.51	13.55	12.24	14.73	19.18
	3	18.20	14.19	15.69	13.10	22.02	19.63	16.08	11.41	19.69	13.82
	Sum	18.79	19.93	30.29	29.31	39.83	45.52	31.72	30.19	33.57	40.10

Discussion

Wine is a commodity with a long tradition, culture, production style or consumption style or ratio. Each country that grows grapes and produces wine is specific in its own way, and wine can never be produced in such a way that it is identical to the previous one or from another country. In the countries of the Visegrad Four, there is currently an increasing supply of wine. For many residents, wine consumption is a prestigious thing and belongs to a higher lifestyle. Beer is and was considered a substitute for wine. When beer consumption per capita in all four analyzed countries exceeds the average beer consumption in the EU-15 countries, it is possible to claim that wine has a special position on the market of alcoholic beverages.

Due to its uniqueness as an alcoholic beverage, wine is suitable for leisure time as well as for culinary specialties. (Chládková, Tomšík, Gurská, 2009). It is the same with consumers who are specific in the amount and frequency of consumption and whether wine is an important element of their culture and nationality. The countries of the Visegrad Four are closely linked countries with a long and so to speak common history, and wine is an inseparable part of them. According to the results from various national databases such as (DATACube., n.d.), (Verejná Databáze VDB, n.d.),

(Wine Consumption per Capita in Hungary, 2021), (Wine Consumption per Capita in Poland, 2021) and WHO (Indicator Details, n.d.-b) and (Ritchie, 2024) it was confirmed that Hungary, the Czech Republic and the Slovak Republic in particular are important consumers of wine, specifically in this order. Hungary is an important producer of wine and has the highest per capita consumption of pure alcohol among the countries of the Visegrad Four, an average of 3.2 liters over the last decade, which was also confirmed by our findings compared to Indicator Details, (n.d.-b), Ritchie (2024) and Wine Consumption per Capita in Hungary (2021). The Czech Republic and the Slovak Republic formed one state for a long time, and after their division, these young states are still very similar in various spheres, and wine consumption is no exception. Likewise, our research confirmed that over the last decade, the consumption of wine in pure alcohol had a value in the Czech Republic (2.3 liters) and in the Slovak Republic (2.0 liters) per inhabitant. While these two countries, despite their size, have a significant position in the production and consumption of wine within the Visegrad Four. In Poland,the consumption of wine for the last decade was also confirmed to be 0.7 liters of wine in pure alcohol per inhabitant, according to the available databases. Even though Poland is a large country and does not have very good conditions for wine production, their efforts to produce it are still growing, and there are also fans of wine consumption there. Although the countries of the Visegrad Four are similar in various respects they still show certain deviations from the point of view of wine consumption. This is also confirmed by the publication of Moskale-wicz et al. (2016) which talks about the specificity of individual countries in alcohol consumption which were revealed in the results of research in 19 EU countries. According to Kilian et al. (2021) the overall intensity of alcohol drinking is decreasing while if we break down individual alcohol into specific types and into individual countries our research proved that in terms of the intensity of wine drinking in individual countries, it is balanced. The frequency of drinking alcohol proved that there is a statistically significant difference between countries in terms of the frequency of drinking alcohol. According to age categories a difference was noted in all compared countries, only between Slovakia and Hungary there are differences in the frequency of drinking wine and between the Czech Republic and Poland. The only exception was the third age category from 45-64 years where there was a significant difference between the frequency of wine drinking between Poland and Slovakia. The analysis proved that of the monitored countries of the Visegrad Four, the Czech Republic, Poland, Slovakia, and Hungary regularly drink from the most to the least. Overall, all countries show that the largest share of people who drink alcohol occasionally is in age category 2 (35-44 years old). An important aspect is also the gender and frequency of alcohol consumption in the individual countries of the Visegrad Four but also as a whole. Our research operated with a balanced proportion of men and women in individual countries, and the analysis shows that there is no significant difference in the frequency of drinking wine between the sexes in the Visegrad Four, nor in individual countries, except for the Czech Republic, where higher differences were demonstrated. A study by Petriashvili et al also speaks about this fact (2023) that women drink more wine than men who prefer beer to wine. This study says that out of the total number of respondents, 65.62% of women drank wine.

Conclusion

1. Along with water wine is the most widespread and popular drink in the world and it can be said that it accompanies the human race on its life journey almost all the time until today. The aim of the article was to evaluate the consumption and frequency of wine drinking in the countries of the Visegrad Group, which was fulfilled.
2. Hungary has achieved the highest consumption in the last period where the average consumption of wine in pure alcohol for the last decade (2010–2021) is 3.2 liters. The Czech Republic took second place with a value of 2.3 liters. Compared to Hungary it is almost one liter more which proves that Hungary is a wine powerhouse and the inhabitants like this alcoholic drink. The Slovak Republic is not too different from the Czech Republic where on average 2.0 liters are drunk in Slovakia.
3. When evaluating all age categories in terms of frequency of wine drinking the analysis shows that the largest group (18.42%) are wine consumers who drink wine 2-5 days a year. The second largest group (16.58%) are wine consumers who drink wine 1-2 days a week. The third group are consumers (16.38%) with a frequency of drinking 2-3 days a month. From the point of view of the comparison of individual countries it follows that in all four analysed countries consumers consume wine occasionally but the most in Poland. From the point of view of regular consumption, the Czech Republic stands out. The results show that wine consumers in Hungary and Slovakia have the same behaviour in contrast to the Czech Republic and Poland. On the other hand, wine consumers in the Czech Republic and Poland have similar consumer behaviour. Among other comparisons of the states, there are significant differences in wine consumption in terms of the frequency of alcohol drinking. Drinking wine is no longer the privilege of connoisseurs in Poland. Its consumption is gradually increasing, new wine shops and wine bars are appearing. Czech, Slovak and Hungarian winemakers who are starting to expand into the Polish market have a chance. Due to the globally decreasing area of vineyards, the demand as well as the prices of wine will increase.

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Analysis of Hungarian consumers' food consumption and wastage patterns in times of the crisis

Tímea Juhász[1], Péter Huszka[2], Péter Karácsony[3]

1 – Budapest Business University, Budapest, Hungary
2 – Széchenyi István University, Győr, Hungary
3 – University of Óbuda, Budapest, Hungary

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Corresponding author:

Tímea Juhász
E-mail: juhasz.timea@uni-bge.hu

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Abstract

Introduction. The present research studies the extent to which recent crises have affected the food purchasing habits of Hungarian consumers and whether the financial difficulties caused by the crisis have changed their food wastage patterns.

Materials and methods. The research method was a questionnaire survey, and the authors evaluated the data based on the opinions of a total of 798 respondents. The evaluation was carried out using SPSS version 28 and Smart PLS software.

Results and discussion. The results showed that the current economic crises have a significant impact on the purchasing habits of the Hungarian consumers surveyed and the amount of food they buy. During a crisis, Hungarian consumers are more conscious and buy less. Consumers are less optimistic about the future (mean: 3.00 standard deviation: 1.248). The study shows that optimism has no effect on whether they waste food. Anxiety has a strong effect on perceptions of crisis and wastage. The study also shows that Hungarian consumers are not satisfied with the way the economic crisis is being handled (mean: 2.29, standard deviation: 1.185). The responses also show that consumers surveyed are typically careful about how much they buy (mean: 3.70, standard deviation: 1.113), what they put away and what they throw away. More conscious thinking about waste and consumption is well outlined (mean 3.62, standard deviation 1.205). Less food is thrown away, with 18.7% of respondents never throwing away food waste and one in five respondents buying only as much food as they can afford to take away. When food is left over, around 18% of survey respondents compost the leftovers.

However, there was no difference in the extent to which those affected by economic impacts abandoned their previous consumption habits or stuck to their previous lifestyle.

Conclusion. Wastage is affected by the experience of the crisis and a sense of uncertainty about the future. Similarly, the experience of the crisis is influenced by our knowledge of eating habits and our environmental awareness.

Introduction

Since the second half of the 20th century, the world has experienced relative economic, social and financial stability. However, this period of socio-political equilibrium and economic prosperity was shattered by the attacks on the Twin Towers and the bankruptcy of Lehman Brothers – the largest bank failure in US history – both of which played major roles in the financial crisis of 2007–2008 (Fernandez and Wigger, 2016; Lukács and Völgyi, 2018). Following the bankruptcy, global markets immediately plummeted, national currencies worldwide depreciated and inflation and unemployment soared. Before a full recovery from this global crisis, the world was already facing a new challenge: the coronavirus. Infectious diseases, which have been a part of people's lives throughout history, have taken on a new level of significance, profoundly affecting the world economy. The coronavirus crisis, declared a global pandemic by the World Health Organization on 11 March 2020, inflicted severe economic damage worldwide (Kumar Jaiswal, 2021), affecting not only national economies but also people's daily lives (Grueso-Hinestroza et al., 2022). Several economic sectors, including trade, tourism, hospitality and retail, have been more severely affected by the crisis (Kohus et al., 2022; Tóth et al., 2023; Yang et al., 2024). We had barely emerged from the Covid crisis when another crisis erupted across Europe as a result of the Russian conflict (Sandoval Velasco et al., 2022). This conflict caused a significant decline in consumer and business confidence in the European Union and also carried serious inflationary consequences (Sohag et al., 2022). According to the Central Statistical Office, in 2022, the average annual domestic inflation rate was 14.5%, more than double the EU average. However, it can also be noted that Hungarian inflation has not yet reached its peak at this point, despite the Central Statistical Office reporting an even higher inflation rate of 25.7% in January 2023. The high inflation in Hungary is largely due to the substantial increase in food prices. The most expensive items were eggs (79.2%), dairy products (76.2%), butter and buttercream (75.1%), cheese (72.1%), bread (71.1%), confectionery (68.6%), dry pasta (57.3%), milk (53.7%), and pastry products (51.9%) (KSH, 2023).

The present research explores the context of Hungarian consumers' food purchasing and wasting decisions in the current crisis period, taking into account the opinions of 799 respondents.

The primary objective of the study was to evaluate how Hungarian consumers perceive their financial security and their attitudes towards food consumption and potential food waste in the current economic context.

Literature review

In consumer society, both wealth and poverty, thrift and waste, obesity and starvation, the need for awareness and the predominance of emotional choices coexist. Within this complex system of consumer behaviour, a series of rational and irrational decisions can be observed (Rybaczewska et al., 2023; Sułkowski et al., 2022; Trandafilovic et al., 2015). When consumers exchange money for a service or product they aim to secure the best possible deal and satisfy a need. However, the role of emotional motivations in the realm of purchasing decisions is also crucial (Ngo et al., 2023). Throughout the process of purchasing and utilizing a product, the buyer is surrounded by pleasant feelings, and emotions can become so significant that the 'pleasure-giving function' of the product transforms into a goal during the purchase (Kotler and Keller, 2013).

During times of economic crisis, consumer awareness increases, leading individuals to be more aware of what they buy, how much they buy and at what cost. Consumer awareness

refers to the fact that consumers make decisions based on certain criteria when making purchases (Alimi and Workneh, 2015; Bednarz et al., 2023). Changes in consumer behaviour were also observed during the coronavirus epidemic, as the fear of the virus pushed consumers towards online shopping, thereby increasing the frequency of online grocery purchases (Dias et al., 2023; Nguyen et al., 2024; Oláh et al., 2023; Rybaczewska et al., 2021; Susanti et al., 2022; Semenenko et al., 2022). In this respect, Semerádová and Weinlich distinguished eight factors that emerged as consumers' risk-reduction expectations during the coronavirus crisis. These factors encompassed, among others, the reduction of shopping occasions or the emphasis on food safety factors (Semerádová and Weinlich, 2022).

The concept of consumer awareness, similar to various aspects within marketing, is not uniform, as different authors approach the concept from various perspectives, such as the awareness and enforcement of consumer rights or thorough information-seeking behaviour. In the literature, an informed consumer is generally identified as a well-informed individual who knows, asserts and uses their consumer rights (Febriandika et al., 2023; Mnerie et al., 2015; Kotler and Keller, 2013). Among today's consumers, the concept of informed consumption can be interpreted more broadly. According to the approach of the informed consumer, awareness not only implies being aware of one's rights and the ability to assert them but also emphasises the importance of being a consumer who is not only aware of oneself but also others, displaying moral and environmental consciousness.

Since the Second World War, there has been a significant increase in mass consumption, primarily driven by the easier availability of material goods and the rise in living standards. Mass consumption has been accompanied by other consequences besides wastefulness, including overconsumption, which is increasingly threatening resources (Mian and Ramana, 2011). The result of overconsumption is the rapid depletion of natural resources, their swift conversion into waste and the disruption of ecological balances (Bublyk et al., 2023; Håkansson, 2014; Hudayah et al., 2023). It is widely known that food wastage remains an unresolved problem across all points in the food chain, from production, harvesting and processing to trade and final consumption (Okobo et al., 2022; Schneider, 2008).

Over the past decade, more and more countries around the world have recognised the problem of food waste and realised the need to take decisive action to tackle it. The prevention of food waste, the management of food surpluses and the reduction of food waste have, therefore, become global challenges. Today, the problem has reached unprecedented proportions, and tackling it is a major challenge (Corrado and Sala, 2018). While developed countries are experiencing significant overproduction, overconsumption, 'unhealthy consumption patterns' and wastage, other parts of the world are facing food crises and even contiguous hunger zones. According to Fanelli and Di Florio (2016), the causes of food waste vary depending on attitudes, eating habits, culture and the level of development in countries. In rich and developed countries such as Italy, food is most wasted at the consumption stage, partly due to the availability of cheap and abundant food in developed countries. In Italy, an individual spends 20% of their income on food, while an Egyptian spends 43% of their money on food. Therefore, consumers in developed countries have a lower appreciation of the true value of food and buy more than they need without much thought (Flanagan et al., 2019; Mnerie et al., 2016). Thus, the more developed the economy and the standard of living in a country, the more food is wasted at the end of the supply chain at the consumer level (Corrado and Sala, 2018). However, the less developed the economy, the more food waste is shifted towards agriculture. This is due to the use of inadequately developed technology for cultivation in such areas, inadequate harvesting methods and an inability to store and transport crops properly, resulting in a large amount of wasted produce right from the beginning (Kumar and Kalita, 2017).

Alongside government interventions and legislation, NGOs, programmes and even individual households are increasingly determined to curb food overproduction and reduce waste. Consumer awareness can also be related to when, where and how much a conscious consumer buys. According to FAO (2011) studies, one-third of food produced for human consumption worldwide will be wasted, equivalent to approximately 1.6 billion tonnes of food and 8% of global greenhouse gas emissions. The production of unconsumed food contributes to more than 20% of global pressures on biodiversity. However, this waste also represents a significant resource in terms of land, water, energy and labour, for example (FAO, 2011). Parfitt et al., (2010) distinguish three types of household wastage: avoidable losses, refers to food that is thrown away but otherwise consumable (e.g. leftovers, untimely use); it refers to food or parts of food that some people consume but others do not (e.g. bread crusts, potato peelings, etc.); and inedible losses, which are those parts of food that are inedible (unfit for human consumption) (e.g. bones, eggshells, coffee grounds, vegetable peelings, apple cores, etc.).

The total amount of food waste in the European Union in 2020 was almost 57 million tonnes, of which more than 31 million tonnes were food discarded in households. According to Eurostat, households, therefore, produced nearly 55% of food waste, amounting to approximately 70 kilograms per inhabitant, while supply chain operators generated approximately 60 kilograms of food waste. Among supply chain actors, around 14 kilograms of food waste per inhabitant was generated in production, 23 kilograms in manufacturing, 12 kilograms in catering and 9 kilograms in food distribution (Eurostat, 2022).

The European Commission attaches the utmost importance to tackling food waste, as reducing waste has huge potential to decrease the resources used in producing the food we consume (e.g. 1 kilogram of beef requires around 15,000 liters of water to produce, and if we throw it away, not only is the food itself wasted but all the resources needed to produce it are wasted as well). More efficient use conserves food for human consumption, saves money and reduces the environmental impact of food production and consumption. Food loss and waste increase the risks associated with food insecurity, malnutrition and excessive water usage, while global hunger continues to rise. EU Member States have committed to the UN Sustainable Development Goal of halving per capita food waste at retail and consumer levels by 2030 and reducing food losses in the production and distribution chain (European Commission, 2022).

On the other side of the food waste coin, food insecurity has become a global problem, with many parts of the world experiencing it to a greater or lesser extent (Ortega Ibarra, 2021). Food insecurity and hunger are not only found in underdeveloped and remote countries but are present all over the world (Aiyedogbon et al., 2022; Dubanych et al., 2023; Nyambayo, 2015; Zegar, 2009). In Hungary, many people do not have access to three meals a day. Qualitative hunger is the most common form of hunger in our country, but quantitative hunger can also occur in families on the table living in extreme poverty. Qualitative hunger means that there is enough food, and they eat regularly, but the quality and nutritional content of this food are inadequate. Quantitative hunger is the reverse, with little food per person, but mostly of adequate energy and nutritional value (Pollard and Booth, 2019). Hunger is a complex problem with many causes, including low agricultural productivity, population growth and poverty.

Materials and methods

Questionnaire

This study is not a random sampling study, but the authors have endeavoured to make the method as close as possible to random sampling. To this end, 798 respondents were included in the sample. The authors published the questionnaire on social media platforms; as a result, it was not possible to measure the willingness to respond. The research was conducted over a period of six months and the questionnaire was anonymous, voluntary and specifically designed for research purposes. The researchers respected the personal data of the respondents, i.e., they interviewed the sample in compliance with GDPR regulations. The questionnaire comprised closed questions and was mainly based on nominal and metric variables.

The questions in the questionnaire were divided into three groups.

In Table 1, the authors present the questionnaire questions grouped accordingly:

Table 1

Structure of the questionnaire

Specific questions	Features of the domestic economic and energy crisis	Food consumption and wastage in the current economic situation
No Age School education Residence Average monthly income per person	The impact of the situation on everyday life Assessment of crisis-induced micro- and macroeconomic measures Characterisation of the financial situation of respondents	How has the food consumption of the respondents changed in the last six months? Wasteful practices of given foods Income expenditure according to the concerned foodstuffs Changes in the financial situation of the concerned families

Before the final questionnaires were completed, the authors carried out a test survey. This meant that around 10 respondents had pre-completed the questionnaire, and as they encountered no problems with interpretability, the authors used the questions in their unchanged from throughout the research. The questions were closed in nature, based on metric and categorical variables. On one hand, the questions were the authors' own, and on the other hand, they were based on a lavish evaluation of the authors' questions. Since the questionnaire was the authors' own, its reliability was tested by the researchers through a small group re-survey. The results were similar to those of the original questionnaire, and the authors of the study rated the questionnaire as reliable.

Questions and the hypothesis

The following questions were raised during the research:

1. How do respondents perceive what foods are typically not used and thrown away?
2. What factors influence whether respondents might throw out food?
3. How do respondents see their financial situation and how does this affect their consumption?

In line with the above objectives, the study focused on proving the following hypotheses:

Hypothesis 1.
For this particular study sample, food waste is strongly influenced by the current economic situation, and respondents are concerned about their present and future living situation.

Hypothesis 2.
The sense of crisis is strongly influenced by respondents' concerns about the economic situation and their future.

Hypothesis 3.
In the Hungarian sample, the feeling of optimism is strongly influenced by the economic situation created by the crisis and the respondents' concern about their future.

Results and discussion

Sample

A total of 798 people answered the questions. The Hungarian population was around 9.711 million in 2022. To calculate the minimum sample size, the authors used Yamane's formula (Yamane, 1965), which, at the 95% confidence level, is: p=0.05

$$N = N/(1+N\times(e^2))$$

N = 9.711 million inhabitants, 9 711 000/ (1+ 9 711 000·0.05·0.05) = 400. As the number of respondents was more than this, the authors accepted the sample number.

For the analysis, the authors used SPSS version 28 and SMART PLS 4 for SEM modelling.

The study sample could be described by the following characteristics:

Table 2

Some characteristics of the sample

Features	N	%
No	Men: 396 pers. Women: 402 pers.	Men: 49.6%% Women: 50.4%
School education	Primary: 44 pers. No secondary school leaving certificate: 124 pers. Graduates: 274 people Tertiary level: 356 pers.	Primary level: 5.5% No secondary school leaving certificate: 15.5 % Graduates: 34.3% Tertiary level: 44.6%
Residence	Village 284 pers. City: 315 pers. County town: 199 pers.	Village 35.6% City: 39.5% County town: 24.9%

Cross tabulation analyses showed that 5.3% of men had primary education, 18.9% had secondary education without a high school diploma, 33.8% had a high school diploma or less and 41.9% also had tertiary education.

For women, it was found that 5.7% had only completed primary school, 12.2% had attended secondary school but did not have a school leaving certificate, while 34.8% had a school leaving certificate as their highest level of education; furthermore, 47.3% held a diploma. The Chi-square test showed that there was no correlation between gender and the highest educational attainment in the sample (Chi-square: 7.247, df:3 sig.: 0.063 $p>0.05$).

It was found that 42.4% of men lived in villages, 33.8% in towns and 23.7% in cities. The proportions for women were as follows: 28.9% lived in a village, 45% lived in a town and 26.1% lived in a city with county status. Based on the place of residence and gender, the Chi-square test showed a significant correlation for this sample (Chi-square: 17.098, df: 2 sig.: 0.001, $p<0.05$).

In terms of educational attainment, the majority of those with primary education lived in rural areas (63.6%), which is similar to those with secondary education lacking a school leaving certificate, who also mostly lived in rural areas (41.9%). Those with a high school diploma were mostly urban residents (42.7%), and the same applied to those with a diploma (41.9%). The Chi-square test confirmed the association between educational attainment and the place of residence (Chi-square: 25.402, df: 6 sig.: 0.001, $p<0.05$).

Model and the results

In the research, the authors created a model to investigate the factors that influence potential food waste and savings. To analyse this, they employed the structural equation model (SEM), including the variance-based method. In the SEM model, the latent variables constructed from indicators and the relationship between them can be presented.

The SEM models consist of two parts: the measurement model and the structural model. The measurement model depicts the relationship between the latent variables and the indicators, while the structural model depicts the relationship between the latent variables. The direction of the relationship between the indicators and the latent variable showed a reflective relationship in the authors' model.

The latent variables in a model can be endogenous, dependent and exogenous, as well as independent (Sajtos-Fache, 2005). In PLS SEM path analysis, the metric measurement-level variables, known as items, are not required to be normally distributed (Kazár, 2014). This was tested by the authors using the Kolmogorov-Smirnov and Shapiro-Wilk tests. For all variables, the p-value was less than 0.01, indicating that the measurement variables under study are not normally distributed.

During the research, the authors made several claims about the current economic situation, food consumption and possible wastage. On a five-point Likert scale, respondents were asked to rate their level of agreement with the statements, where one indicated strongly disagree and five meant strong agreement. The mean and standard deviation of the items used and the variables constructed are summarised in Table 3.

Table 3

Constructs and items

Construct	Item	N		M	SD
		Valid	Missing		
DI Disposal	DI1 We should pay more attention to our environment.	798	0	4.36	0.991
	DI2 I spend a significant proportion of my income on food, so I always try to waste less.	798	0	3.70	1.113
	DI3 I was brought up as a child to throw food away only when I had to.	798	0	4.26	1.090
OP Optimism	OP1A Addressing the economic crisis, the energy crisis is good.	798	0	2.29	1.185
	OP2 I am optimistic about the future.	798	0	3.00	1.248
	OP3 It is good to live in Hungary.	798	0	2.74	1.266
	OP4 By 2030, my standard of living at home will rise significantly.	798	0	2.39	1.241
CR Crisis	CR1 I am buying less because of the economic crisis and the energy crisis.	798	0	3.30	1.247
	CR2 I buy cheaper products because of the economic and energy crisis.	798	0	3.38	1.219
	CR3 I am in a bad financial situation because of the energy crisis.	798	0	2.86	1.292
KN Knowledge	KN1 We live in an environmentally responsible way, so we don't throw food away.	798	0	3.66	1.078
	KN2 I watch my eating habits, throwing out less food than before.	798	0	3.62	1.205
	KN3 I am aware of the concept of functional food.	798	0	2.93	1.380
AN Anxiety	AN1They are often worried about the future.	798	0	3.41	1.251
	AN2 Poverty is a serious problem in Hungary today.	798	0	4.14	1.041
	AN3 There are significant income disparities in today's Hungarian society.	798	0	4.24	1.061

The mean and standard deviation data in the table indicate that respondents are typically careful about how much they buy, what goes into surplus and what they throw away. Regarding this question, the respondents were relatively homogeneous in their opinions based on the standard deviation values. Survey respondents were not optimistic and satisfied with their present circumstances and they were not particularly optimistic about their future. They were worried about the years ahead and perceived poverty and income inequality as an existing problem. The responses suggest that the economic crisis is affecting both the financial situation of the sample and, consequently, their purchasing habits and the amount of food they buy. The majority are aware of their eating habits and environmental awareness.

Based on the given items and constructs, the authors have created the following model. The following five latent variables were incorporated into the model by the researchers: disposal, optimism, crisis, knowledge and anxiety. All latent variables are low-level constructs, which means that they cannot be further subdivided into sub-dimensions. There are exogenous and endogenous variables in the model. Exogenous variables include anxiety and knowledge, while endogenous variables include disposal, optimism, crisis, etc. The model includes mediator variables, which act as mediators between constructs.

The authors employed the model to explore two main aspects. Firstly, the model delved into the factors shaping food accumulation and wastage within the ongoing economic crisis. Secondly, it investigated respondents' optimism regarding the current state and their future outlook. Furthermore, the analysis aimed to ascertain how individuals evaluate crisis management and identify the factors that amplify or diminish its impact.

Figure two illustrates the model created by the authors:

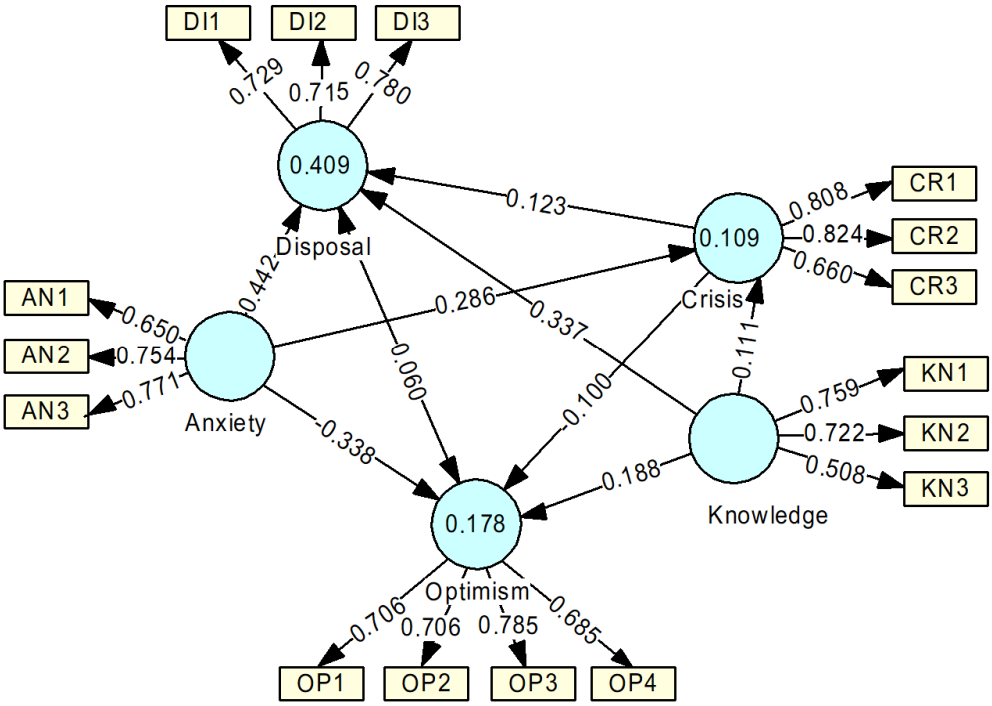

Figure 2. Food waste during the crisis

Proceeding, the authors analyse the model, which they begin by presenting the measurement model.

The figure clearly shows that the standardized factor weights for all items were above 0.5 (Haier et al., 2016).

To analyse the multicollinearity of the indicators, they used the VIF value, which should be below 5 (Hair et al., 2016). The variables met this requirement. The authors also analysed the composite reliability (CR) value, which should be above 0.7 (Werts et al., 1974). All constructs met this criterion. To measure convergent validity, the authors checked the average variance extracted (AVE), which is considered acceptable above 0.5 (Fornell-Larcker, 1981).

One construct had a lower value, but this latent variable was deemed important by the authors and was not excluded from the model. The above results are presented in Table 4. In this, items and constructs are now labelled by the authors using only the alphabetic characters in Table 4.

Table 4

Standardised factor weight, VIF, CR and AVE

Construct	Changing	Standardised factor weight	VIF	CR	AVE
DI	DI1	0.729	1.194	0.786	0.550
	DI2	0.715	1.180		
	DI3	0.780	1.202	0.813	0.521
OP	OP1	0.706	1.370		
	OP2	0.706	1.200		
	OP3	0.785	1.421		
	OP4	0.685	1.341		
CR	CR1	0.808	1.306	0.810	0.589
	CR2	0.824	1.361		
	CR3	0.660	1.213		
KN	CN1	0.759	1.080	0.706	0.452
	CN2	0.722	1.077		
	KN3	0.508	1.033		
AN	AN1	0.650	1.039	0.770	0.528
	AN2	0.754	1.404		
	AN3	0.771	1.402		

It was employed several methods to test the validity of discriminant validity. One of the most popular techniques is the Fornell-Larcker criterion (Fornell-Larcker, 1981). According to this criterion, the square root of the mean variance extracted by a construct should be greater than the correlation between the construct and any other construct. This criterion was met for the model. Another possibility is cross-loading, where items can express content more strongly in their own construct than in another latent variable. This condition was also fulfilled.

Table 5

Fornell-Larcker criterion

	AN	CR	DI	KN	OP
AN	**0.727**				
CR	0.313	**0.768**			
DI	0.521	0.304	**0.742**		
KN	0.244	0.180	0.467	**0.672**	
OP	-0.374	-0.188	-0.096	0.075	**0.722**

Table 6

Cross loading

	AN	CR	DI	KN	OP
AN1	0.650	0.370	0.292	0.085	-0.341
AN2	0.754	0.117	0.374	0.209	-0.278
AN3	0.771	0.170	0.469	0.245	-0.187
CR1	0.238	0.808	0.331	0.199	-0.060
CR2	0.278	0.824	0.253	0.129	-0.166
CR3	0.198	0.660	0.067	0.068	-0.249
DI1	0.402	0.125	0.729	0.318	-0.079
DI2	0.310	0.349	0.715	0.332	-0.054
DI3	0.439	0.210	0.780	0.385	-0.078
CN1	0.142	0.078	0.375	0.759	0.139
CN2	0.250	0.177	0.332	0.722	-0.035
KN3	0.084	0.118	0.210	0.508	0.038
OP1	-0.273	-0.054	-0.127	-0.010	0.706
OP2	-0.273	-0.268	-0.038	0.092	0.706
OP3	-0.282	-0.137	-0.066	0.110	0.785
OP4	-0.250	-0.037	-0.054	-0.005	0.685

The authors found the measurement model to be appropriate. To analyse the structured model, the authors used bootstrap sampling. The sub-sample size was 5000, and the p-value had a significance level of 0.05. The authors checked whether the independent variables had a significant effect on the dependent variables. Another test was for the beta coefficient, which indicates how much one variable influences the other. The R-squared values indicate the extent to which the magnitude of the change in the endogenous variable is explained by the exogenous variables. In the authors' model, the numbers in the blue circles represent the R-squared values, which behave as endogenous variables. The number in the arrows represents the β value. The significance values are summarised in Table 7. The authors have also included mediator variables in the model. Through these, we can discuss the indirect effect between latent variables. If there is no mediating variable, we talk about a direct effect. The direct and indirect effects together represent the total effect in the model. These relationships are summarised in Tables 7 and 8, encompassing beta values and T-statistics, with values exceeding 1.96 deemed acceptable (Fawad, 2022).

Table 7

Direct contacts (p:0.05)

	β value	T statistics	P value
Anxiety -> Crisis	0.286	7.791	0.000
Anxiety -> Disposal	0.422	11.231	0.000
Anxiety -> Optimism	-0.388	12.432	0.000
Crisis -> Disposal	0.123	4.125	0.000
Crisis -> Optimism	-0.100	2.325	0.020
Knowledge -> Crisis	0.111	2.759	0.006
Knowledge -> Disposal	0.337	11.449	0.000
Knowledge -> Optimism	0.188	4.913	0.000
Optimism -> Disposal	0.060	1.868	0.062

The data in the table show that optimism does not affect whether we waste food. However, there is a strong effect of worry on the perception of crisis (β: 0.286, t: 7.791, p: 0.000), wastage (β: 0.422, t: 11.231, p: 0.00), as well as a negative effect on optimism (β: -0.388, t: 12.432, p: 0.000) and negatively affects optimism (β: -0.100, t: 2.325, p: 0.020). Knowledge and knowledge of relevant information affect the crisis (β: 0.111, t: 2.759, p: 0.006), significantly impact wastage (β: 0.337, t: 11.449, p: 0.000) and optimism (β: 0.188, t: 4.913, p: 0.000).

Table 8

Indirect links (p: 0.05)

	β value	T statistics	P values
Anxiety -> Crisis -> Disposal	0.035	3.601	0.000
Knowledge -> Crisis -> Disposal	0.014	2.179	0.029
Anxiety -> Crisis -> Optimism -> Disposal	-0.002	1.295	0.195
Anxiety -> Crisis -> Optimism	-0.029	2.165	0.030
Knowledge -> Crisis -> Optimism	-0.011	1.754	0.079
Crisis -> Optimism -> Disposal	-0.006	1.350	0.177
Anxiety -> Optimism -> Disposal	-0.023	1.836	0.066
Knowledge -> Crisis -> Optimism -> Disposal	-0.001	1.143	0.253
Knowledge -> Optimism -> Disposal	0.011	1.679	0.093

Table 8 shows that anxiety has an impact on disposal through the crisis, both indirectly (indirect link) and directly. In this case, the total effect is the sum of the direct effect (β: 0.422) and the indirect effect (0.286*0.123): (β: 0.458). Since the indirect relationship is positive and significant for these three variables, the total effect is also positive and significant, and is therefore considered a complementary partial mediation.

In another example, there is a similar mediation process between knowledge, crisis and disposal. The direct relationship between knowledge and disposal is significant and positive (β: 0.337), and crisis also serves as a significant mediator between the latent variables (indirect effect: 0.111*0.123). In this case, as well, we can speak of an additional partial mediation, where the total effect is (β: 0.351).

The authors also examined what can be concluded about R squares with respect to endogenous variables. The 40.9% change in wastage is explained by the crisis and concern about the future. The 17.8% difference in optimism is explained by knowledge, worry and crisis, while 10.9% of changes in the crisis can be attributed to knowledge and worry.

Finally, the authors looked at which foods are least likely to be thrown away by respondents. They examined several staple foods. The table shows the proportion of foods that are never thrown away.

It can be seen that even for less durable foods, such as bread, fresh vegetables and fresh meat, the proportion of respondents who never throw them away is very high, while for durable goods the proportion is even higher. True, Hungarian food waste is still very high. In 2020, 905067 tonnes of food waste (about 92 kilograms per capita) will be generated in Hungary, of which 16 587 tonnes in the processing industry, 187 391 tonnes in the manufacturing industry, 41 952 tonnes in food distribution and 19 331 tonnes in catering. In addition, households disposed of 639,806 tonnes of food waste (about 65 kg/person). Prepared meals, bakery products, fresh vegetables, fruits and dairy products continue to account for a significant share (83.5%) of total food waste (Szabó-Bódi et al., 2018).

Table 9

I never throw away the food I have

Food	%
Bread parties	22
Chocolates	66
Canned food	71
Frozen meat, vegetables	65
Dairy products	26
Fats (butter and margarine)	59
Jam	66
Fresh meat	67
Fresh fruit and vegetables	43
Clippings	38
Eggs	66

However, it can be said that, in parallel with global economic events, we are also witnessing a continuous change in human nutrition and eating habits (Kolte et al, 2022; Marinković and Lazarević, 2021). Dietary culture and habits are among the earliest evolved behaviours, influenced not only by personal motives and family dynamics, but also by external forces that surround us, such as the Covid epidemic (Yamamoto and Uenishi, 2021) and the food price hike of 2022 (Elhajjar, 2023). Consumer behaviour is undergoing a major transformation as carefully planned marketing activities prove ineffective when an unexpected food price explosion caused by a crisis impacts on all aspects of our lives. These unforeseen factors have underlined the growing importance of studying the factors that influence consumer behaviour in both domestic and international research.

Conclusion

1. The consumer behaviour is undergoing a significant transformation, as exemplified by the coronavirus epidemic and the aftermath of the Russian-Ukrainian war in 2022. These events caused panic buying, and the freedom of choice was replaced by consumer vulnerability (lack of goods), similar to the prevailing situation during the years of regime change.
2. In the first half of 2023, Hungary will experience a decrease in solvent demand, primarily due to high inflation (retail sales volume down by 9.5% year-on-year). Therefore, companies need to be aware of changes in consumer demand, such as emphasizing higher value for money or offering smaller pack sizes, to avoid food waste.
3. The research looked at the factors that influence potential food wastage and saving, and how optimistic Hungarian consumers are about their future in the current economic downturn.
4. The responses suggest that the economic crisis is having an impact on both the financial situation of the sample and, consequently, on their purchasing habits and the amount of food they buy.
5. Based on the above results, the acceptance and rejection of the research hypotheses are summarized in the following table:

Table 10

Hypothesis table

Hypothesis	Acceptance-Decline	Reasons
Hip.1.	Adoption	The effect of Anxiety is significant-indirect, and significant-indirect through the crisis. The overall effect: β: 0.458. The effect of the crisis is direct and significant on wastage (β: 0.123).
Hip.2.	Adoption	The direct effect of Anxiety on Crisis is significant (β: 0.286).
Hip.3.	Adoption	The effect of Anxiety is significant-directly, and significant-indirectly through the crisis. The overall effect: β: -0.417. The effect of the crisis is direct and significant on optimism (β: -0.100).

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(Drobot, 2008); (Qi and Zhou, 2012); (Bolarinwa et al., 2019; Rabie et al., 2020; Sengev et al., 2013).

Reference list should be alphabetized by the last name of the first author of each work.

If available, please always include DOIs links in the reference list.

Reference style

Journal article

Please follow this style and order: author's surname, initial(s), year of publication (in brackets), paper title, *journal title (in italic)*, volume number (issue), first and last page numbers, DOI. e.g.:

Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science*, *Technique and Technologies*, 2(2), pp. 104-108, https://doi.org/11.1016/22-33-85

Journal names should not be abbreviated.

Book

Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

Book chapter in an edited book

Fordyce F.M. (2013), Selenium deficiency and toxicity in the environment. In: O. Selinus (Ed.), *Essentials of Medical Geology*, Springer, pp. 375–416, https://doi.org/10.14453/10.1007/978-94-007-4375-5_16

Online document

Mendeley J.A., Thomson, M., Coyne R.P. (2017), *How and When to Reference*, Available at: https://www.howandwhentoreference.com

Conference paper

Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018,* NUFT, Kyiv, pp. 52–57, https://doi.org/11.1016/22-33-85

Figures

All figures should be made in graphic editor using a font Arial.

The font size on the figures and the text of the article should be the same.

Black and white graphic with no shading should be used.

The figure elements (lines, grid, and text) should be presented in black (not gray) colour.

Figure parts should be denoted by lowercase letters (a, b, etc.).

All figures are to be numbered using Arabic numerals.

Figures should be cited in text in consecutive numerical order.

Place figure after its first mentioned in the text.

Figure captions begin with the term Figure in bold type, followed by the figure number, also in bold type.

Each figure should have a caption describing what the figure depicts in bold type.

Supply all figures and EXCEL format files with graphs additionally as separate files.

Photos are not advisable to be used.

If you include figures that have already been published elsewhere, you must obtain permission from the copyright owner(s).

Tables

Number tables consecutively in accordance with their appearance in the text.

Place footnotes to tables below the table body and indicate them with superscript lowercase letters.

Place table after its first mentioned in the text.

Ensure that the data presented in tables do not duplicate results described elsewhere in the article.

Suggesting / excluding reviewers

Authors are welcome to suggest reviewers and/or request the exclusion of certain individuals when they submit their manuscripts.

When suggesting reviewers, authors should make sure they are totally independent and not connected to the work in any way. When suggesting reviewers, the Corresponding Author must provide an institutional email address for each suggested reviewer. Please note that the Journal may not use the suggestions, but suggestions are appreciated and may help facilitate the peer review process.

Submission

Email for all submissions and other inquiries:

ufj_nuft@meta.ua

Шановні колеги!

Редакційна колегія наукового періодичного видання «**Ukrainian Food Journal**» запрошує Вас до публікації результатів наукових досліджень.

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**.**
2. **Назва статті.**
3. Автори статті (ім’я та прізвище повністю, приклад: Денис Озерянко).
4. *Установа, в якій виконана робота.*
5. Анотація. **Обов’язкова** структура анотації:
   - Вступ (2–3 рядки).
   - Матеріали та методи (до 5 рядків)
   - Результати та обговорення (пів сторінки).
   - Висновки (2–3 рядки).
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
   - Матеріали та методи
   - Результати та обговорення
   - Висновки
   - Література.

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).
9. Контактні дані автора, до якого за необхідності буде звертатись редакція журналу.

Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути **співрозмірним** (!) тексту статті. **Фотографії можна використовувати лише за їх значної наукової цінності.**

Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

Рисунки та графіки EXCEL з графіками додатково подаються в окремих файлах.

Скорочені назви фізичних величин в тексті та на графіках позначаються латинськими літерами відповідно до системи СІ.

У списку літератури повинні переважати англомовні статті та монографії, які опубліковані після 2010 року.

Оформлення цитат у тексті статті:

Кількість авторів статті	Приклад цитування у тексті
1 автор	(Arych, 2019)
2 автора	(Kuievda and Bront, 2020)
3 і більше авторів	(Bazopol et al., 2022)

Приклад тексту із цитуванням: It is known (Arych, 2019; Bazopol et al., 2022), the product yield depends on temperature, but, there are some exceptions (Kuievda and Bront, 2020).

У цитуваннях необхідно вказувати одне джерело, звідки взято інформацію.

Список літератури сортується за алфавітом, літературні джерела не нумеруються.

Правила оформлення списку літератури

В Ukrainian Food Journalвзято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

1. Посилання на статтю:

Автори А.А. (рік видання), Назва статті, *Назва журналу (курсивом)*, Том (номер), сторінки, DOI.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies,* 2(2), pp. 104–108, https://doi.org/5533.935-3.

2. Посилання на книгу:

Автори (рік), *Назва книги (курсивом)*, Видавництво, Місто.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

3. Посилання на розділ у редагованій книзі:

Автори (рік), Назва глави, In: Редактори, *Назва книги (курсивом)*, Видавництво, Місто, сторінки.

Приклад:

Fordyce F.M. (2013), Selenium deficiency and toxicity in the environment. In: O. Selinus (Ed.), *Essentials of Medical Geology*, Springer, pp. 375–416, https://doi.org/10.14453/10.1007/978-94-007-4375-5_16

4. Тези доповідей конференції:

Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018,* NUFT, Kyiv, pp. 52–57, https://doi.org/5533.935-3.

5. Посилання на електронний ресурс:

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова **Available at:** та вказується електронна адреса.

Приклад:

Cheung T. (2011), *World's 50 most delicious drinks*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт.

Зручний сайт для транслітерації з української мови: http://translit.kh.ua/#lat/passport

Стаття надсилається за електронною адресою:
ufj_nuft@meta.ua

УДК 663/664

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

Тематика публікацій в Ukrainian Food Journal:

Харчова інженерія	Процеси та обладнання
Харчова хімія	Нанотехнології
Мікробіологія	Економіка та управління
Фізичні властивості харчових продуктів	Автоматизація процесів
Якість та безпека харчових продуктів	Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

Ukrainian Food Journal індексується наукометричними базами:

- Index Copernicus (2012)
- EBSCO (2013)
- Google Scholar (2013)
- UlrichsWeb (2013)
- CABI full text (2014)
- Online Library of University of Southern Denmark (2014)
- Directory of Open Access scholarly Resources (ROAD) (2014)
- European Reference Index for the Humanities and the Social Sciences (ERIH PLUS) (2014)
- Directory of Open Access Journals (DOAJ) (2015)
- InfoBase Index (2015)
- Chemical Abstracts Service Source Index (CASSI) (2016)
- FSTA (Food Science and Technology Abstracts) (2018)
- Web of Science (Emerging Sourses Citaton Index) (2018)
- Scopus (2022)

Рецензія рукопису статті. Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний учений.

Детальна інформація про Журнал, інструкції авторам, приклади оформлення статті та анотацій розміщені на сайті:

http://ufj.nuft.edu.ua

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