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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
Київ 01601, **Україна**

e-mail: **ufj_nuft@meta.ua**

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Editorial

For three years Ukraine has been on fire – the Russian aggressor is destroying our land, industry and infrastructure, hospitals, schools, demolishing historical and cultural heritage, bringing death, killing our future and a flower of Nation.

For three years, Ukrainian scientists have been continued to work fruitfully, appreciating the support of partners and people who are not silent now and are contributing to the approach of a just peace.

Three years ago, members of the authoritative international association the Global Harmonization Initiative and personally its ex-president dr. h.c. Lelieveld Huub joined the Editorial Board of the Journal and are working together on its development now. Observation of the results of the work demonstrates that interest in the Journal and its influence are growing.

At present, issues of academic integrity and objective assessment of the results of one's own research are of paramount importance. Our Journal in the conditions of Russian aggression remains an open access journal and does not require payment for the publication of articles. Accordingly, the Journal is interested in high-quality, professionally prepared articles. We value authors, who incorporate modern scientific content into their writing, strive for a critical view of the state of the problems in the materials of articles, taking into account an innovative scientific approach, and expect that the articles will be interesting not only to scientists of a particular university, but to the global scientific community.

Deep respect for experienced scientists who pass on their experience to young people and, by personal example, educate a new, advanced and integrity generation of scientists and educators.

Sincerely,
Editor-in-Chief
Olena Stabnikova

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Effect of cryostabilizing mixtures on quality of cooked sausages

Olena Tunik, Iryna Shevchenko

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Olena Tunik
E-mail:
tunikov@nuft.edu.ua

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Abstract

Introduction. The aim of the study was to investigate the effect of binary cryostabilizing mixtures on the functional, technological structural, mechanical and sensory characteristics of cooked sausages.

Materials and methods. The cryostabilizing mixtures differed in the content of animal protein, vegetable fiber, and sodium alginate. The minced meat for sausages consisted of pork, chicken fillet, minced chicken, an emulsion of beef vein and lard (20% each). The test was performed by replacing 2.5% of the fillet in the recipe with cryostabilizers. The minced meat was cut, formed into a casing, heat-treated (to a temperature of 72 °C in the center of the product), frozen and stored at –18 °C for 30 days.

Results and discussion. Studies of the cryoprotective properties of sodium alginate, animal protein, fiber, sodium tripolyphosphate, and maltodextrin have shown their ability to reduce the cryoscopic temperature of model sausage minced systems to –2.8 °C. To enhance the effectiveness of these ingredients, their properties in binary composite mixtures were considered.

Five variants of the mixtures were used, which differed in the content of animal protein, vegetable fiber, and sodium alginate. Studies have shown that mixtures with a high content of protein and alginate provide the best functional and technological indicators: moisture binding (524–864%) and emulsifying ability (78.18–89.88%), emulsion stability (95.30–96.20%) and increased product yield (122.22–124.02%). Also, these mixtures demonstrate the lowest cryoscopic temperature (–4.58 °C and –4.62 °C) and the best sensory characteristics (juiciness, density, taste). The sensory evaluation confirmed that the sausage samples with this mixture had juiciness, dense structure, harmonious taste and aroma. Other mixtures showed certain shortcomings: samples with the highest protein and alginate content had excessive density, samples with low protein and alginate content had insufficient juiciness, and samples with the lowest protein and alginate content had poor elasticity.

Based on the results obtained, it is recommended to use a blend with an optimal protein and alginate content for the production of frozen long-term storage meat products.

Conclusions. The optimal composition of a binary cryostabilizing mixture that preserves the structure and improves the quality of cooked sausages after freezing and thawing because of the synergistic interaction between protein and polysaccharide has been determined.

Introduction

The freezing process is accompanied by the formation of ice crystals, which leads to structural changes in tissues, destruction of cell membranes and loss of product quality (Castro-Giraldez et al., 2014; Savinok et al., 2017). These changes are irreversible, reduce the sensory properties of meat products and affect their consumer value (Hlushakova et al., 2018; Ramadhan et al., 2012). This is especially true for cooked sausages, which, due to their high moisture content, are vulnerable to changes caused by freezing and thawing (Dromenko et al., 2020).

To minimize these effects, cryoprotectants are used to promote the formation of small, evenly distributed ice crystals (Ivanenko et al., 2018; Sher Ali et al., 2015). Studies have shown that combining functional ingredients such as sodium alginate, plant fibers, animal proteins, tripolyphosphate, and maltodextrin can reduce the cryoscopic temperature to –2.8°C (Shevchenko et al., 2020; Stabnikova et al., 2022; Xianbao Sun et al., 2022). Penetrating cryoprotectants slow the growth of ice crystals inside cells, while impermeable ones act as ice blockers, reducing water diffusion across the membrane (Boutron et al., 1979; Smirnova and Boiko, 2020).

However, for cooked sausage products that are stored in a frozen state for a long time, the peculiarities of combining functional ingredients in cryostabilizing mixtures have not yet been sufficiently studied. It is important to investigate how individual ingredients (polysaccharides, proteins, phosphates, and vegetable fibers) and mixtures developed on their basis affect the functional, technological, structural, and mechanical properties of minced meat systems and ensure synergy between the recipe components. The use of optimized cryostabilizing mixtures will stabilize the fractional composition of water, promote the formation of a fine crystalline ice structure and reduce the effect of salt hyperconcentration.

The development of new functional mixtures with cryoprotective properties will also stabilize the sensory properties and improve the structure of sausages, which is especially important for products stored for a long time at temperatures below – 18°C.

The aim of the study is to investigate the effect of binary cryostabilizing mixtures based on animal protein, fiber, and alginate on the structural, mechanical, and sensory properties of cooked sausages.

Materials and methods

Materials

Cryostabilizing mixture. Composition of cryostabilizing mixtures: animal protein "ScanPro™ A-95" (Essentia) – high-functional cold-brewed animal protein made from natural food pork raw materials by mechanical and heat treatment, dietary fiber: bamboo and wheat (Shandong Jianyuan Foods Co., Ltd.), sodium alginate (E401) (Shandong Jiejing Group Corporation), tripolyphosphate, maltodextrin.

Formulation composition of the tested samples of cooked sausages. Model sausage stuffing systems were made on the basis of pork (20%), chicken fillet (20%), minced chicken (20%), beef vein emulsion (20%) and lard (20%). In the manufacture of minced meat for the experimental samples of cooked sausages, the developed cryostabilizing mixtures with different compositions of functional ingredients (Table 1) were added in the amount of 2.5% to replace 2.5% of chicken fillet, respectively.

Table 1

Composition of binary cryostabilizing mixtures

Ingredients	Blend 1	Blend 2	Blend 0	Blend 3	Blend 4
Sodium alginate	0.034	0.067	0.1	0.134	0.167
Animal protein	0.067	0.167	0.267	0.367	0.467
Vegetable fiber	0.05	0.08	0.12	0.15	0.180
Sodium tripolyphosphate	0.09	0.09	0.09	0.09	0.09
Maltodextrin	0.759	0.596	0.423	0.259	0.096
Total	**1**	**1**	**1**	**1**	**1**

Preparation of sausage samples. Model minced cooked sausage systems were cut with ice in a cutter to 12 °C for 15 minutes, formed into a protein sausage casing with a diameter of 60 mm and subjected to heat treatment in a thermal chamber. The program for heat treatment of cooked sausages in a natural protein casing was used until the temperature in the center reached 72 °C. The finished sausage products were cooled to 12°C for 8 hours and frozen at minus 18°C. The shelf life of the experimental sausage samples at minus 12 °C was 30 days. The heat treatment of the experimental sausage samples, until the temperature in the center reached 70 °C, was carried out after defrosting. Defrosting was made at a temperature of 20 ± 2 °C for 4 hours.

Methods for studying the properties of the cryostabilizing mixture

Determination of the pH of the mixture. The pH value was determined according to ISO 2917:1999 on a laboratory pH meter in a water extract prepared at a mixture: water ratio of 1:10. For this purpose, 5 g of the mixture was taken into a 250 ml conical flask, filled with 50 ml of distilled water and extracted for 30 min with periodic stirring. After the extraction was completed, the extract was filtered through filter paper and the pH of the filtrate was determined using a laboratory pH meter (Puolanne and Kivikari, 2000).

Determination of water-binding capacity (WBC). The moisture binding capacity of the study objects was determined by the Grau-Hamm press method in the modification of V.I. Volovinska and B.Y. Kelman (Shevchenko et al., 2021). The method is based on the release of water from a 300 mg sample during a 10-minute pressing with a 1 kg weight. The determination is made by the size of the spot that remains on the filter paper after sorption of the released moisture, outlining the contour of the spot of compressed meat with a pencil. The size of the wet spot (external) is calculated by the difference between the total spot area and the spot area formed by the meat (product). The content of the WBC was calculated by the formula:

$$WBC = \frac{(A-8.4B)\times 100}{A},$$

where: WBC – the water-binding capacity, % of total moisture; A – total moisture content in the sample, mg; B – wet spot area, cm².

Determination of moisture holding capacity (MHC). A sample of sausage product weighing 0.3-0.5 g is cut into thin slices (2 – 3 mm thick). The sample is taken directly from the middle of the product to avoid the influence of the outer layers. The sample is placed

between two sheets of filter paper to ensure uniform absorption of moisture released under pressure. A weight is placed on the sample with the paper or placed in a special press. The pressure is usually 5 kg/cm² and lasts for 10 minutes. After the pressing is complete, the filter paper is weighed to determine the amount of moisture that has been released from the sample. The difference in weight between the paper before and after pressing reflects the amount of moisture lost (Barbut, 2024).

Determination of emulsion stability. The emulsion stability (ES) of the coarse raw material was determined by heating at 80°C for 30×60s and cooling with water for 15×60s. Then, four 50 ml calibrated centrifuge tubes were filled with the emulsion and centrifuged at a rotational speed of 500 s^{-1} for 5×60 s. The volume of the emulsified layer was then determined. The stability of the emulsion was calculated by the formula:

$$S = \frac{V_1}{V_2} \times 100,$$

where: V_1 – the volume of emulsified oil, ml; V_2 – total volume of the emulsion, ml.

Determination of emulsifying capacity. The emulsifying capacity (EC) was determined after centrifuging a mixture of oil, water, and emulsion at a rotation speed of 500 s^{-1} for 10×60 s. The volume of the emulsified oil was then measured, and the emulsifying capacity was calculated using the following formula:

$$EC = \frac{V_1}{V_2} \times 100,$$

where: V_1 – volume of emulsified oil, ml; V_2 – total volume of oil, ml.

Determination of the penetration stress. Penetration stress of sausage products was determined according to ISO 11036:1994 using a Brookfield DV1 digital viscometer by the depth of the indenter immersion in the test sample at 20 °C. Three measurements were made on the open surface of the sample at a distance of at least 10 mm from the edge of the product and at the maximum distance from the points of other measurements so that the deformed part of the surface did not enter the measurement area, after which the penetration value was converted to the penetration stress value.

Determination of sensory characteristics of sausages. Sensory characteristics of sausages: the method is to evaluate the quality of sausage products according to five criteria: appearance, consistency, color in the cut, smell and taste. Each of these indicators is rated on a five-point scale, where 5 is excellent and 1 is unsatisfactory. The assessment is carried out by a group of experts who analyze the product according to established parameters to determine its compliance with quality standards (Fudali et all., 2021).

Determination of cryoscopic temperature. The cryoscopic temperature was determined by the classical cryoscopic method, which is a method of determining the freezing point of a solution compared to a pure solvent (Fikiin et al., 1998). The test sample with cryostabilizers was cooled in a cryoscope. The temperature difference between the freezing of the pure solvent and the solution was used to evaluate the effect of cryostabilizing additives. The calculations were performed according to Raoul's formula:

$$\Delta T_f = K_f \cdot m,$$

where: ΔT_f is the decrease in freezing point, °C; K_f is the cryoscopic constant of the solvent, °C·kg/mol; m is the mole fraction of the solution, mol/kg.

Statistical analysis

The statistical analysis of the results was carried out with Microsoft Excel program. All determinations were performed at least in triplicate. Values of different parameters were presented as the average (mean) ± standard deviation.

Results and discussion

Analysis and development of ingredient composition of cryostabilizing mixtures

At the first stage, the cryoprotective ability of various functional ingredients (sodium alginate, animal protein, vegetable fiber, tripolyphosphate, maltodextrin) was investigated when added to cooked sausage minced meat before freezing. The results of studies characterizing the change in cryoscopic temperature in model minced sausage systems under the influence of functional ingredients are shown in Figure 1.

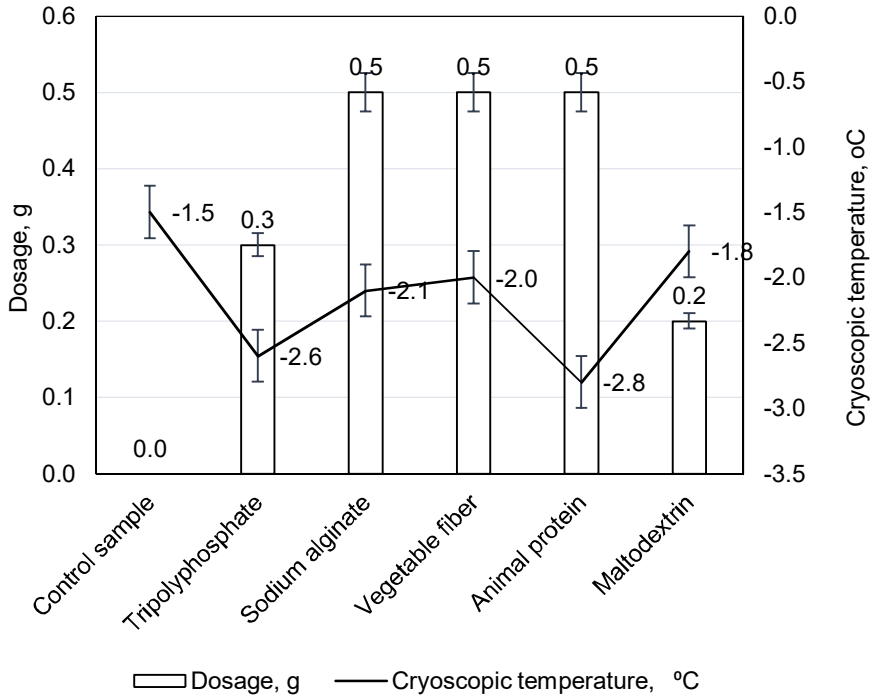

Figure 1. Changes in cryoscopic temperature in minced sausage systems for cooked sausages depending on the influence of functional ingredients

As can be seen from the data in Figure 2, all the studied ingredients are capable of reducing the cryoscopic temperature of model meat systems compared to the control sample, with values ranging from –1.8 °C to –2.8 °C. The use of cryoprotective complex mixtures in model meat systems reduces the cryoscopic temperature by 2.09–2.81 °C, decreases the mass fraction of frozen moisture by 0.9%, and increases water-holding capacity by 5.3–9.7%

compared to the control sample, which positively affects the quality indicators of the final products (Skochko et al., 2018). The obtained results indicate the potential of using the selected functional ingredients as substances aimed at stabilizing meat systems and providing cryoprotection against the effects of low temperatures.

However, the use of individual functional ingredients can reduce the cryoscopic temperature of the meat mixture during freezing to values close to –2 °C, which is insufficient for the long-term storage of cooked sausages in a frozen state. Therefore, based on previous studies (Keniyz, 2014; Shevchenko and Tunik, 2024), we conditionally adopted the formulation of five binary cryostabilizing mixtures with different ratios of ingredients based on animal protein, plant fibers, phosphates, and sodium alginate (Table 1). Protein ingredients of animal and plant origin in cryostabilizing mixtures, as high-molecular compounds, are capable of reducing the rate of crystal growth and protecting muscle tissue cells from osmotic and temperature fluctuations (Skochko et al., 2018). In addition, the cryoprotective effect of polysaccharides can stabilize the functional properties of meat systems during freezing and thawing (Yancheva et al., 2014a).

Study of functional and technological properties of cryostabilizing mixtures

At the next stage, the functional properties of the cryostabilizing mixtures of the proposed composition were studied to evaluate their possible impact on the functional and technological properties of model minced meat systems and finished cooked sausage products. The obtained research results are shown in Table 2.

Table 2

Functional and technological properties (FTP) of cryostabilizing mixtures of different composition

Indicators	Samples of crystallizing compound with different formulations				
	Blend 1	Blend 2	Blend 0	Blend 3	Blend 4
pH mixtures	9.57±0.14	9.83±0.21	9.63±0.17	9.32±0.19	9.15±0.27
Moisture-binding capacity, %.	104±3.62	270±6.14	320±9.60	524±13.70	864±23.50
Penetration stress, g	48.3±1.75	62.1±1.95	68.1±2.16	78.32±3.08	69.2±2.07

The study of functional and technological properties (FTP) of models of sausage minced systems using 2.5% cryostabilizing mixtures to assess their effect on the technological properties of minced systems is presented in Table 3.

According to the results of FTP studies, model minced meat systems with 2.5% cryostabilizing mixtures of different ingredient composition had higher moisture binding, emulsifying ability, and emulsion stability compared to the control. This is due to the fact that at the gelling temperature of the selected polysaccharides close to the denaturation temperature of meat proteins, the water separated from the proteins is absorbed by the protein-polysaccharide complexes that make up the cryostabilizing mixtures (Tomaniak et al., 1998; Shevchenko and Skochko, 2018).

Table 3

FTP of model minced meat systems and samples of cooked sausages with 2.5% cryostabilizing mixtures of different ingredient composition

Indicators	Cryostabilization mixtures				
	Blend 1	**Blend 2**	**Blend 0**	**Blend 3**	**Blend 4**
pH emulsions	6.1±0.14	6.1±0.21	6.3±0.17	6.2±0.19	6.2±0.18
Moisture binding capacity, %	10.6±0.32	18.2±0.64	22.1±0.92	28.6±0.96	33.8±1.02
Moisture retention capacity, %	41.7±1.05	48.9 ±1.16	69.7±2.12	72.01±2.80	83.6±3.04
Fat retention holding capacity, %	66.82±2.18	70.60±2.82	74.40±3.10	77.32±2.46	78.68±2.94
Emulsifying ability, %	69.90±2.24	72.10±3.36	76.46±2.82	78.18±3.14	89.88±3.34
Emulsion stability, %	82.40±3.15	86.20±3.10	91.20±3.56	95.30±3.75	96.20±3.81
Penetration stress, %	44.1±0.95	50.2±1.95	62.8±2.06	80.2±3.18	63.9±2.07
Output, %	110.02±3.1	110.84±4.3	118.16±4.9	122.22±5.1	124.02±5.3

An important indicator that determines the quality characteristics of cooked sausages is the stability of minced meat systems. Crystallizing mixtures are a key tool in preserving the structure and properties of meat products (Petracci et al., 2013). The presence of hydrophilic and hydrophobic groups in protein molecules causes the orientation of polar groups to water and non-polar groups to fat, which results in the formation of an interfacial adsorption layer in the minced protein emulsion (Boutron et al., 1979). This indicator characterizes the strength of the formed interfacial layers at the fat-water interface (Castro-Giraldez et al., 2014). The results of the study indicate an increase in the stability of minced meat systems in sausage samples using 2.5% cryostabilizing mixtures Blend 0, Blend 3 and Blend 4, which contain a higher amount of animal protein, sodium alginate and plant fibers in the composition.

The determination of losses during heat treatment of the experimental samples of cooked sausages showed that the highest yield (122.22 – 124.02 %) was characteristic of sausage samples with 2.5% cryostabilizing mixtures Blend 3 and Blend 4, which contain a larger amount of protein-polysaccharide complexes. Due to their ability to reduce the formation of large ice crystals, stabilize proteins and retain moisture, they minimize product quality losses during long-term storage at low temperatures (Yancheva et all., 2014b).

Significant changes in physicochemical and sensory properties after freezing and thawing, including reduced juiciness, fragility of the structure, and lower product yield (110.02 – 110.84%), were characteristic of sausage samples containing a smaller amount of components with moisture-retaining properties Blend 1 and Blend 0.

Studies have confirmed that the use of a mixture with a high content of proteins and polysaccharides significantly improves the quality of cooked sausages in terms of functional and technological indicators, in particular moisture and fat retention, which contributes to an increase in the yield of finished products.

Study of structural and mechanical properties of model sausage samples

The shear stress of the model samples of cooked sausages with cryostabilizing mixtures of different ingredient composition was measured using a Brookfield DV1 viscometer (Figure 2).

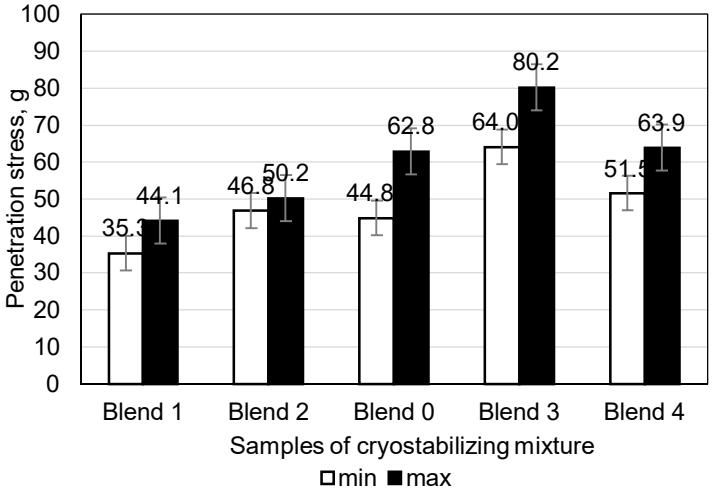

Figure 2. Changes in penetration stress of model samples of cooked sausages depending on the composition of cryostabilizing mixtures

Sausages with the cryostabilizing mixture Blend 3 had the highest values of shear stress – 80.2 g, which significantly exceeded the results of the other samples. Samples of sausages with the cryostabilizing mixture Blend 2 also had high values of shear stress – 50.2 g, although slightly lower than those with the mixture Blend 3, which indicates the ability of both mixtures to increase the stability of the minced systems of cooked sausages. Samples of sausages with the cryostabilizing mixture Blend 4 had average values of shear stress – 63.9 g, but significantly better than those of samples of sausages with cryostabilizing mixtures Blend 1 and Blend 0 – 50.2 g and 44.1 g, respectively. This indicates the insufficiently high functionality of the ingredient composition of the composite mixtures Blend 1 and Blend 0 and their low ability to stabilize the minced systems of cooked sausages, as well as the lower efficiency of the ingredient composition compared to the composition of the cryostabilizing mixtures Blend 3, Blend 4 and Blend 2.

Thus, the results of measuring the shear stress of the experimental samples of cooked sausages on the Brookfield DV1 viscometer showed that the most optimal is the ingredient composition of the composite mixture Blend 3, the use of which in the composition of cooked sausages contributes to the formation of their best structural properties, which confirms the high functionality of its composition as a cryostabilizing mixture for cooked sausages.

Sensory properties of model cooked sausage samples

According to the results of the sensory study of model samples of cooked sausages with different formulations of the cryostabilizing mixture, it was found that the best sensory characteristics were observed in sausage samples with the Blend 3 mixture, which contains a sufficient amount of proteins and polysaccharides. This confirms the high results of the functional, technological and rheological properties of the model sausage samples, and indicates that this composition of the mixture is the most optimal for ensuring high sensory quality indicators of sausage products.

This is in line with studies Tomaniak et al. (1998), which showed that polysaccharides improve the texture and juiciness of meat products. However, studies Yancheva et al. (2014b) have shown that a combination of polysaccharides and proteins can reduce the cryoscopic temperature to – 5.0 ºC. This suggests that there is a potential for further improvement of the mixtures. However, it is important to keep in mind that excessive amounts of polysaccharides, such as sodium alginate, can lead to an excessively thick texture and a deterioration in the product's taste. Therefore, optimizing the composition of mixtures should take into account the balance between the effectiveness of cryoprotective properties and sensory characteristics.

Samples of sausages made from the cryostabilizing mixture Blend 3 were characterized by a satisfactory appearance, uniform color on the cut, dense structure and juicy texture. They also had an expressive aroma and harmonious taste, which ensured their overall high rating (Figure 3). The optimal dosage of sodium alginate in Blend 3 is a key factor in ensuring high quality sausages. It gives the product a good structure, juiciness and stability, without adversely affecting the taste. An excessive amount of sodium alginate could lead to an overly dense or "rubbery" texture, as well as an unpleasant aftertaste. This makes Blend 3 an ideal choice for the production of cooked sausages with high sensory characteristics.

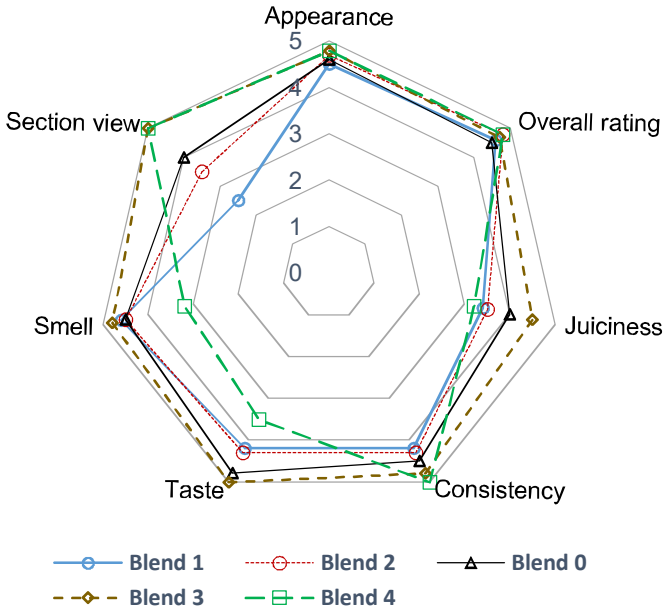

Figure 3. Quality profiles of model cooked sausages samples by sensory characteristics and recipe composition

At the same time, other samples had certain shortcomings: samples of sausages with Blend 4 had an excessively dense texture and less pronounced taste, which negatively affected the overall score; samples of sausages with Blend 1 and 2 were characterized by a split structure, insufficient juiciness and weaker flavor, which made them less attractive to consumers. Samples of sausages with Blend 1 had a pleasant odor, but their texture was not dense and the taste did not meet expectations. Samples of sausages with Blend 0, which were used as a base recipe, demonstrated average performance in all characteristics, but did not stand out with any particular advantages.

Thus, samples of cooked sausages with a cryostabilizing mixture containing sodium alginate 0.134 g and protein 0.367 g (Blend 3) best meet the requirements for sensory quality indicators, combining sufficient juiciness, structural strength, elastic texture, distinct aroma, and balanced taste (Figure 4).

Figure 4. Model cooked sausages samples after defrosting

Water activity and cryoscopic temperature

The cryoscopic temperature and water activity (a_w) are important indicators that allow us to assess the stability of the product during storage, its consistency and susceptibility to microbiological spoilage. The use of cryostabilizing mixtures Blend 4 and Blend 3 in model minced sausage systems for cooked sausages contributed to the maximum reduction of the cryoscopic temperature from – 4.62 °C to – 4.58 °C, which correlated with rather low values of water activity (0.940 and 0.948, respectively) in the experimental sausage samples. This indicates the ability of these mixtures to positively affect the preservation of the structure of sausages in the frozen state and after defrosting and greater microbiological stability during storage. This effect is achieved due to the synergy between the components of the mixtures (protein, alginate, phosphates), which provides better water binding, emulsion stabilization and the formation of a fine crystalline ice structure.

The cryostabilizing blend Blend 0 ranks in the middle in terms of its properties, demonstrating a compromise between the two parameters. The use of cryostabilizing mixtures Blend 2 and Blend 1 in model minced meat systems is less effective due to the relatively low values of cryoscopic temperature (– 3.82 ºC and – 3.76 ºC) and water activity of cooked sausages (0.936 and 0.928) (Figure 5).

The use of more effective cryostabilizing mixtures (Blend 4 or Blend 3) ensures better quality of cooked sausages during freezing and storage, contributing to the preservation of their structure, as evidenced by the results of the sensory evaluation (Figure 4). Due to the optimally selected composition of the mixture: alginate, animal protein, phosphate and fiber, which reduce the freezing point of water in the minced meat system, water binding and the formation of small ice crystals occur, which affect the cryoscopic temperature and water activity (a_w) in minced meat systems.

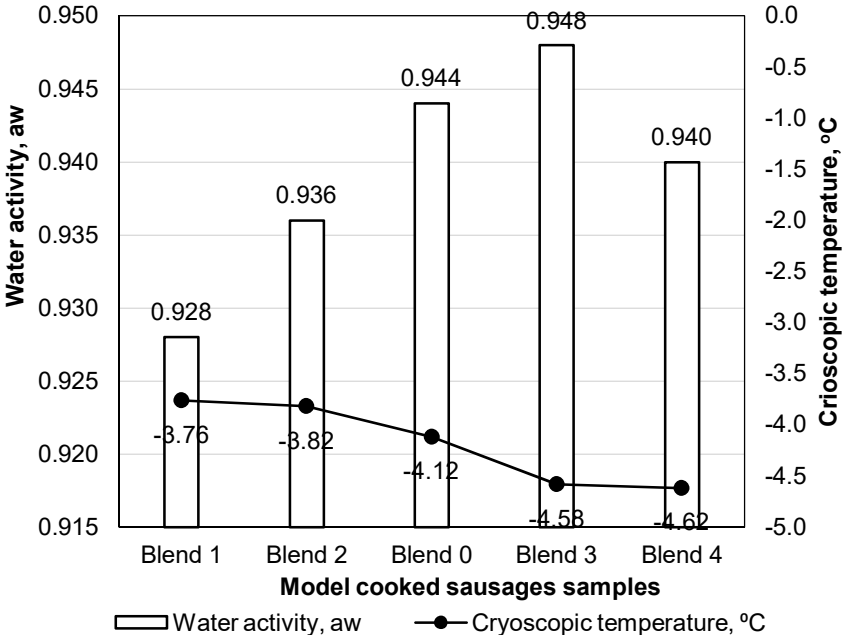

Figure 5. Cryoscopic temperature and water activity of cooked sausage with 2.5% cryostabilizing mixtures of different ingredient compositions

Effectiveness of the cryostabilizing mixture

The use of cryostabilizing mixtures, in particular Blend 3, improves the quality of minced meat systems during freezing and thawing. The blend helps to reduce moisture loss and structure stratification, which has a positive effect on the texture, emulsion stability and sensory properties of cooked sausages.

The synergy of protein and polysaccharide components ensures the formation of protein-polysaccharide complexes, stabilizes the water-fat emulsion and improves the consistency of the products. The cryostabilizing mixture also demonstrates high functional and technological properties, such as moisture retention, stability and emulsifying ability, which is confirmed by positive results of sensory tests.

The results showed a decrease in cryoscopic temperature to – 4.62 ºC and – 4.58 ºC for Blend 4 and Blend 3. These values are in line with the research of Skochko et al. (2018), who also found that the use of cryoprotective blends reduces the cryoscopic temperature by 2.09 – 2.81 ºC. The practical use of a cryostabilizing mixture based on the optimal ratio of

components in the Blend 3 recipe in the production of cooked sausages can increase the yield of finished products by 10%. Due to the stability of the water-fat emulsion and preservation of the product texture, the finished products meet high quality standards, retain a uniform color, harmonious taste and aroma, which makes them competitive in the market. Thus, Blend 3 cryostabilizing mix can be recommended for wide application in the industrial production of frozen sausage products, as it improves quality, reduces losses and increases consumer appeal.

The use of the composite mixtures of the proposed composition can be recommended for the industrial production of cooked sausages, sausages and bratwursts. The use of the proposed mixtures increases the stability of sensory characteristics: the products have an elastic consistency, harmonious taste and aroma, even after prolonged storage at low temperatures and subsequent defrosting, which increases their competitiveness in the market.

Thus, the research results confirm the feasibility of using the cryostabilizing mixture in the production of sausages. Its use improves the stabilization of minced meat systems and ensures effective cryostability of products during freezing and thawing and long-term storage at low temperatures; also, the cryostabilizing mixture avoids the formation of large ice crystals that destroy the structure of minced meat, reducing its moisture retention and sensory properties.

Based on this study, two main areas of implementation of the cryostabilizing mixture in the production of cooked sausages for long-term storage can be identified: stabilization of the functional and technological properties of minced meat and raw meat during production; cryoprotection of finished sausage products during storage and export in a frozen state, and preservation of sensory characteristics after defrosting and further use.

Conclusions

1. The cryostabilizing mixture with a high content of animal protein and alginate (Blend 3) contains the most preferable combination of proteins, polysaccharides and other functional ingredients and demonstrates the best balance between the stability of minced meat systems of cooked sausages and their sensory properties. Its formulation is promising for use in the production of industrial cryostabilizing mixtures.
2. The complex use of the cryostabilizing mixture, using a combination of ingredients (sodium alginate, animal protein, vegetable fiber, tripolyphosphate, and maltodextrin) has proven effective in improving the functional properties of cooked sausages. The combination of animal protein and polysaccharides ensures the stability of the product structure and minimizes moisture and fat loss during freezing and thawing.
3. The use of the cryostabilizing mixture Blend 3 improves the quality of cooked sausages for export and long-term storage at low temperatures, ensuring the stability of their structure and sensory characteristics.

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References

Barbut S. (2024), Measuring water holding capacity in poultry meat, *Poultry Science*, 103(5), 103577, https://doi.org/10.1016/j.psj.2024.103577

Boutron P., Kaufmann A. (1979), Stability of the amorphous state in the system water-glycerol-ethylene glycol, *Cryobiology*, 16(1), pp. 83–89. https://doi.org/10.1016/0011-2240(79)90015-4

Castro-Giraldez M., Balaguer N., Hinarejos E., Fito P.J. (2014), Thermodynamic approach of meat freezing process, *Innovative Food Science & Emerging Technologies,* 23, pp. 138–145, https://doi.org/10.1016/j.ifset.2014.03.007

Dromenko O., Potapov V., Yancheva M., Onishchenko V., Bolshakova V., Inzhyyants A. (2021), Research into the thermophysical characteristics of muscle and adipose tissues in the freezing-thawing process, *Food Science and Technology,* 15(3), pp. 40–51, https://doi.org/10.15673/fst.v15i3.2120

Fudali A., Chełmecka I., Salejda A.M., Krasnowska G. (2021), Microbiological safety and sensory quality of homogenized sausages manufactured with commercial functional additives, *Applied Sciences,* 11(11), 11662, https://doi.org/10.3390/app112411662

Fikiin K.A. (1998), Ice content prediction methods during food freezing: A survey of the Eastern European literature, *Journal of Food Engineering*, 38(3), pp. 331–339, https://doi.org/10.1016/S0260-8774(98)00120-4

Hlushakova Y.V., Petrenko I.O. (2018), Research of cryoprotective properties of biopolysaccharide mixtures in meat systems, *Scientific Works of NUFT*, 5, pp. 25–30.

Ivanenko O.V., Petrova L.M. (2018), Influence of cryoprotectors on the quality of meat products after freezing, *Scientific Works of NUFT*, 24, pp. 45–52.

Keniyz N.V. (2014), *Technology of frozen semi-finished products using cryoprotectants,* Saarbrücken, Germany, Palmarium Academic Publishing, pp. 112–119.

Petracci M., Bianchi M., Mudalal S., Cavani C. (2013), Functional ingredients for poultry meat products, *Trends in Food Science & Technology,* 33(1), pp. 27–39.

Puolanne E., Kivikari R. (2000), Determination of the buffering capacity of postrigor meat, *Meat Science,* 56, pp. 7–13, https://doi.org/10.1016/S0309-1740(00)00007-3

Ramadhan K., Huda N., Ahmad R. (2012), Freeze-thaw stability of duck surimi-like materials with different cryoprotectants added, *Poultry Science*, 91(7), pp. 1703–1708, https://doi.org/10.3382/ps.2011-01926

Savinok O., Pastyukov S., Kuzelov A., Shepelenko D. (2017), Sensory and functional parameters of sausages with reduced sodium nitrite content, *Scientific Works of University of Food Technologies*, 64(1), pp. 32–40.

Semenenko O. M., Ivanenko A. P. (2018), Scientific and practical bases of freezing and cold processing of meat and meat products, *Bulletin of Sumy National Agrarian University,* 12, pp. 89–95.

Stabnikova O., Danylenko S., Kryzhska T., Shang F., Duan Z. (2022), Effects of different phosphate content on the quality of wheat bran chicken sausage, *Ukrainian Food Journal,* 11(4), pp. 588–600, https://doi.org/10.24263/2304-974X-2022-11-4-8

Shevchenko I., Polishchuk G., Kotliar Y., Osmak T., Skochko A. (2020), Prospects of using the crystobilizing protein-polysaccharide composition to manufacture semi-finished chopped meat products, *Food Science and Technology*, 14(1), pp. 134–141, https://doi.org/10.15673/fst.v14i1.1642

Shevchenko I., Polishchuk G., Topchii O., Kotliar Ye., Osmak T. (2021), Improving the technology of restructured ham-type products from turkey meat and pse pork, *Food Science and Technology,* 15(4), pp. 106–115, https://doi.org/10.15673/fst.v15i4.2125

Shevchenko I., Skochko A. (2018), Advantages of using proteins in the production of truncated semi-finished products, *Journal of Faculty of Food Engineering*, Stefan cel Mare University of Suceava, 12(3), pp. 272–277

Shevchenko I., Tunik O. (2024), Cryoprotective properties of functional mixtures in cooked sausage products, *Ukrainian Journal of Food Science*, 13(3), pp. 33–44, https://doi.org/10.24263/2310-1008-2024-12-1-5

Sher Ali, Zhang W., Rajput N., Khan M.A., Li C., Zhou G. (2015), Effect of multiple freeze–thaw cycles on the quality of chicken breast meat, *Food Chemistry*, 173, pp. 808–814, https://doi.org/10.1016/j.foodchem.2014.09.095

Skochko O., Druhoveiko V., Shevchenko I., Maslikov M. (2018), The study of cryoprotective properties of protein-polysaccharide mixtures in the composition of chopped semi-finished products, *Scientific Works of NUFT,* 24(5), pp. 203–207.

Skochko, O., Shevchenko, I., Polichuk, G., Paska, M. (2018), The estimation of effects of food cryoprotectors on quality indicators of semi-finished products,*Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Food Technologies*, 20(90), pp. 27–31. https://doi.org/10.32718/nvlvet9006

Smirnova T.A., Boiko D.Yu. (2020), Use of combined cryoprotectors to improve the stability of meat products during low-temperature storage. *Meat and Meat Products Technology,* 5, pp. 78–85.

Tomaniak A., Tyszkiewicz I., Komosa J. (1998), Cryoprotectants for frozen red meats, *Meat Science*, 50(3), pp. 365–371, https://doi.org/10.1016/S0309-1740(98)00043-6

Yancheva M.O., Bolshakova V.A., Zheleva T.S. (2014a), Study of hydrocolloids properties during the freezing-thawing process, *Kharchova Promyslovist (Food Industry),* 16, pp. 42–45, http://nbuv.gov.ua/UJRN/Khp_2014_16_11

Yancheva M., Zheleva T., Pogozhych N., Hrynchenko A. (2014b), Cryoscopy research of solutions of food ingredients of polysaccharide nature, *Eastern-European Journal of Enterprise Technologies,* 2(12), pp. 84, https://doi.org/10.15587/1729-4061.2014.23513

Sun X., Wu Y., Song Z., Chen X. (2022), A review of natural polysaccharides for food cryoprotection: Ice crystals inhibition and cryo-stabilization, *Bioactive Carbohydrates and Dietary Fibre,* 27, 100291, https://doi.org/10.1016/j.bcdf.2021.100291

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Content of phenolic substances in grapes and wine of the Vranec variety (*Vitis vilifera* L.) grown at different altitude

Dushko Nedelkovski[1], Venelin Roytchev[2], Dimitar Dimitrov[3]

1 – Ss. Cyril and Methodius University Skopje, Institute of Agriculture, Skopje, North Macedonia
2 – Agricultural University, Plovdiv, Bulgaria
3 – Agricultural Academy, Institute of Viticulture and Enology, Pleven, Bulgaria

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Corresponding author:

Dimitar Dimitrov
E-mail: dimitar_robertov@abv.bg

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Abstract

Introduction.The aim of this study was to determine the content of phenolic substances in grapes and wine of the Vranec variety grown at three different altitudes in the area of Macedonia.

Materials and methods. The total phenols content, antocyanins and flavan-3-ols in grape extracts and wines from the control (without application of summer pruning operations) and experimental variants (with application of three different pruning operations) were determined by spectrophotometric analysis.

Results and discussion. The highest levels of anthocyanins (10.74 mg/g FW) were found in the berries skin extract when the pruning operation "normation to 6 bunches per vine" was applied in the Veles region (280 m altitude). The concentration of anthocyanins in the other variants from the Veles region were lower and ranged from 7.26 mg/g FW (control) to 8.39 mg/g FW (variant 10 bunches per vine). The highest levels of total phenols were found in the berries skins and seeds of the "6 bunches per vine" variant in the Skopje region (595 m altitude), accumulated in amounts of 22.82 mg GAE/g FW (berries skin) and 101.04 mg GAE/g FW (berry seeds), respectively. The highest content of total flavan-3-ols was found in the berries skins and seeds of the "6 bunches per vine" variant from the Veles region, accumulated in amounts of 2.33 mg CE/g FW (berries skin) and 29.64 mg CE/g FW (berries seeds), respectively. In the wines from the experimental variants, the highest levels of anthocyanins were found in the wine of the variant "6 bunches per vine" from the Veles region (593.56 mg/L). In the experimental wines from this variant of the same Veles region, the highest amounts of total phenols were also found (2925.61 mg GAE/L). Flavan-3-ols were found in the highest amount (441.46 mg CE/L) in the wine of the variant "6 bunches per vine", but from the Skopje region.

Conclusions. The higher altitude showed to increase the amount only of total phenols in the berries skins and seeds.There were no significant differences in the content of phenolic substances – total anthocyanins and total phenols in the wine of the Vranec variety by variants and regions, as their quantities were higher in the variants with normation of grape bunches.

Introduction

Phenols are a significant group of substances found in the grapes and wine, which have great influence on their organoleptic characteristics, biological potential and quality (Zhang et al., 2023). They determine the colour of the berries skin and also the wine's colour (anthocyanins) and density (phenols increase the wine extract) and taste (Margaryan et al., 2017). These substances are transferred from grapes into the wine during the alcoholic fermentation and are directly involved in the formation of its quality (Gutiérrez-Escobar et al., 2021; Landrault et al., 2001). As biologically active substances, they have different effects on human health – antimicrobial, anticancer, cardioprotective, anti-inflamation, neuroprotective, antiaging, antidiabetic, and antioxidant (Milinčić et al., 2025; Neira-Opsina et al., 2024; Renaud and Lorgeril, 1992; Xia et al., 2010;). Their quantity in the grapes depends on many factors, which are divided into two groups: biological, including the ampelographic features of the variety; environmental – soil and climatic conditions of the region, applied agricultural techniques, plant protection measures, etc. Increased rainfall, as well as excessive irrigation, have a negative impact on the content of phenolic substances in grapes (Valdés et al., 2009). The lower content of total anthocyanins in grapes can be due to a later harvest and advanced technological maturity (Andjelkovic et al., 2013). The reduction of the anthocyanins in the skin of the berry provokes not only inhibition of the biosynthetic processes, but also a number of accompanying high temperatures factors such as the chemical and/or enzymatic degradation (Morri et al., 2007). At the beginning of the alcoholic fermentation, the extraction of anthocyanins from the berry skin in the must is slow, but gradually it increases and reaches its maximum when the alcohol content increases from 3 to 6 vol. %, after which their amount slowly decreases (Nagel and Wulf, 1979; Watson et al., 1995). At the end of the fermentation, the decrease in the concentration of anthocyanins in wine is due to adsorption by solid parts, the interaction of anthocyanins with tannins and the formation of polymer pigments (Singleton and Trousdale, 1992). The extraction of tannins from the skins and seeds increases even after free anthocyanins have reached their maximum concentrations (Ozmianski et al., 1986).

There are a number of studies in the scientific literature on the changes occurring in the phenolic profile of grapes grown at different altitudes and their corresponding wines. Jin et al. (2017) investigated the changes in the phenolic profile of red wines from Merlot and Cabernet Sauvignon varieties produced from grapes grown at three different altitudes – 2282 m, 2435 m and 2608 m in a mountainous region north of the Hengdnan Mountains, China. The team found that altitude had a significant effect, leading to an increase in the content of total phenolic compounds, total flavonoids and total anthocyanin content in the wines of both varieties. It was also reported that the tannin content of Cabernet Sauvignon wine increased proportionally with increasing altitude. Brighenti et al. (2017) determined the total polyphenol content of grapes from 13 red grapevine varieties grown in the São Joaquim region of Brazil at 1400 m above sea level. The team found a range of total polyphenol content from 523.87 mg/L to 4929.57 mg/L, with the highest levels in grapes from Ancelota, Uva di Troia and Croatina.

The aim of this study was to determine the content of phenolic substances in grapes and wine of the Vranec variety grown at three different altitudes in the area of Macedonia.

Materials and methods

Materials

The experimental work was conducted during the period 2012 – 2015, with vines of the local grape variety Vranec grown in three regions of the R. N. Macedonia with different altitudes: Gevgelija – 50 m, Veles – 280 m and Skopje – 595 m. The row and interlinear planting distances in the vineyard was 3.20/1.00 m in Gevgelija, while in Veles and Skopje – 3.20/1.20 m. The vines pruning system was double-Guyot loaded with 20 winter buds in total – two spurs with 2 buds and two fruit sticks with 8 buds.

In the study, 150 vines were included on each vineyard; four variants were formed with 35 vines of each variant:

- control – without application of summer pruning operations;
- with application of defoliation from the base of the fruiting shoots to the area of the bunches, carried out in the middle of August at 80% version of the berries;
- with normation of grape bunches by leaving 6 grape bunches per vine in the middle of July;
- with normation of grape bunches by leaving 10 grape bunches per vine in mid-July.

In order to determine the influence of altitude on the content of phenolic substances, the grapes from the three regions were harvested at the same time. The moment of harvesting was determined by visual control, measurement of sugar content with a refractometer and monitoring the appearance of wilting in 5 – 10 % of the berries bunch of the variant with normation of 6 bunches per vine.

For the analysis of the total phenols, total anthocyanins, total flavan-3-ols and the colour characteristics was used spectrophotometer Agilent 8453 UV/VIS (Agilent Technologies, California, USA)

Methods

Extraction of berries skins and seeds. Berries from couple of grape bunches were skinned with laboratory tweezers. The seeds were separated from the pulp, washed with distilled water and the water was removed with filter paper. Skins were blotted on filter paper to remove any residual pulp. The skins and seeds were ground in lab mortar. Approximately 1g of grape berry skins and 1g seeds were weight on analytical balance and were extracted twice for 15 min with10 mL acetone/water (80/20, v/v) containing HCl (0.1/10, v/v) to prevent oxidation of the polyphenolic substances in an ultrasonic bath at room temperature and then stirred for 30 min on a magnetic agitator. The samples were centrifugated (3000 rpm for 10 min) and the supernatants from both extractions were combined and made up to a final volume of 25 mL with distilled H_2O. All extracts were filtered before spectrophotometric determination of phenol compounds (Ivanova-Petropulos et al., 2010).

Total polyphenols content. The total polyphenols content in grape extracts and wines from the control and experimental variants were determined by spectrophotometric analysis at a wavelength of 765 nm by the Folin-Ciocalteu method (Singleton et al., 1999). The obtained results were expressed as mg/g FW (fresh weight) gallic acid equivalent (for grape extracts) and mg/L gallic acid equivalent (for wine). The obtained grape extracts or the tested wines were diluted (1:25). A quantity of 1 ml of this sample was transferred to a 10 ml flask, in which 5 ml of distilled water was previously poured. 5 ml of Folin-Ciocalteu reagent was added and 3 min were waited, then 1.5 ml of 20% Na_2CO_3 was added. At the next stage, the

flask was filled to the mark. The samples were placed in a water bath at a temperature of 50 ºC for 16 min. After that, they were cooled with cold water and the absorption of the spectrophotometer was established at a wavelength of 765 nm. In the control sample, 1 ml of distilled water was used instead of the tested solution. The result was established from a standard curve with gallic acid.

Total anthocyanins content. The grapes extracts or wines for analysis were centrifuged at 3000 rpm for 20 min. The centrifuged sample was diluted (1:100) and 0.1 ml of this sample was made up to 10 ml with acidified ethanol (96% ethanol: HCl). The absorbance of the sample was determined at 550 nm wavelength, and acidified ethanol was used as a control. Total anthocyanins content were determined by the formula: (15xD0) x R, where D0 – absorbance recorded at 550 nm wavelength; R = sample dilution. The content was expressed as mg/l (for wine sample) and mg/g FW (grapes extracts) (Di Stefano et al., 1989).

Total flavan-3-ols content. The quantification of total flavan-3-ols was performed with p-dimethylamino-cinnamaldehyde (p-DMACA) method and the obtained results were expressed as catechin equivalent (mg CE/L) (Di Stefano et al., 1989). In a 10 ml flask, 0.1 ml of red wine or extract was poured, three drops of glycerol were added, 5 ml of p-DMACA reagent flask was poured to the mark with distilled water. After 7 min, measuring of the absorbance at 640 nm wavelength was made. Methanol was used as a control sample. The result was determined from a standard curve of catechin hydrate.

Wine colur characteristics. The wine colour characteristics were determined according to the method of Ivanova-Petropolus (2013) and were expressed as Absolute Units (AU).

Colour intensity of the wines was determined by the level of the anthocyanins present in the wine and was defined as the sum of the absorbances at 420, 520 and 620 nm. In this study, the absorbance of wines was directly measured at 420, 520 and 620 nm using 2 mm cuvette. From the obtained results colour intensity (*CI*), hue or tint (*T*), proportion of red colour (% *Rd*), blue colour (% *Bl*), yellow colour (% *Ye*) were calculated.

Colour intensity (CI) is sum of $A420+A520+A620$

The hue or tint (T) is defined as the ratio of $A420/A520$.

The colour composition of the wines was expressed as percentage and it was calculated according to the following equations:

$$\% Ye \text{ or } \% Rd \text{ or} \% Bl = 100(A\lambda/CI)$$

where: % *Ye* is the percentage of yellow colour (λ = 420 nm), %*Rd* is thepercentage of red colour (λ = 620 nm) and % *Bl* is the percentage of blue colour (λ = 520 nm) in the overall wine colour.

Statistical analysis

The software package IBM SPSS Statistics 2015 was used for the statistical analysis. In order to verify if there is a statistical difference between the parameters, Duncan's multiple range test was used with significance level of 0.05

Results and discussion

Content of phenolic substances in the grapes of the Vranec variety for all investigated variants and regions, average for the period 2013–2015

The statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety in all variants and regions, on average for the period 2013–2015 showed the lowest amount of total anthocyanins in the berries skins from the control variant which was statistically proven – 6.40 mg/g FW compared to the others (Table 1).

The highest anthocyanin amount was found in the variant with 10 bunches per vine – 9.05 mg/g FW in the berries skins of the Vranec variety for all investigated variants and regions, average for the period 2013–2015**.** The differences in the anthocyanin content in berries skins between the other studied variants (defoliation, 6 grape bunches and 10 grape bunches) were not statistically proven. The total phenols in the berries seeds were again statistically proven with the highest content in variant with 10 grape bunches per vine – 93.26 mg GAE/g FW, compared to the control – 77.21 mg GAE/g FW. The established increasing tendency for the total flavan-3-ols amount was the same, but statistically not proven, which again confirmed the positive impact of the yield normation on the quality of the obtained grapes.

Content of phenolic substances in the grapes of the Vranec variety by variants and regions, average for the period 2013–2015

The same trend was also established by comparing of the data for anthocyanins in the berries skins by variants and regions (Table 2). It should be noted that the vineyard in Gevgelija was characterized with higher temperature values than the vineyards of the other two regions, and respectively lower absolute values for the amount of total anthocyanins in all variants of the study, although statistically proven only in the variant with 6 grape bunches per vine.

The highest values for this indicator (total antocyanins content) in the studied variants (one control without pruning operations and the three variants with different pruning operations) were obtained in the berries skin from the vineyard located around Veles – control (7.26 mg/g), defoliation (8.35 mg/g), with 6 bunches per vine (10.74 mg/g) and with 10 bunches per vine (8.39 mg/g). The amount of total phenols was the highest in the grapes variants (one control without pruning operations and the three variants with different pruning operations) from the Skopje region. Their content varied from 14.84 mg GAE/g FW (defoliation) to 22.82 mg GAE/g FW (6 bunches per vine), and the lowest of total phenolic content was established in the variants from Gevgelija region with variation from 9.77 mg GAE/g FW (defoliation) to 12.39 mg GAE/g FW (6 bunches per vine). All established differences between the variants were statistically proven. The changes in the content of total flavan-3-ols were almost identical with those of the total anthocyanins, as the statistical differences between the variants were proved only in the variant with 10 bunches per vine compared to the grapes from Veles region, where the highest values for this indicator were reported. The total phenols in the berries seeds were statistically proven and had a higher values in the grapes from the Skopje region – 83.42 mg GAE/g FW (control), 88.62 mg GAE/g FW (defoliation), 101.04 mg GAE/g FW (6 bunches per vine) and 94.03 mg GAE/g FW (10 bunches per vine). The content of total flavan-3-ols was statistically proven in almost all variants, more in the berries seeds of grapes form Veles region – 23.42 mg CE/g FW (control), 20.82 mg CE/g FW (defoliation), 29.64 mg CE/g FW (6 bunches per vine) and 28.35 mg CE/g FW (10 bunches per vine). The higher altitude showed the influence to increase the amount only of total phenols in the berries skins and seeds, while the values of all other indicators were the highest in the grapes from the Veles region.

Content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

The statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety by variants showed that during the year with the most precipitation – 2014, the total anthocyanins in the berries skins were the lowest and this was statistically proven (control – 4.85 mg/g, defoliation – 4.75 mg/g, 6 grape bunches per vine – 7.64 mg/g and 10 grape bunches per vine – 4.77 mg/g) (Table 3). Only in the variant with 6 grape bunches per vine there was no statistically significant difference with the others.

In the case of total phenols, the emerging trend related to the influence of humidity on their accumulation was maintained, and again in 2014 their content was the lowest. There was diversity in this indicator, which was expressed by proven higher amounts in the berries skins from the Gevgelija region – 19.11 mg GAE/g FW (control), 23.75 mg GAE/g (6 grapes bunches per vine) and 22.11 mg GAE/g FW (10 grapes bunches per vine). Only in the variant with defoliation the value of this indicator was the highest in Skopje region – 15.28 mg GAE/g FW. In general, the flavan-3-ols also had the highest content in the grapes from Gevgelija region, with exception again for the variant with defoliation. Their accumulation starts at the beginning with the berry formation and continues until the phenophase verison. Then, in the period from verison to reaching the phenolic maturity, the synthesis of the monomeric flavan-3-ols – (+) catechin, (-) epicatechin and (-) epicatechin gallate are slowed or in some cases stopped, leading to a decrease in their amount (Kennedy et al., 2000). This process in the present study was caused by yield normation of the vine. There was a statistically significant difference in the total phenols and flavan-3-ols content in the seeds between the vineyard located in Veles region and the other two. Yield normation definitely increased the proven average values of these two groups of phenolic substances. The total flavan-3-ols content in 2014 reached the highest and statistically proven levels in the seeds, and the reason of this result was related with the bad weather conditions that caused difficulties in the proper course of the grapes ripening process. These results showed that the studied indicators in the grapes skins and seeds from all three studied regions were strongly depended of the external climatic conditions for the individual years and it was not possible to identify clearly any trend related to the importance of the altitude for their formation.

Content of phenolic substances in the wine of Vranec variety by variants, average for the period 2013–2015

The statistical comparative analysis of the phenolic substances content in the wine of the Vranec variety by variants and regions showed that there were no significant differences between the variants for the total anthocyanins and total phenols indicators (Table 4). It was clearly noticed that in the variants with grape bunches normation, higher quantities were achieved compared to the control and defoliation variants. The wines obtained from the variants with normalized yield with 6 bunches and 10 bunches per vine had superior values compared to the others variants, but had no statistically proven differences.

The same analysis applied for the content of total flavan-3-ols, showed differences in the values between the studied variants. The yield normation variants had statistically proven difference with the others. The highest values of all three indicators were reported for the variant with 6 grape bunches per vine – total anthocyanins (571.44 mg/L), total phenols (2610.04 mg GAE/L) and total flavan-3-ols (364.24 mg CE/L), compared to the control – 425.74 mg/L, 2035.07 mg GAE/L and 277.36 mg CE/L, respectively. The defoliation, as a summer pruning operation, also showed higher values compared to the control, but there was no statistically significant difference. The highest colour intensity value was determined in the variant with 6 grape bunches per vine. It had statistically proven differences from the others. The wine from the control variant had the lowest colour intensity. The levels of the other indicators – hue, yellow colour, red colour and blue colour had very similar values that were not statistically proven.

Table 1

Content of phenolic substances in the grapes of the Vranec variety for all investigated variants and regions, average for the period 2013–2015

Variants	Phenolic fractions				
	Total anthocyanins, mg/g FW	Total phenolics, mg GAE /g FW	Total flavan-3-ols, mg CE/g FW	Total phenolics, mg GAE/g FW	Total flavan-3-ols, mg CE/g F
	Berry skins			Berry seeds	
Control	6.40 ^{b}	14.01 ^{a}	1.45 ^{a}	77.21 ^{b}	16.63 ^{a}
Defoliation	6.82 ^{ab}	12.52 ^{a}	0.83 ^{a}	80.16 ^{b}	17.58 ^{a}
6 grape bunches	7.89 ^{ab}	15.96 ^{a}	1.37 ^{a}	86.33 ^{ab}	21.78 ^{a}
10 grape bunches	9.05 ^{a}	17.93 ^{a}	1.65 ^{a}	93.26 ^{a}	23.28 ^{a}

Note: a, b, c – Duncan significance level of 0.05, GAE – gallic acid equivalent; CE – catechin equivalent; FW – fresh weight

Table 2

Content of phenolic substances in the grapes of the Vranec variety by variants and regions, average for the period 2013–2015

Vineyard location		Variants and phenolic fractions				
		Total anthocyanins, mg/g FW	Total phenolics, mg GAE /g FW	Total flavan-3-ols, mg CE/g FW	Total phenolics, mg GAE /g FW	Total flavan-3-ols, mg CE/g FW
		Berry skins			Berry seeds	
Control	Gevgelija	5.79 ^{a}	10.49 ^{b}	1.02 ^{a}	66.19 ^{b}	12.53 ^{b}
	Veles	7.26 ^{a}	14.15 ^{ab}	1.73 ^{a}	82.02 ^{a}	23.42 ^{a}
	Skopje	6.14 ^{a}	17.36 ^{a}	1.61 ^{a}	83.42 ^{a}	13.94 ^{b}
Defoliation	Gevgelija	5.22 ^{a}	9.77 ^{b}	0.64 ^{a}	69.82 ^{b}	17.11 ^{a}
	Veles	8.35 ^{a}	12.95 ^{ab}	0.96 ^{a}	82.06 ^{a}	20.82 ^{a}
	Skopje	6.91 ^{a}	14.84 ^{a}	0.88 ^{a}	88.62 ^{a}	14.81 ^{a}
6 bunches per vine	Gevgelija	8.13 ^{b}	12.39 ^{b}	1.24 ^{a}	86.43 ^{b}	20.95 ^{b}
	Veles	10.74 ^{a}	18.57 ^{ab}	2.33 ^{a}	92.32 ^{ab}	29.64 ^{a}
	Skopje	8.29 ^{b}	22.82 ^{a}	1.37 ^{a}	101.04 ^{a}	19.28 ^{b}
10 bunches per vine	Gevgelija	7.23 ^{a}	11.01 ^{b}	1.03 ^{b}	80.47 ^{b}	18.79 ^{b}
	Veles	8.39 ^{a}	16.23 ^{ab}	2.14 ^{a}	84.48 ^{b}	28.35 ^{a}
	Skopje	8.07 ^{a}	20.64 ^{a}	0.95 ^{b}	94.03 ^{a}	18.22 ^{b}

Note: *a, b, c – Duncan significance level of 0.05;* GAE – gallic acid equivalent; CE – catechin equivalent; FW – fresh weight

Table 3

Statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

Years	Variants and phenolic fractions				
	Total anthocyanins, mg/g FW	Total phenolics, mg GAE/g FW	Total flavan-3-ols, mg CE/g FW	Total phenolics, mg GAE/g FW	Total flavan-3-ols mg CE/g FW
	Berry skins			Berry seeds	
Control					
2013	6.29 ^{b}	19.11 ^{a}	2.53 ^{a}	83.68 ^{a}	19.62 ^{a}
2014	4.85 ^{c}	8.88 ^{c}	1.07 ^{b}	76.36 ^{ab}	18.41 ^{a}
2015	8.01 ^{a}	13.44 ^{b}	0.44 ^{b}	71.02 ^{b}	9.95 ^{b}
Defoliation					
2013	6.83 ^{b}	12.52 ^{b}	0.83 ^{b}	80.16 ^{a}	17.58 ^{b}
2014	4.75 ^{c}	9.76 ^{c}	1.13 ^{a}	81.89 ^{a}	23.75 ^{a}
2015	8.89 ^{a}	15.28 ^{a}	0.52 ^{c}	78.43 ^{a}	11.41 ^{c}
6 bunches per vine					
2013	8.81 ^{a}	23.75 ^{a}	2.32 ^{a}	89.35 ^{b}	22.69 ^{b}
2014	7.64 ^{a}	14.46 ^{b}	1.94 ^{a}	101.42 ^{a}	30.45 ^{a}
2015	9.83 ^{a}	13.44 b	0.44 ^{b}	86.57 ^{b}	13.81 ^{c}
10 bunches per vine					
2013	8.30 ^{b}	22.11 ^{a}	2.14 ^{a}	88.59 ^{a}	22.28 ^{a}
2014	4.77 c	9.24 ^{c}	1.30 ^{b}	84.19 ^{a}	27.43 ^{a}
2015	9.89 a	14.94 ^{b}	0.49 ^{b}	84.01 ^{a}	12.87 ^{b}

Note: a, b, c – Duncan significance level of 0.05; GAE – gallic acid equivalent; CE – catechin equivalent; FW – fresh weight

Table 4

Content of phenolic substances in the wine of Vranec variety by variants, average for the period 2013–2015

Variants	Phenolic fractions							
	Total anthocyanins, mg/L	Total phenolics, mg GAE/L	Total flavan-3-ols, mg CE/L	Color intensity, AU	Hue, AU	Yellow color, %	Red color, %	Blue color, %
Control	425.74 [a]	2035.07 [a]	277.36 [b]	1.85 [c]	0.443 [a]	30.48 [a]	57.36 [a]	12.15 [a]
Defoliation	463.89 [a]	2042.16 [a]	293.36 [ab]	2.08 [bc]	0.435 [a]	30.06 [a]	59.16 [a]	10.78 [a]
6 grape bunches	571.44 [a]	2610.04 [a]	364.24 [a]	2.87 [a]	0.443 [a]	30.45 [a]	57.17 [a]	12.37 [a]
10 grape bunches	531.88 [a]	2470.01 [a]	373.86 [a]	2.52 [ab]	0.430 [a]	30.21 [a]	57.93 [a]	11.82 [a]

Note: a, b, c – Duncan significance level of 0.05, GAE – gallic acid equivalent; CE – catechin equivalent;

Table 5

Content of phenolic substances in the wines of Vranec variety by variants and regions, average for the period 2013–2015

Vineyard location		Phenolic fractions			Colour characteristics				
		Total anthocyanins, (mg/L)	Total phenolics, mg GAE/L	Total flavan-3-ols, mg CE/L	Color intensity, AU	Hue, AU	Yellow color, %	Red color, %	Blue color, %
Control	Gevgelija	405.44[a]	1702.13 [a]	230.51 [a]	1.71[ab]	0.423[a]	30.05 [a]	57.36 [a]	11.84 [a]
	Veles	446.44 [a]	2141.04 [a]	308.78 [a]	2.49 [a]	0.417[a]	29.10 [a]	58.51 [a]	11.77 [a]
	Skopje	425.33 [a]	2262.07 [a]	292.77 [a]	1.35 [b]	0.484[a]	31.71 [a]	55.44 [a]	12.85 [a]
Defoliation	Gevgelija	444.66 [a]	1628.57 [a]	226.12 [a]	1.73 [a]	0.415 [a]	29.46 [a]	59.97 [a]	10.56 0[a]
	Veles	483.50 [a]	2332.90 [a]	321.77 [a]	2.48 [a]	0.410[a]	29.03 [a]	60.95 [a]	10.020 [a]
	Skopje	463.50 [a]	2165.00 [a]	332.21 [a]	2.06 [a]	0.480[a]	31.68 [a]	56.56 [a]	11.750 [a]
6 bunches per vine	Gevgelija	537.44 [a]	2216.67 [a]	281.66 [b]	2.49 [b]	0.410	29.71 [a]	58.64 [a]	11.64 [a]
	Veles	593.56 [a]	2925.61 [a]	369.58 [ab]	3.44 [a]	0.487	31.19 [a]	56.15 [a]	12.66 [a]
	Skopje	583.33 [a]	2687.84 [a]	441.46 [a]	2.67 [ab]	0,430[a]	30.46 [a]	56.73 [a]	12.81 [a]
10 bunches per vine	Gevgelija	503.44 [a]	2075.55 [a]	295.52 [b]	2.16 [a]	0.430[a]	30.36 [a]	57.96 [a]	11.67 [a]
	Veles	555.66 [a]	2656.67 [a]	359.90 [ab]	2.98 [a]	0.415[a]	29.24 [a]	60.59 [a]	10.17 [a]
	Skopje	541.33 [a]	2672.53 [a]	439.91 [a]	2.55 [a]	0.433[a]	30.58 [a]	56.57 [a]	12.77 [a]

Note: a, b, c – Duncan significance level of 0.05; GAE – gallic acid equivalent; CE – catechin equivalent.

Table 6

Statistical comparative analysis of the content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

Years	Phenolic fractions			Colour characteristics				
	Total anthocyanins, mg/L	Total phenolics, mg GAE/L	Total flavan-3-ols, mg CE/L	Color intensity, AU	Hue, AU	Yellow color, %	Red color, %	Blue color, %
Control								
2013	435.44 ^{ab}	2414.44 ^{a}	313.83 ^{a}	2.09 ^{a}	0.406 ^{a}	30.55 ^{ab}	54.92 ^{ab}	14.53 ^{a}
2014	365.66 ^{b}	1625.96 ^{b}	224.94 ^{a}	1.57 ^{a}	0.463 ^{a}	32.64 ^{a}	54.46 ^{b}	12.89 ^{b}
2015	476.11 ^{a}	2064.82 ^{ab}	293.29 ^{a}	1.89 ^{a}	0.453 ^{a}	28.27 ^{b}	62.69 ^{a}	9.04 ^{c}
Defoliation								
2013	463.88 ^{ab}	2042.16 ^{a}	293.36 ^{a}	2.08 ^{a}	0.435 ^{a}	30.06 ^{ab}	59.16 ^{ab}	10.78 ^{ab}
2014	397.66 ^{b}	1883.49 ^{a}	271.62 ^{a}	2.13 ^{a}	0.423 ^{a}	31.75 ^{a}	55.78 ^{b}	12.46 ^{a}
2015	530.11 ^{a}	2200.82 ^{a}	315.11 ^{a}	2.04 ^{a}	0.447 ^{a}	28.36 ^{b}	62.55 ^{a}	9.09 ^{b}
6 bunches per vine								
2013	557.33 ^{b}	3033.66 ^{a}	400.76 ^{a}	3.17 ^{a}	0.467 ^{a}	32.41 ^{a}	54.00 ^{b}	13.59 ^{a}
2014	487.33 ^{c}	2188.46 ^{b}	390.44 ^{a}	2.76 ^{a}	0.393 ^{a}	30.12 ^{a}	55.77 ^{b}	14.11 ^{a}
2015	669.66 ^{a}	2607.98 ^{ab}	301.50 ^{a}	2.67 ^{a}	0.467 ^{a}	28.84 ^{a}	61.75 ^{a}	9.41 ^{b}
10 bunches per vine								
2013	494.00 ^{b}	2749.66 ^{a}	401.28 ^{a}	2.79 ^{a}	0.430 ^{a}	31.27 ^{a}	54.83 ^{b}	13.90 ^{a}
2014	466.33 ^{b}	2179.60 ^{a}	346.01 ^{a}	2.38 ^{a}	0.403 ^{a}	31.14 ^{a}	56.22 ^{b}	12.65 ^{a}
2015	635.33 ^{a}	2480.76 ^{a}	374.31 ^{a}	2.36 ^{a}	0.450 ^{a}	28.22 ^{a}	62.74 ^{a}	8.93 ^{b}

Note: a, b, c – Duncan significance level of 0.05; GAE – gallic acid equivalent; CE – catechin equivalent.

Content of phenolic substances in the wines of Vranec variety by variants and regions, average for the period 2013–2015

The statistical comparative analysis of the content of phenolic substances in the wine of the Vranec variety analysed by regions showed that for most of the indicators the established differences were not statistically significant (Table 5).

The values for the total anthocyanins content was higher in the variants with yield normation, 6 and 10 grape bunches per vine. In the variants, the highest anthocyanins content were found in the wine obtained from grapes grown in the Veles region – control (446.44 mg/L), defoliation (483.50 mg/L), with 6 grapes bunches per vine (593.56 mg/L) and with 10 grape bunches per vine (555.66 mg/L). In the Veles region the highest anthocyanins accumulation was achieved in the normation variant with 6 grape bunches per vine. The total phenols content in the control and the variant with 10 grapes bunches per vine was the highest in the wine from the Skopje region (2262.07 mg GAE/L and 2672.53 mg GAE/L), and in the variants with defoliation and 6 grapes bunches per vine in Veles (2332.90 mg GAE/L and 2925.61 mg GAE/L). The wine variant (Veles region) with 6 grapes bunches per vine had the highest total phenols and total flavan-3-ols content, more than all the other normation variants. All the differences were statistically proven. In the control variant, their quantity was the highest in Veles region – 308.78 mg CE/L and in the other variants in Skopje region (defoliation – 332.21 mg CE/L, with 6 bunches per vine – 441.46 mg CE/L and with 10 bunches per vine – 439.91 mg CE/L). The highest values for the colour intensity was determined in the wines from Veles region in all studied variants. The proven differences were determined only in the control and 6 grapes bunches per vine variants. The obvious trends in the change of the quantities of the analyzed studied indicators were significantly weaker in the others – hue, yellow colour, red colour, blue colour, but since the differences between the variants and the regions were unproven and as absolute values very similar so it was assumed that they did not change the wine quality.

Content of phenolic substances in the grapes of the Vranec variety by variants and years, average for the period 2013–2015

The experimental data results, related with the content of phenolic substances in the wine of the Vranec variety presented by years and variants, for all indicators (total anthocycnins, total phenols and total flavan-3-ols) had the lowest obtained values in 2014 (Table 6). Again, over the years, the largest amounts of these indicators were achieved in the wine samples obtained from the variants with grape normation per vine.

Total content of anthocyanins was the highest in wines from 2015 harvest in all variants – control (476.11 mg/L), defoliation (530.11 mg/L), with 6 bunches per vine (669.66 mg/L) and with 10 bunches per vine (635.33 mg/L). The total phenols content was more in 2013 harvest, except for the defoliation variant – 2015 harvest. For this indicator, the differences in the obtained results between the years were statistically proven for the control and 6 bunches per vine variants. The same trend was observed of the total flavan-3-ols amount. The data for the colour intensity and hue of the wine had very close values. The statistical analysis showed unproven difference in any of the variants. Over the years, there were some differences in the percentage of the wine colours – yellow, red and blue, which were not statistically proven only for yellow colour in the variants with 6 and 10 bunches per vine. Red predominated, followed by yellow and blue colour. Depending on the year (harvest), the highest percentage of red colour was achieved in 2015 in all variants, percentage of yellow

colour in 2014 for control and defoliation, and percentage of blue colour in 2013 for control and 10 bunches per vine.

The data in the present study were in correlation with the study of Mateus et al. (2001), who found that grapes grown at higher altitudes synthesized higher levels of anthocyanins in grape skins, while procyanidins in skins and seeds were accumulated in higher amounts at lower altitudes. Coklar (2017) also found that as altitude increased from 1000 m to 1500 m, the accumulation of phenolics and antioxidant activity increased in berries, seeds and skins of Ekşikara grape (*Vitis vinifera* L) from the Konya region, Turkey. The data of our study correlate also with the research of Nascimento et al. (2016), who investigated the phenolic profile of tropical wines produced from grapes grown in northeastern Brazil at an altitude of 1100 m. Red wines from four varieties (Cabernet Franc, Pinot Noir, Malbec and Cabernet Sauvignon) were studied and it was found a range of total phenols from 1024.21 mg GAE/L to 2958.96 mg GAE/L. The total anthocyanins ranged from 28.47 mg/L to 260.92 mg/L. Osorio-Macias et al. (2018) studied the phenolic profile of 34 red wines from different countries and found a variation in the total phenolic content from 1600.00 to 3500.00 mg GAE/L, with results confirming that wines obtained from grapes grown at altitudes above 1500 m showed higher levels of total phenolic compounds. De Oliveira et al. (2019) found that Syrah grapes grown at an altitude of 1100 m in the Bahia region of Brazil showed high levels of anthocyanins and condensed tannins in the skins.

Conclusions

Such conclusions from the conducted research, the following conclusions could be made:

- The content of phenolic substances in the grape berries skins and seeds of the Vranec variety in all studied variants and regions, expressed as total anthocyanins, total phenols and flavan-3-ols had statistically proven the highest content when the vine had normated yield with 10 grapes bunches per vine. The higher altitude showed to increase the amount only of total phenols in the berries skins and seeds, while the content of all other indicators were the highest in the grapes from the Veles region. The studied indicators in the berries skins and seeds of the grapes from all three regions were strongly depended on the external climatic factors in the individual years.
- There were no significant differences in the content of phenolic substances – total anthocyanins and total phenols in the wine of the Vranec variety by variants and regions, as their quantities were higher in the variants with normation of grape bunches. The wine colour intensity was characterized by the highest value in the variant with 6 grape bunches per vine. In the different years in the levels of the indicators hue, yellow colour, red colour and blue colour some differences were noticed. The wines colour intensity and hue indicators had very close values and the differences between them were not statistically proven in any variant. The average altitude in the Veles region provided the best soil and climatic conditions for the Vranec variety growing and for obtaining of the highest quality grapes and wine.

References

Andjelkovic M., Radovanović B., Radovanović A., Andjelkovic A. M. (2013), Changes in polyphenolic content and antioxidant activity of grape cv Vranac during ripening, *South African Journal of Enology and Viticulture*, 34(2), pp. 147-156, https://doi.org/10.21548/34-2-1090

Bighenti E., Casagrande K., Cardoso P., Pasa M., Ciotta M., Brighenti F. (2017), Total polyphenols contents in different grapevine varieties in highlands of southern Brazil, *Bio Web of Conferences*, 9, 01024, https://doi.org/10.1051/bioconf/20170901024

Coklar H. (2017), Antioxidant capacity and phenolic profile of berry, seed, and skin of Ekşikara (*Vitis vinifera* L.) grape: Influence of harvest year and altitude, *International Journal of Food Properties*, 20(9), pp. 2071-2087, https://doi.org/10.1080/10942912.2016.1230870

De Oliveira J., Egipto R., Laureano O., De Castro R., Pereira G., Da Silva J. (2019), Climate effects on physicochemical composition of Syrah grapes at low and high altitude sites from tropical grown regions of Brazil, *Food Research International*, 121, pp. 870-879, https://doi.org/10.1016/j.foodres.2019.01.011

Di Stefano R., Cravero M.C., Gentilini N. (1989), Metodi per lo studio deipolifenoli dei vini, *L'enotecnico I. Maggio*, 25(5), pp. 83-89. (IT),

Gutiérrez-Escobar R., Aliaño-González M., Cantos-Villar E. (2021), Wine polyphenol content and its influence on wine quality and properties: A review, *Molecules*, 26(3), pp. 718, https://doi.org/10.3390/molecules26030718

Ivanova-Petropoulos V. (2013), *Practicum in sensory and analytical evaluation of wine*, "Goce Delchev" University – Shtip, Macedonia. (MK)

Jin X., Wu X., Liu X. (2017), Phenolic characteristics and antioxidant activity of Merlot and Cabernet Sauvignon wines increase with vineyard altitude in a high-altitude region, *South African Journal of Enology and Viticulture*, 38(2), pp. 132-143, https://doi.org/10.21548/38-2-1068

Kennedy J., Troup G., Pilbrow J., Hutton D., Hewitt D., Hunter C., Ristic R., Iland P., Jones G. (2000), Development of seed polyphenols in berries from *Vitis vinifera* L. cv. Shiraz,*Australian Journal of Grape and Wine Research*, 6, pp. 244-254, https://doi.org/10.1111/j.1755-0238.2000.tb00185.x

Landrault N., Poucheret P., Ravel P, Gasc F, Teissedre P. L. (2001), Antioxidant capacities and phenolic levels of French wines from different varieties and vintages, *Journal of Agricultural and Food Chemistry*, 49, pp. 3341-3348, https://doi.org/10.1021/jf010128f

Margaryan K., Melyan G., Vardanyan D., Devejyan H., Aroutiounian R. (2017), Phenolic content and antioxidant activity of Armenian cultivated and wild grapes, *Bio Web of Conferences*, 9, 02023, https://doi.org/10.1051/bioconf/20170902029

Mateus N., Proença S., Ribeiro P., Machado J. M., De Freitas V. (2001), Grape and wine polyphenolic composition of red *Vitis vinifera* varieties concerning vineyard altitude, *Ciencia y Tecnologia Alimentaria*, 3(2), pp. 102-110, https://doi.org/10.1080/11358120109487653

Milinčić D., Stanisavljević N., Pešić M., Kostić A., Stanojević S., Pešić M. (2025), The bioaccessibility of grape-derived phenolic compounds. An overview, *Foods*, 14(4), 607, https://doi.org/10.3390/foods14040607

Mori K., Goto-Yamamoto N., Kitayama M., Hashizume K. (2007), Loss of anthocyanins in red-wine grape under high temperature, *Journal of Experimental Botany*, 58(8), pp. 1935–1945, https://doi.org/10.1093/jxb/erm055

Nagel C., Wulf L. (1979), Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet sauvignon, *American Journal of Enology and Viticulture*, 30, pp. 111-116, https://doi.org/10.5344/ajev.1979.30.2.111

Nascimento A., De Souza J., Costa A., Santos S., Silva G., Pereira G. (2016), Characterization of phenolic composition of altitude tropical wines in the Brazilian Northeast, *Bio Web of Conferences*, 7, 02014, https://doi.org/10.1051/bioconf/20160702014

Neira-Opsina E., Rojas J., Santa-Gonzalez G., Garzón M. (2024), Phenolic compounds in grapes (genus *Vitis*): A review of their antioxidant activity, antiproliferative capacity, and cytotoxic effect on colorectal cancer, *Journal of Applied Pharmaceutical Science*, 14(6), pp. 71-89, https://doi.org/10.7324/japs.2024.169381

Osorio-Macias D., Vásquez P., Carrasco C., Bergenstahl B., Peñarrieta J.M. (2018), Resveratrol, phenolic antioxidants and saccharides in South American red wines, *International Journal of Wine Research*, 2018(10), pp. 1-11, https://doi.org/10.2147/ijwr.s152026

Ozmianski J., Romeyer F., Sapis J., Macheix J. (1986), Grape seed phenolics: Extraction as affected by some conditions occurring during wine processing, *American Journal of Enology and Viticulture*, 37, pp. 7-12, https://doi.org/10.5344/ajev.1986.37.1.7

Peña-Neira A., Cáceres A., Pastenes C. (2007), Low molecular weight phenolic and anthocyanin composition of grape skins from cv. Syrah (*Vitis vinifera* L.) in the Maipo Valley (Chile): Effect of clusters thinning and vineyard yield, *Food Science and Technology International*, 13(2), pp. 153–158, https://doi.org/10.1177/1082013207077920

Renaud S., Lorgeril M. (1992), Wine, alcohol, platelets and the French paradox for coronary heart disease, *Lancet*, 339, pp. 1523-1526, https://doi.org/10.1016/0140-6736(92)91277-f

Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999), Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, *Methods in Enzimology*, 299, pp. 152-178, https://doi.org/10.1016/s0076-6879(99)99017-1

Singleton V.L., Trousdale E.K. (1992), Anthocianin – tannin interaction explaining differences in polymeric phenols between white and red wines. *American Journal of Enology and Viticulture*, 43, pp. 63-70, https://doi.org/10.5344/ajev.1992.43.1.63

Valdés E. M., Moreno D., Gamero E., Uriarte D., Prieto H. M., Manzano R., Picon J., Intrigliolo D.S. (2009), Effects of cluster thinning and irrigation amount on water relations, growth, yield and fruit and wine composition of Tempranillo grapes in Extemadura (Spain), *Journal International des Sciences de la Vigne et du Vin*, 43(2), pp. 67-76, https://doi.org/10.20870/oeno-one.2009.43.2.799

Watson B., Price S., Chen H., Valladao M. (1995), Effect of fermentation practices on anthocyanin and phenolic composition of Pinot noir wines, *American Journal of Enology and Viticulture*, 46, pp. 404-412,

Xia E., Deng G., Guo J., Li H. (2010), Biological activities of polyphenols from grapes, *International Journal of Molecular Sciences*, 11, pp. 622-646, https://doi.org/10.3390/ijms11020622

Zhang K., Yin H., Wang J., Cao J., Xi Z. (2023), Differences and genesis of grape phenolic compounds among different altitudes in Yunnan Shangri-la, *Scienta Agricultura Sinica*, 56(19), pp. 3879-3893,

Zhishen J., Mengeheng T., Jianming W. (1999), The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chemistry*, 64, 555, https://doi.org/10.1016/s0308-8146(98)00102-2

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Effect of ground psyllium on the quality characteristics of dairy-plant concentrates

Larysa Chubenko, Viktor Grek, Alla Tymchuk, Nikita Solovyov

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Alla Tymchuk
E-mail: 589112@ukr.net

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Abstract

Introduction. The aim of research – to determinate of the comprehensive effect of ground psyllium on the characteristics dairy-plant concentrates obtained by enzymatic coagulation of goat milk proteins with *Plantago major* L. juice.

Materials and methods. The study utilized goat milk, *Plantago major* L. juice as a coagulant, ground psyllium powder, and calcium chloride. The granulometric composition of psyllium and its swelling capacity were determined using sieve analysis, rheological properties were measured using a Kinexus Pro+ rheometer, and water-holding capacity was assessed using a gravimetric method.

Results and discussion. The granulometric composition of psyllium was determined. The particle size of the most significant fraction of psyllium, which constitutes 46.2%, ranges from 251 to 335 μm, while for ground psyllium (51.2%), it is 126–200 μm. It was shown that under identical conditions of the enzymatic coagulation process of milk proteins with heating in the presence of the active complex of *Plantago major*, the use of ground psyllium in amounts ranging from 0.25% to 0.75% contributed to an increase in the utilization rate of goat milk dry matter by 2.15–18.71% compared to the control. The optimal amount of calcium chloride for obtaining coagulates with high organoleptic and physicochemical properties in goat milk was 0.3–0.4 g/L. With an increase in the amount of ground psyllium, the viscosity of dairy-plant concentrates decreased, indicating a reduction in the mechanical strength of model systems.

Considering organoleptic limitations and quality indicators of dairy-plant concentrates, the optimal amount of ground psyllium addition was determined as 0.5±0.1% of goat milk mass during enzymatic coagulation of proteins with the active complex of *Plantago major*. The samples had a milky taste with a slight herbal aftertaste and aroma, a firm, moderately dense coagulate with a greenish color uniformly distributed throughout the mass.

The effect of ground psyllium in enzymatic coagulation of goat milk proteins with the active complex of *Plantago major* was confirmed by the transition indicators of dry substances into the dairy-plant concentrate and whey. The loss of goat milk dry matter into whey varies within the range of 4.55–7.00%, depending on the influence of various factors. The studies revealed a similar trend in changes in the water-holding capacity of dairy-plant concentrates for all samples, indicating interactions between proteins and a decrease in hydrated water content around casein micelles when using the active complex of *Plantago major* and ground psyllium.

Conclusions. The use of psyllium during the fermentation of goat milk with *Plantago major* juice ensures the stabilization of the physicochemical properties of dairy-plant coagulates and their enrichment with soluble and insoluble dietary fibers, proteins, micro- and macroelements.

Introduction

The use of dairy and plant raw materials under traditional technological processes, including protein precipitation and concentrate formation, can impart new qualities to the system (Onopriichuk et al., 2022). A promising direction in the development of multi-component protein products is the combination of goat milk and local plant raw materials (Chechetkina et al., 2016; Shah et al., 2014). Craft enterprises actively use such dairy and plant raw materials for the production of beverages, frozen desserts with high protein content, and curd-based products (Ahmed et al., 2024; Guyomarc'h et al., 2020). Plant processing products in the form of juice, extracts, concentrates, and dry powders are introduced at different stages of the technological process. It is proposed to add plant-based raw materials to milk or dairy products in various percentage ratios depending on the technological function (enrichment, coagulation or color correction) (Grek et al., 2020; Pavlyuk et al., 2019).

Plantain is used in food technologies as an enriching agent to increase biological value in minced meat semi-finished products in the form of green mass (Luo et al., 2018; Ukrainets et al., 2017) and in fermented dairy productsin the form of husks (Arabshahi et al., 2022).

Processed products from *Plantago major* L., commonly known as great plantain, including psylliumjuice and powder, are promising as coagulants and enrichers (Dülgeroğlu et al., 2017), but comprehensive study on their combined application in the production of dairy-plant concentrates are limited. The nutritional composition of psyllium, %, is as follows: fats, 1.2±0.01; proteins, 3.9±0.05, insoluble carbohydrates, 20.23±0.4, soluble carbohydrates, 78.2±4.01; ash, 4.1±0.01; moisture, 4.9±0.01. The mineral content, mg/100 g, includes potassium, 805; calcium, 104; sodium, 62.3, and magnesium, 19.7 (Chong et al., 2019). The scientific substantiation of their effectiveness in influencing the rheological and physicochemical properties of concentrates is a relevant area of applied scientific research.

Conditions for the industrial implementation of a technology using plantain juice with a dry matter content of 6.00±0.23 % for enzymatic and thermal-acid coagulation of cow's milk proteins were formulated (Grek et al., 2018, 2019), and specific physicochemical parameters of the corresponding protein-plant concentrates were identified. There is no information on the use of goat milk as a raw material in this technology. Possible corrections to the yield of dairy-protein concentrate and the application of technological approaches, such as the inclusion of plant ingredients (dietary fibers) to strengthen the curd, have been considered. The necessity of controlling the proteolytic activity of aspartic proteases has been proven, as high activity can lead to excessive cleavage of peptide bonds and the formation of a significant amount of soluble proteins, resulting in a weak curd (Shah et al., 2014). Potential losses of dry matter beyond regulatory limits and a slowdown in the syneresis process, as well as the appearance of defects in taste and consistency, are possible.

Wild plants are already being used to obtain safe enzyme preparations, as well as dietary fiber powders for different purposes. The limitation is the seasonality and regional specificity of raw materials (Khan et al., 2024). The industrially manufactured product derived from *Plantago major* is psyllium in the form of powder and husk is used in prepatatoion of certain foods (Franco et al., 2022; Neveen and Eelbassiony; Znamiriwska-Piotrowskaet al., 2023). Psyllium contains proteins, polysaccharides, glycosides, vitamin B1, choline, and fiber, which mainly consists of hemicellulose. Psyllium is obtained by processing the outer seed coat of *Plantago*. There are different types of plantain husk that vary in particle size (Agrawal, 2021).

It was shown that goat's milk coagulates worse than cow's milk when enzymes are used (Nayik et al., 2022). This is due to the peculiar protein structure, a reduced content of kappa-casein, low acidity, and high dispersion of fat globules with diameters ranging from 0.5 to 15 μm. The high content of β-casein and the low content of α-caseins present a technological challenge, as they complicate protein coagulation and dense curd formation (Pokhyl et al.,

2023; Ryzhkova et al., 2019). It is likely that adding psyllium during the fermentation of goat's milk with *Plantago major* juice will increase the yield of milk-plant concentrate.

However, at present there is no comprehensive approach to solving the problems outlined above. Therefore, it was advisable to determine the comprehensive effect of ground psyllium powder on the characteristics of milk-vegetable concentrates obtained by enzymatic coagulation of goat milk proteins with plantain juice.

Materials and methods

Materials

As raw materials, goat milk was used with the following composition, %: fats – 4.14, proteins – 3.6 (including casein – 3.0), carbohydrates – 4.3–4.5, mineral substances – 0.85, ash – 0.82.

Psyllium (TDS No. BF/SP/007), the husk (shell) of plantain seeds, was used in the research. The appearance of psyllium is husk powder ranging from pale yellow to light yellow, odorless, with a moisture content of 12%, and total ash content not exceeding 4%. According to the manufacturer, psyllium is a natural dietary fiber that is hygroscopic, mucilaginous, capable of retaining water, and forms a homogeneous gel (Gebremeskal et al., 2024). Mucilage consists of approximately 15% non-polysaccharide substances, such as fat and protein, while the remaining 85% is a single polysaccharide containing D-xylose (~62%), L-rhamnose (~9%), L-arabinose (~20%), and D-galacturonic acid (~9%). β-D-xylose residues in the pyranose ring form the linear framework of the polysaccharide. Another disaccharide chain is linked to the terminal α-D-galacturonic acid and O-2-α-L-rhamnose. The total protein fraction consists of approximately 23.9% globulin, 35.8% albumin, and 11.7% prolamin (Agrawal, 2021; Guo et al., 2008). The water-holding capacity of psyllium is 59.2% (Aldughpassi et al., 2021). Country of origin - India.

Juice extraction from *Plantago major*

As a coagulant, direct-pressed juice from the aerial part of *Plantago major* was used, obtained using the technology (Grek et al., 2021), which includes the following operations: washing and drying fresh plant raw materials, passing them through rollers to obtain a mush-like mixture. Under laboratory conditions, the grinding process of wild plants was carried out using a MOULINEX ME626132 device with a power of 2000 W. The juice was used for research immediately after extraction. In the juice of *Plantago major L.,* the dry matter content was 6.00±0.23%, and the active acidity index was 5.85±0.18 pH units.

Preparation of model samples of dairy-plant concentrates

Model samples of dairy-plant concentrates were prepared for research by adding psyllium in amounts ranging from 0.25% to 0.75%, followed by enzymatic coagulation. The samples were obtained in laboratory conditions as follows: goat milk was pasteurized at a temperature of 74±2 °C for 15...20 s, cooled to the coagulation temperature, calcium chloride was added as a 40% aqueous solution, psyllium powder, and direct-pressed juice from the aerial part of *Plantago major* L. in the amount of 8.0±0.1%. Coagulation conditions: temperature 50-65 °C with an incubation time of 75±5 min. For the control sample without psyllium, the curd was also cut, heated for densification, whey was removed, and the concentrate was subjected to self-pressing and cooling.

Methods

Standard and well-known methods for studying the technological-functional properties and quality indicators were used to ensure the fulfillment of the research objectives.

Functional-technological properties of psyllium

The granulometric composition of psyllium was determined as follows. From the average sample, a 100 g portion was taken and sieved using silk sieves No. 35 and No. 43. To clean the sieves, 5 rubber discs with a diameter of 1.0 cm, a thickness of 0.3 cm, and a weight of 0.5 g each were placed on each sieve. The psyllium powder sample was poured onto the upper sieve, covered with a lid, the sieve set was secured on the platform of the sieve shaker, and sieving was started. After 8 minutes, the shaker was turned off, the sieve frames were tapped, and sieving was continued for another 2 minutes. The rubber discs were removed from the sieves, and the residue on the upper sieve and the passage through the lower sieve were weighed. The result was expressed as a percentage of the psyllium sample weight.

The swelling capacity of psyllium was determined by the gravimetric method, which involves measuring the mass change of the plant ingredient after immersion in a liquid for a certain period. This indicator is quantitatively characterized by the swelling degree (K), which shows the relative increase in system mass, mg/mg. The swelling degree was calculated using the formula:

$$K = \frac{m_1 - m_0}{m_0} = \frac{m_p}{m_0}$$

where m_0, m_1 are the mass of the system before and after swelling, mg; m_p is the mass of the absorbed solvent, mg.

Effect of ground psyllium on fermentation process of goat milk

The degree of utilization of milk solids (As) was determined by the formula:

$$As = 100\frac{Cz(Cm - Cwhey)}{Cm(Cz - Cwhey)}$$

where Cz is mass fraction of dry matter in the dairy-plant concentrate, %; Cm is mass fraction of dry matter in goat milk, %; Cwhey is mass fraction of dry matter in whey, %.

The calcium content in the experimental samples, %, was measured by the titrimetric method (Prylipko et al., 2020).

Complexometric titration was used to determine the calcium content in the products (dairy-plant concentrate). The method utilizes the molecule called EDTA, which forms a complex with calcium ions. Murexide dye was used as an indicator. This dye also forms a complex with calcium ions, changing color from pink/red to violet. However, the dye-metal ion complex is less stable than the metal ion-EDTA complex. As a result, when titrating the calcium-Mux complex with EDTA, Ca^{2+} ions react to form a stronger complex with EDTA. For titration, the indicator was added to the sample solution containing calcium ions, forming a pink-red calcium ion-indicator complex (Ca-Mux). This solution was then titrated with EDTA. The endpoint occurred when the solution turned violet, indicating that the Ca-Mux complex was completely replaced by the calcium ion-EDTA complex and the Mux indicator returned to its violet color.

The calcium content in the experimental samples, %, was calculated using the formula:

$$W = 0.4 \cdot (V - V_0) \cdot f / m,$$

where: W is a calcium content, %; V is a volume of potassium permanganate solution used for the test sample, ml; V_0 is a volume of potassium permanganate solution used for the control experiment, ml; m is a mass of the test sample, g; f is correction factor for the volume of precipitate obtained after precipitation with trichloroacetic acid depending on the fat content in the sample.

The degree of protein utilization (%) is determined by the formula:

$$B = 100\frac{Bdpc(Bм - Bwhey)}{Bdpc(Бmilk - Bwhey)}$$

where Bdpc is mass fraction of protein in the dairy-plant concentrate, %; Bmilk is mass fraction of protein in goat milk, %; Bwhey is mass fraction of protein in whey, %.

The appearance and color of dairy-plant concentrates and whey were evaluated visually, while the consistency was assessed organoleptically at a temperature of 18-20°C.

Rheological characteristics of dairy-plant concentrates

The rheological characteristics of dairy-plant concentrates were measured using a Kinexus Pro+ rheometer (Malvern Instruments Ltd, United Kingdom). A 40 mm round plate geometry (PU40 SR5040 SS:PL61 ST) was used, fixed on the vertical shaft. The sample was placed on the lower platform, and the shaft with the plate was lowered to a gap of 1 mm between them. Viscosity and flow curves were obtained by varying the shear rate in the range of 0.1-1000 s^{-1} with 10 measurement points per decade. Each step was maintained until a stable state was reached. The study was conducted at a temperature of 20±1 °C (Alvarez et al., 2015).

Physicochemical and organoleptic characteristics of dairy-plant concentrates

The organoleptic characteristics of dairy-plant concentrates were evaluated according to ISO 22935-3:2009 (2009). A panel of 10 experts assessed the quality of dairy-plant concentrate samples using a hedonic scale (1 – low quality, unacceptable taste/odor/color/appearance/consistency; 2 – below average quality, noticeable defects; 3 – average quality, generally acceptable for most consumers; 4 – high quality, pleasant taste/odor/color/appearance/consistency; 5 – exceptionally high quality, impressive taste/odor/color/appearance/consistency) (Elsamani, 2016) based on the following individual sensory attributes: appearance, consistency, taste, odor, and color. Scores for individual attributes were plotted on diagram axes to obtain sensory profiles of each dairy-plant concentrate sample.

The moisture content of dairy-plant concentrates was measured using an accelerated method on a KVARTS-21M-33 moisture analyzer by drying the sample to a constant mass, as well as a thermogravimetric method using ADS series electronic laboratory moisture analyzer scales produced by "AXIS" (Poland).

The dry matter content of dairy-plant concentrates was determined using the formula:

$$C = 100 – B,$$

where S is the dry matter content, %, and W is the moisture content, %.

The water-holding capacity (WHC) of dairy-plant concentrates was studied using the Grau-Hamm gravimetric method, based on determining the amount of water released from the product under light pressing and absorbed by filter paper (Grek et al., 2024).

The active acidity (pH) of dairy-plant concentrates and whey was determined potentiometrically using a Sartorius PB-20 universal pH meter.

The dry matter content in whey was analyzed using a refractometric method based on light refraction indices (Grek et al., 2024).

Statistical analysis

Data were expressed as means ± standard deviations for triplicate determination. Statistical analysis was performed using Microsoft Excel 2007. Differences were considered to be significant at validity of α=0.95.

Results and discussion

Functional-technological properties of psyllium

The granulometric composition of psyllium, along with its chemical composition, influences the rate of biochemical and colloidal processes. The use of psyllium in the fermentation of goat milk with *Plantago major* juice is likely to affect the qualitative characteristics of dairy-plant concentrates and the coagulation time of the curd. The granulometric composition of psyllium is a particle size characteristic that expresses the distribution function or particle density. Psyllium grinding was performed using a device with a rotation speed of approximately 3000 rpm and a power of 300 W. The duration of the process was 3–5 minutes.

The determined granulometric composition of psyllium is presented in Figure 1.

According to the diagram (Figure 1), the particle size of the most significant fraction of psyllium, accounting for 46.2±1.1%, is 251-335 μm, while for ground psyllium (51.2±1.2%), it is 126-200 μm.

Grinding psyllium increases the availability and digestibility of nutrients and enhances the potential technological effect associated with water binding in dairy-plant concentrates. The interaction of ground psyllium with different dispersion media—goat milk (raw material for dairy-plant concentrates) and water (control)—was studied to determine the impact of psyllium on the qualitative and quantitative characteristics of the samples.

For better distribution of ground psyllium, (a powder composed of soluble and insoluble fiber) within the structure of dairy-plant concentrates, pre-dissolution is advisable. The relative increase in system mass due to solvent absorption is characterized by the swelling degree (K) and depends on the type of dispersion medium. Swelling of ground psyllium was conducted in water and goat milk at temperatures of 20±2 °C, 60±2 °C, and 90±2 °C for 10±1 minutes. The swelling ability of ground psyllium at different temperatures in water and goat milk is presented in Figure 2.

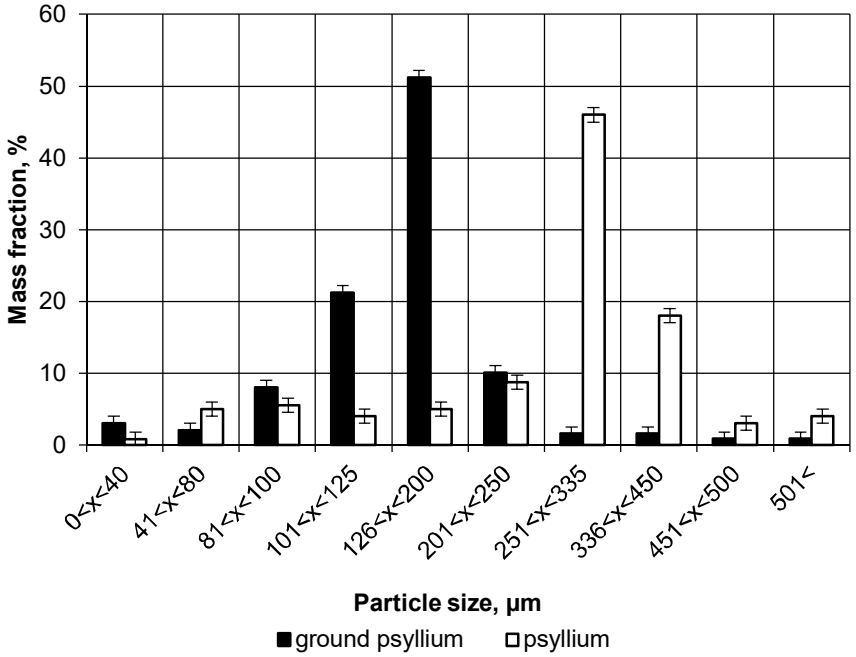

Figure 1. Granulometric composition of psyllium

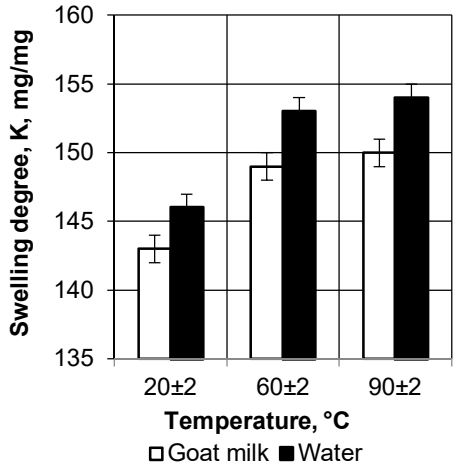

Figure 2. Swelling ability of ground psyllium at different temperatures in water and goat milk

It was found that the swelling degree of ground psyllium in goat milk was 2.1–2.7% lower than in water within the specified temperature range. When the process temperature increased to 90±2°C, the swelling ability increased slightly (by 0.7% in goat milk and by

0.8% in water) compared to 60±2°C. Pre-mixing ground psyllium with water before adding it to goat milk reduces the nutritional value of the product. Given the relatively minor deviations in swelling degree indicators in milk and water, the process should be conducted directly in goat milk. The optimal swelling temperature was determined to be 60±2°C, as it is the closest to the pasteurization regime required to ensure the necessary microbiological parameters.The obtained data correlate with the water-holding capacity of psyllium, which is 59.2±0.1% (Aldughpassi et al., 2021).

Psyllium consists of 35% soluble fractions and 65% insoluble polysaccharides such as cellulose, hemicellulose, and lignin. Psyllium is a hydrophilic mucilloid with a highly branched arabinoxylan polysaccharide structure, possessing a high water-holding capacity and gel-forming ability. The gel-forming fraction of polysaccharides consists of xylose, arabinose, and small amounts of other sugars (Agrawal, 2021; Chong et al., 2019).

Effect of ground psyllium on fermentation process of goat milk

The degree of dry matter utilization in goat milk during enzymatic coagulation with heating in the presence of an active *Plantago major* complex and different amounts of ground psyllium is presented in Figure 3.

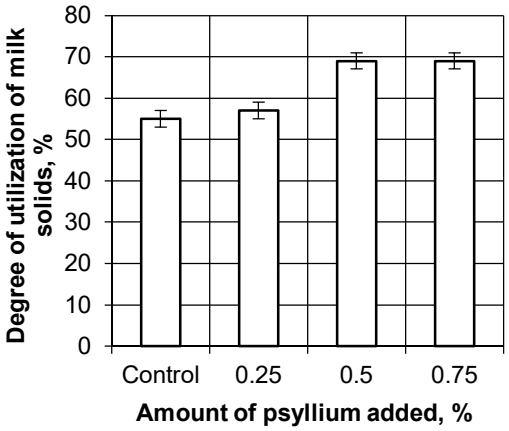

Figure 3. The degree of utilization of goat milk solids during enzymatic coagulation with heating in the presence of the active complex of *Plantago major* and different amounts of psyllium

For the control sample, the degree of utilization of dry matter from goat's milk was 50.93±0.05 %. The highest value of the aforementioned indicator, 69.64±0.01 %, was observed in the dairy-plant concentrate obtained with the addition of 0.5% ground psyllium. When 0.75% psyllium was added, the aforementioned indicator did not change. It is likely that the coagulation process of goat milk proteins with *Plantago major* juice and the thickening of the curd with the addition of ground psyllium occur due to the cleavage of an unstable peptide bond in χ-casein – Phe 105 – Met 106. This can be explained by the hydrophobic nature of serine, leucine, and isoleucine, which participate in the initial stage of the process. As a result, the χ-casein chain is split into two unequal segments: 1-105, which forms para-χ-casein, and 106-169, which forms caseinomacropeptide. Further, para-χ-casein binds with α- and β-caseins and remains part of the micelle, exhibiting pronounced alkaline

and hydrophobic properties. Caseinomacropeptide, having an acidic and hydrophilic nature, is released into the whey. Consequently, para-χ-casein molecules transition into an unstable state. The next phase of coagulation, associated with thickening, occurs under the technological influence of dietary fibers from psyllium. This technological approach neutralizes the specificity of the fractional composition of goat milk proteins (dominance of β-casein and α-lactoglobulin) and their low ability to form dairy curds (Ryzhkova et al., 2019).

At the next stage of the study, a technological method of adding $CaCl_2$ was used to influence the coagulation rate and curd density. Calcium ions play an important role in the aggregation of casein micelles. The addition of $CaCl_2$ to milk, especially thermally treated milk, is the simplest way to reduce sedimentation time and increase curd stability during production. It is likely that adding $CaCl_2$ during enzymatic coagulation with plant proteases in the presence of ground psyllium is appropriate.

The effect of different $CaCl_2$ concentrations on the degree of protein utilization from goat's milk, curd characteristics, and calcium content was studied. For this purpose, a $CaCl_2$ solution was added to goat's milk at concentrations ranging from 0.1 g to 0.6 g of dry salt per liter of milk. The milk was mixed until curd formation and whey separation. The protein curd was drained into lavsan bags and left for self-pressing. The study results are presented in Figure 4 and Table 1.

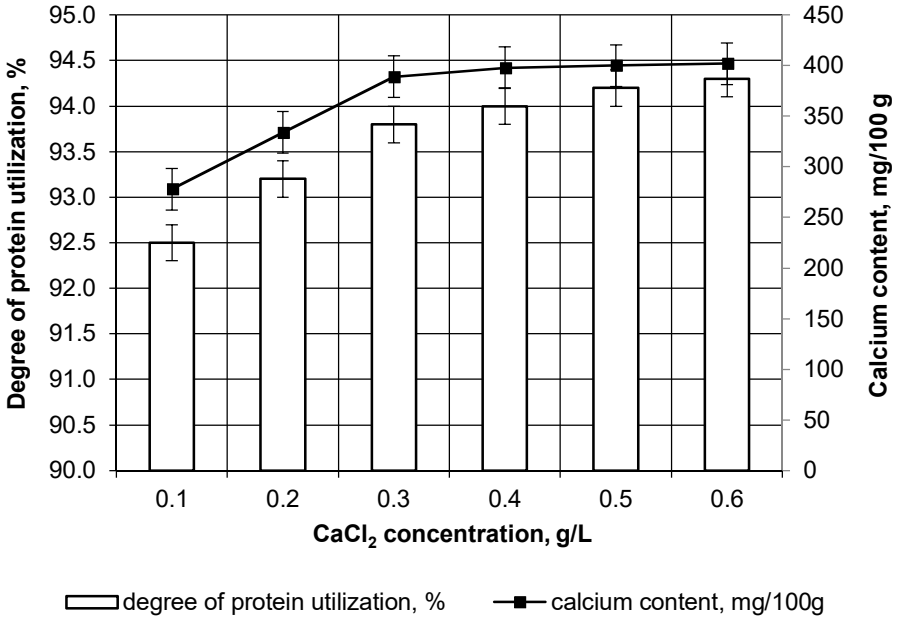

Figure 4. Degree of protein utilization in goat milk and calcium content in curds obtained by enzymatic coagulation with heating in the presence of the active complex of *Plantago major* and 0.5% ground psyllium with the addition of $CaCl_2$.

Table1

Effect of $CaCl_2$ concentration on the characteristics of goat milk curds obtained through enzymatic coagulation with heating in the presence of an active *Plantago major* complex and 0.5% ground psyllium

$CaCl_2$ concentration, g/L	Characteristic	
	Dairy-plant concentrates	Whey
0.1 – 0.2	Flaky soft	Yellow-green, turbid
0.1 – 0.4	Soft curd	Yellow-green, clear
0.5 – 0.6	Soft curd	Yellow-green, clear

According to the results (Figure 4 and Table 1), as the concentration of $CaCl_2$ increased, the degree of protein utilization from goat's milk also increased. When $CaCl_2$ was added at 0.1–0.2 g/L, loose curds and yellow-green turbid whey were formed. Curds with the appropriate organoleptic characteristics were obtained when $CaCl_2$was added at 0.3–0.4 g/L to goat's milk. With an increase in calcium chloride concentration, calcium binding by protein curds also increased. It was established that in goat milk protein curds, the calcium content was 400 mg/100 g when 0.50 g/L of $CaCl_2$was added. Thus, the optimal amount of calcium chloride to be added to goat's milk is 0.35±0.05 g/L. Further additions are unnecessary, as they do not yield superior curd quality in terms of organoleptic and physicochemical properties. The obtained results correlate with the findings of Kethireddipalli and Hill (2015), which indicate that 0.4-0.6 g/L $CaCl_2$ is needed for milk subjected to more intense heat treatment. Similar information is presented in the works of Brutti et al. (2012), Lo Piero et al. (2002), and Mazorra-Manzano et al. (2013). The disruption of enzymatic coagulation (extended coagulation time) in thermally treated milk is primarily explained by the interaction between denatured whey proteins and casein micelles, which hinders the aggregation of the latter.

Rheological characteristics of dairy-plant concentrates

The study of rheological characteristics and the patterns of their changes with the addition of technological ingredients is important for developing new types of dairy-plant concentrates and adapting existing equipment for mechanical processing and transportation.

Due to the chemical structure and ability to swell and gel in the presence of liquid, ground psyllium influences the structural formation of dairy-plant concentrates. The study of the dependence of effective viscosity on shear rate in dairy-plant concentrate samples obtained by fermenting goat's milk with *Plantago major* L.juice and adding different amounts of ground psyllium (Figure 5) showed that the latter affects the shape of the curves.

It was found that the initial shear viscosity of dairy-plant concentrates was higher in the control sample than in dairy-plant concentrates with 0.25% to 0.75% ground psyllium. The shear viscosity value in the concentrate with 0.25% psyllium was the closest to the control sample. Changes in the viscosity of dairy-plant concentrates with increasing shear rate can be explained by the deformed arrangement of polysaccharides, friction between expanded granules, and hemicellulose content (Sasaki et al., 2002).

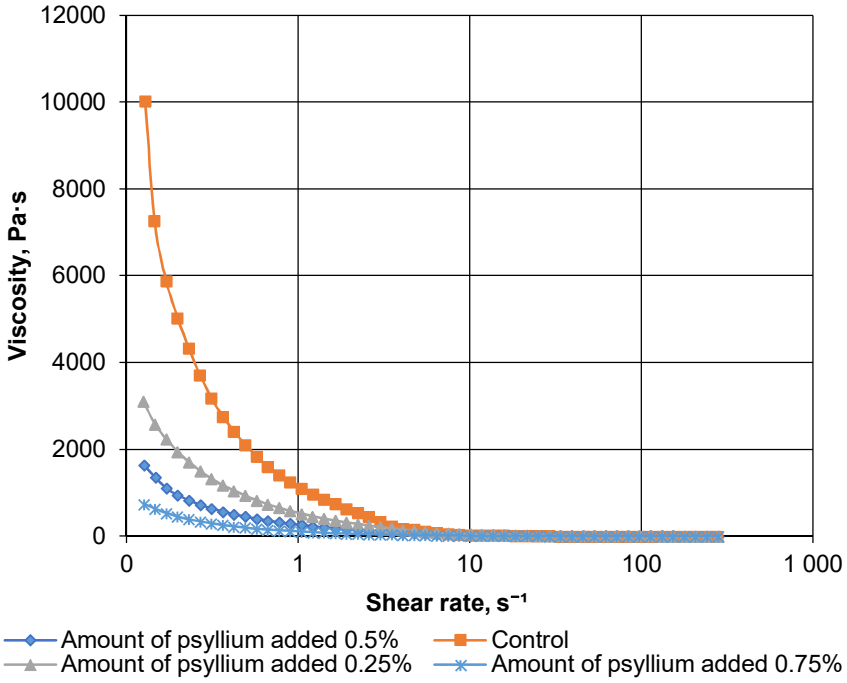

Figure 5. Dependence of effective viscosity changes on shear rate in dairy-plant concentrate samples with different amounts of ground psyllium during fermentation

In samples of dairy-plant concentrates with ground psyllium, the shear viscosity decreased compared to the control, regardless of psyllium concentration. This is due to the effect of liquid on soluble and insoluble dietary fibers and milk proteins, which slows down swelling (Lan et al., 2008). This effect indicates a reduction in the mechanical strength of the model systems. In psyllium, dietary fibers and mucilage are predominantly represented by soluble, highly branched, gel-forming polysaccharides – arabinoxylans (85%), which have a xylose backbone with arabino- and xylose-containing side chains (Craeyveld et al., 2009).

Additionally, dairy-plant concentrate samples with 0.25% ground psyllium reached stable viscosity significantly earlier – at a shear rate of 3 s^{-1}, while samples with 0.5% reached stability at 5 s^{-1}, and those with 0.75% at 7 s^{-1}. The viscosity of dairy-plant concentrates with 0.25% psyllium was slightly higher – about 105 Pas compared to 43 Pas for the 0.75% psyllium sample. This effect is likely related to greater fragmentation of psyllium chains at 0.75% concentration (Zambelli et al., 2018). The concentrates likely exhibited a coagulation structure formed by protein particle adhesion through thin layers of free or adsorbed whey.

The identified rheological characteristics correlate with the organoleptic perception of dairy-plant concentrates obtained by enzymatic coagulation of goat milk proteins with an active *Plantago major* complex and different amounts of ground psyllium. The observed changes in system properties are explained by psyllium's highly hydrophilic nature, forming a molecular solution in the dispersion medium and exerting a significant dehydrating effect on protein molecules by reducing the hydration shell. As a result, moisture is better separated from the curds, and the effective viscosity of dairy-plant concentrates decreases.

Organoleptic properties, water-holding capacity, and moisture content of dairy-plant concentrates

The obtained dairy-plant concentrates represent a polydisperse colloidal system, where the dispersion medium is whey, and the dispersed phase consists of milk proteins, dry substances of *Plantago major*, and psyllium. The formation of the dairy-plant coagulum structure occurs due to the swelling of psyllium dietary fibers at the stage of goat milk protein coagulation during fermentation. The specific structure of the coagulum determines its organoleptic characteristics. The obtained dairy-plant concentrates exhibit a thixotropic coagulation-type structure with liquid interlayers between the particles, which results in lower structural strength but provides plasticity and elasticity (Grek et al., 2019).

The optimal psyllium dosage was determined based on maintaining standard organoleptic characteristics typical of dairy-plant concentrates, which could serve as a basis for producing various types of curd-based products. Figure 6 presents the results of the organoleptic evaluation of dairy-plant concentrates with psyllium.

Organoleptic characteristics are a limiting factor for using dairy-plant concentrate as a base for dairy-protein products due to variations in coagulum color, ranging from light pistachio to intensely pistachio-colored.

The profilogram of the organoleptic evaluation of dairy-plant concentrates obtained by coagulating goat milk proteins with *Plantago major* juice and different amounts of psyllium is presented in Figure 6.

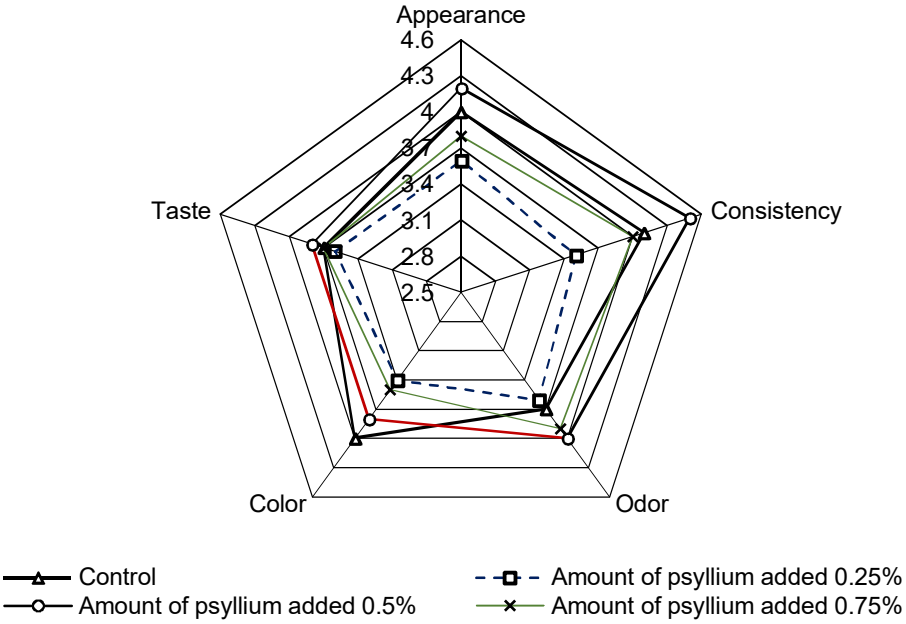

Figure 6. Profilogram of the organoleptic evaluation of dairy-plant concentrates obtained by coagulating goat milk proteins with *Plantago major* juice and different amounts of psyllium

The organoleptic evaluation of the dairy-plant concentrate obtained by coagulating milk proteins with *Plantago major* L. juice and finely ground psyllium at a concentration of 0.5±0.1 % is presented in Table 2.

Table 2

Organoleptic evaluation of the dairy-plant concentrate obtained by coagulating milk proteins with *Plantago major* juice and psyllium at a concentration of 0.5±0.1 %

Parameter	Characteristic
Color	Greenish, uniform throughout the mass with green inclusions
Taste and aroma	Milky with a slight herbal aftertaste and aroma
Consistency	Firm curd, moderately dense

The best taste and consistency of the dairy-plant concentrate were achieved with the addition of 0.5±0.1 % psyllium. The samples exhibited a milky taste with a mild herbal aftertaste and aroma, a firm and moderately dense coagulum, and a greenish color evenly distributed throughout the mass with visible inclusions. The addition of psyllium in amounts greater than 0.75% led to undesirable organoleptic changes in the dairy-plant concentrate, resulting in a jelly-like soft consistency. Adding less than 0.25% psyllium had no significant positive effect on organoleptic properties and minimized the enrichment of the coagulum with biologically active nutrients. Inconsistencies in texture, which complicate the self-pressing process, must be considered.

The color of the dairy-plant concentrate is determined by the presence of chlorophyll in *Plantago major* .juice, which is present in two forms: chlorophyll a (75%) and chlorophyll b (25%). The inclusion of this component in the diet has a positive physiological effect on the human body.

A dairy-plant concentrate containing 0.5±0.1 % finely ground psyllium with appropriate organoleptic characteristics can be used as a base for dairy-protein products with a plastic structure (e.g., creams, desserts, sauces).

The main physicochemical characteristics of dairy-plant concentrates were investigated. The corresponding changes in the mass fraction of dry matter in dairy-plant concentrates and whey, depending on the amount of psyllium added, are presented in Figure 7.

The changes in moisture content and water-holding capacity of dairy-plant concentrates depending on the amount of psyllium added are presented in Figure 8.

The efficiency of the above-mentioned technological solutions can be confirmed by quantitative indicators of dry matter transfer into the dairy-plant concentrate and whey. In the control sample of the dairy-plant concentrate, up to 7.11±0.11% of dry matter transfers into the whey, while 57.36±1.2% remains in the coagulum. The active acidity of the samples decreases with an increasing amount of psyllium, ranging from 6.47±0.12 to 6.25±0.11 pH units, while for the control sample, this value is 6.16±0.11 pH units.The losses of goat milk dry matter into the whey for all model samples varied between 4.53±0.12 % and 7.11±0.11 %, depending on various factors. The water-holding capacity of dairy-plant concentrates decreases as the amount of finely ground psyllium increases. The changes in moisture content and water-holding capacity of dairy-plant concentrates obtained using the active complex of *Plantago major* and finely ground psyllium can be explained by continuous protein interactions, leading to a reduction in hydrated water content around casein micelles.

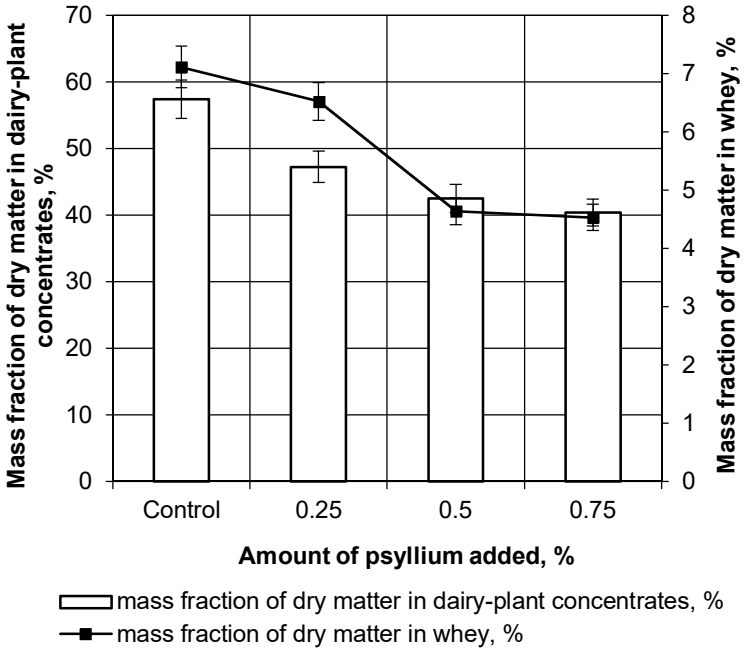

Figure 7. Changes in the mass fraction of dry matter in dairy-plant concentrates and whey depending on the amount of psyllium added

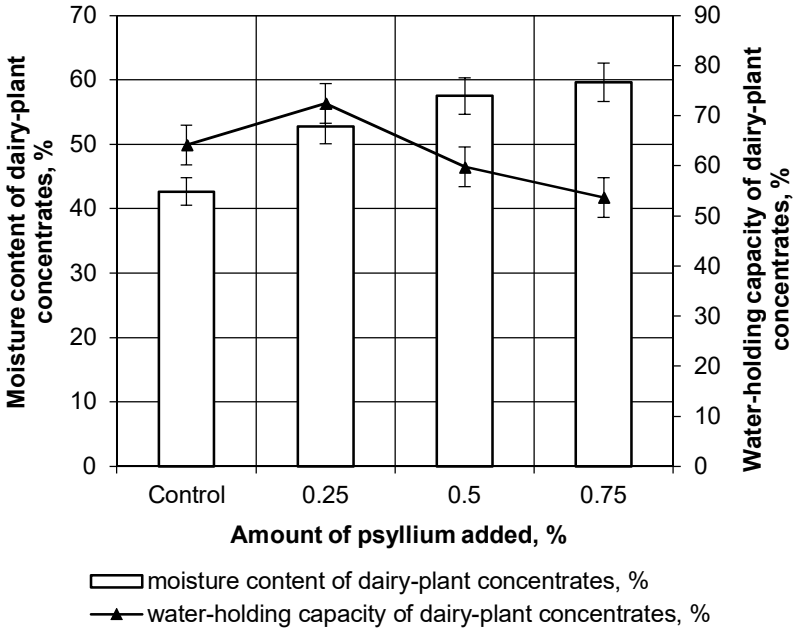

Figure 8. Changes in moisture content and water-holding capacity of dairy-plant concentrates depending on the amount of psyllium added

Conclusions

1. The feasibility of using ground psyllium during the fermentation of goat milk with *Plantago major* L. juice to stabilize the physicochemical parameters of milk-plant coagulates and enrich them with soluble and insoluble dietary fibers, proteins, micro- and macroelements has been substantiated.
2. The granulometric composition of psyllium has been determined, with the main particle size fractions ranging from 126 to 200 μm (51.2±1.2 %) and 251 to 335 μm (46.2±1.1%). The swelling temperature of ground psyllium when added to goat milk has been specified at 60±2°C.
3. Technological solutions have been proposed to increase the yield of milk-plant concentrate from goat milk: the addition of $CaCl_2$ in an amount of 0.35±0.05 g/L and ground psyllium with moisture-retaining properties in an amount of 0.5±0.1 %. Milk-plant concentrates are characterized by a dense spatial structure and syneresis properties, which are regulated by the husk content under thermomechanical influence.
4. The presence of whey layers between protein particles and highly branched gel-forming psyllium polysaccharides results in lower structural strength while ensuring plasticity and elasticity. This has been confirmed by the effective viscosity ranging from 735.7±1.2 Pa·s to 3101.0±2.1 Pa·s in milk-plant concentrates with the addition of ground psyllium.
5. The physicochemical and organoleptic characteristics of milk-plant concentrates and whey have been studied depending on the amount of added ground psyllium. The milk-plant concentrate obtained by fermenting goat milk with *Plantago major* juice with the addition of 0.5±0.1 % ground psyllium had an appropriate consistency and taste properties—a milky flavor with a light herbal aftertaste and aroma, a firm and moderately dense coagulate with a greenish color, uniform throughout the mass with inclusions. It has been established that when the husk addition increased from 0.25 % to 0.75%, the loss of dry substances from goat milk into whey varied in the range of 7.11±0.11 % to 4.53±0.12 %.

References

Agrawal R. (2021), *Psyllium: A source of dietary fiber*, IntechOpen, https://doi.org/10.5772/intechopen.99372

Ahmed S.Y.E., Zubeir I.E.M.El. (2024), Storage quality of goat milk yoghurt mish containing black cumin (*Nigella sativa* L.) and coriander (*Coriandrum sativum* L.) seeds, *Journal of Nutrition and Food Processing*, 7(9), pp. 1-9, https://doi.org/10.31579/2637-8914/243

Aldughpassi A., Alkandari S., Alkandari D., Al-Hassawi F., Sidhu J,. Al-Amiri H., Al-Salem E. (2021), Effect of psyllium fiber addition on the quality of Arabic flatbread (Pita) produced in a commercial bakery, *Annals of Agricultural Sciences*, 66, pp. 115-120, https://doi.org/10.1016/j.aoas.2021.08.002

Alvarez M.D., Fuentes R., Canet W. (2015), Effects of pressure, temperature, treatment time, and storage on rheological, textural, and structural properties of heat-induced chickpea gels, *Foods*, 4(2), pp. 80-114, https://doi.org/10.3390/foods4020080

Arabshahi S.S., Sedaghati M. (2022), Production of synbiotic Doogh enriched with *Plantago psyllium* mucilage, *Journal of Food Science and Technology*, 59(10), pp. 3819–3826, https://doi.org/10.1007/s13197-022-05401-8

Brutti C.B., Pardo M.F., Caffini N.O., Natalucci C.L. (2012), *Onopordum acanthium* L. (Asteraceae) flowers as coagulating agent for cheesemaking, *LWT – Food Science and Technology*, 45(2), pp.172-179, https://doi.org/10.1016/j.lwt.2011.09.001

Chechetkina A., Iakovchenko N., Zabodalova L. (2016), The technology of soft cheese with a vegetable component, *Agronomy Research*, 14, pp. 1562-1572.

Chong R., Ball M., McRae C., Packer N. (2019), Comparing the chemical composition of dietary fibres prepared from sugarcane, psyllium husk and wheat dextrin, *Food Chemistry*, 298, pp. 125032, https://doi.org/10.1016/j.foodchem.2019.125032

Craeyveld V., Delcour J., Courtin C. (2009), Extractability and chemical and enzymic degradation of psyllium (*Plantago ovata* Forsk) seed husk arabinoxylans, *Food Chemistry*, 112, pp. 812-819, https://doi.org/10.1016/j.foodchem.2008.06.035

Dülgeroğlu C., Aksoy A. (2017), Plants used in the rennet of erzincan tulum cheese as adjuvants, *Erzincan University Journal of Science and Technology*, 10(1), pp. 156-167, https://doi.org/10.18185/erzifbed.310338

Neveen A.M.A, Eelbassiony K.R.A. (2024), Using amaranth and psyllium for bread production, *Food Technology Research Journal*, 4, pp. 35-48.

Franco M., Gómez M. (2022), Effect of psyllium on physical properties, composition and acceptability of whole grain breads, *Foods*, *11*(12), 1685, https://doi.org/10.3390/foods11121685

Gebremeskal Y.H., Nadtochii L., Natalia B., Mensah E., Kazydub N., Soliman T., Baranenko D., El-Messery T., Tantawy A. (2024), Comparative analysis of the nutritional composition, phytochemicals, and antioxidant activity of chia seeds, flax seeds, and psyllium husk, *Food Bioscience*, 61, 104889, https://doi.org/10.1016/j.fbio.2024.104889

Grek O., Chubenko L., Kumar A., Khareba V., Tymchuk A., Onopriichuk O. (2019), Polyphenolic compounds transition into protein-plant concentrates during the deposition of milk proteins by *Plantago major* L., *Ukrainian Food Journal*, 8(4), pp. 745-754, https://doi.org/10.24263/2304-974X-2019-8-4-6

Grek O., Krasulya O., Tymchuk A., Chubenko L. (2018), The investigation of the potentional complex from plantago major to coagulate milk proteins, *Food and Environment Safety*, 17(2), pp. 165-175.

Grek O., Onopriichuk O., Chubenko L., Tsygankov S., Savchenko O., Ochkolyas O. (2021), Research of milk proteins coagulation by active complex of *Plantago major* L, *Journal of Hygienic Engineering & Design*, 35, pp. 115-121.

Grek O., Pshenychna T., Tymchuk A., Savchenko O., Ochkolyas O. (2020), Research of quality indicators in protein-blueberry concentrates, *Potravinarstvo Slovak Journal of Food Sciences*, 14, pp. 156-163, https://doi.org/10.5219/1255

Grek V., Pshenychna T., Onopriichuk O., Grek O. (2024), Milk protein clots obtained by the addition of purple corn powder, *Ukrainian Food Journal*, 13(2), pp. 247-261, https://doi.org/10.24263/2304-974X-2024-13-2-4

Guo Q., Cui S., Wang Q., Young J. (2008), Fractionation and physicochemical characterization of psyllium gum, *Carbohydrate Polymers*, 73, pp. 35-43, https://doi.org/10.1016/j.carbpol.2007.11.001

Guyomarc'h F., Arvisenet G., Bouhallab S., Canon F., Deutsch S.M., Drigon V., Dupont D., Famelart M.,H., Gilles G., Guédon E., Guyot T., Hiolle M., Jan G., Le Loir Y., Lechevalier V., Nau F., Pezennec S., Thierry A., Valence F., Gagnaire V. (2021), Mixing milk, egg and plant resources to obtain safe and tasty foods with environmental and health benefits, *Trends in Food Science & Technology*, 108, pp. 119-132, https://doi.org/10.1016/j.tifs.2020.12.010

Kethireddipalli P., Hill A.R. (2015), Rennet coagulation and cheesemaking properties of thermally processed milk: overview and recent developments, *Journal of Agricultural and Food Chemistry*, 63(43), pp. 9389–9403, https://doi.org/10.1021/jf504167v

Khan U.M., Sameen A., Decker E.A., Shabbir M.A., Hussain S., Latif A., Abdi G., Aadil R.M. (2024), Implementation of plant extracts for cheddar-type cheese production in conjunction with FTIR and Raman spectroscopy comparison, *Food Chemistry*, 22, 101256, https://doi.org/10.1016/j.fochx.2024.101256

Lan H., Hoover R., Jayakody L., Liu Q., Donner E., Baga M., Asare E.K., Hucl P., Chibbar R.N. (2008), Impact of annealing on the molecular structure and physicochemical properties of normal, waxy and high amylose bread wheat starches, *Food Chemistry*, 111, pp. 663-675, https://doi.org/10.1016/j.foodchem.2008.04.055

Lo Piero A.R., Puglisi I., Petrone G. (2002). Characterization of "lettucine", a serine-like protease from Lactuca sativa leaves, as a novel enzyme for milk clotting, *Journal of Agricultural and Food Chemistry*, 50(8), pp. 2439–2443, https://doi.org/ 10.1021/jf011269k

Luo J., Xiao C., Zhang H., Ren F., Lei X., Yang Z., Yu Z. (2018), Characterization and milk coagulating properties of *Cynanchum otophyllum* Schneid. proteases, *Journal of Dairy Science*,101(4), pp. 2842–2850, https://doi.org/10.3168/jds.2017-13888

Mazorra-Manzano M.A., Perea-Gutiérrez T.C., Lugo-Sánchez M.E., Ramirez-Suarez J.C., Torres-Llanez M.J., González-Córdova A.F., Vallejo-Cordoba B. (2013), Comparison of the milk-clotting properties of three plant extracts, *Food Chemistry*, 141(3), pp. 1902–1907, https://doi.org/10.1016/j.foodchem.2013.05.042

Nayik G.A., Jagdale Y.D., Gaikwad S.A., Devkatte A.N., Dar A. H., Ansari M. J. (2022), Nutritional profile, processing and potential products: A comparative review of goat milk, *Dairy*, *3*(3), pp. 622-647, https://doi.org/10.3390/dairy3030044

Onopriichuk O., Grek O., Tymchuk A. (2022), Influence of malt properties on the indicators of milk-protein concentrates, In O. Paredes-López, O. Shevchenko, V. Stabnikov, V. Ivanov (Eds.), *Bioenhancement and Fortification of Foods for a Healthy Diet,* pp. 179-202, CRC Press, Boca Raton, https://doi.org/10.1201/9781003225287-12

Onopriichuk O., Skuibida V. (2024), Improvement of fermented dairy-plant concentrate technology, *Ukrainian Journal of Food Science*, 12(2), pp. 117–130, https://doi.org/10.24263/2310-1008-2024-12-2-4

Pavlyuk R., Pogarskaya V., Balaba K., Kravchuk T., Pogarskiy A. (2019), Of sour-milk healthy beverages with the use of natural herbal, *Food Science and Technology*, 13(4), pp. 127-137, https://doi.org/10.15673/fst.v13i4.1566

Pokhyl V.I., Mykolaichuk L.P. (2023), Features and nutritional value of sheep and goat milk, *Bulletin of Sumy National Agrarian University. The Series: Livestock*, 1, pp. 38-43, https://doi.org/10.32782/bsnau.lvst.2023.1.6

Prylipko T.M., Bukalova N.V., Bohatko N.M., Liasota V.P. (2020), Udoskonalennia sposobu vyznachennia vmistu kaltsiiu v molotsi tytrometrychnym metodom, *Podilskyi visnyk: Silske Hospodarstvo, Tekhnika, Ekonomika*, 33, pp. 42-46, https://doi.org/10.37406/2706-9052-2020-2-4

Ryzhkova T., Danylenko S., Kopylova K. (2019), Otsinka fizyko-khimichnykh pokaznykiv kozynoho ta koroviachoho moloka-syrovyny, *Prodovolchi Resursy*, 7(12), pp. 142–151, https://iprjournal.kyiv.ua/index.php/pr/article/view/117.

Sasaki T., Yasui I., Matsuki J., Satake T. (2002), Comparison of physical properties of wheat starch gels with different amylose content, *Cereal Chemistry*, 79, pp. 861-866, https://doi.org/10.1094/CCHEM.2002.79.6.861

Shah M.A., Mir S.A., Paray M.A. (2014), Plant proteases as milk-clotting enzymes in cheesemaking: a review, *Dairy Science and Technology,* 94, pp. 5–16, https://doi.org/10.1007/s13594-013-0144-3

Ukrainets A., Pasichnyi V., Shvedyuk D., Matsuk Y. (2017), Investigation of proteolysis ability of functional destinated minced half-finished meat products*, Scientific Messenger LNUVMBT named after S.Z. Gzhytskyj*, 19(75), pp. 129–133.

Zambelli R.A., Galvao A., de Mendonca L.G., Leao M.V.D., Carneiro S.V., Lima A.C.S., Melo, C.A.L. (2018), Effect of different levels of acetic, citric and lactic acid in the cassava starch modification on physical, rheological, thermal and microstructural properties, *Food Science and Technology Research*, 24, pp. 747-754, https://doi.org/10.3136/fstr.24.747

Znamiriwska-Piotrowska A., Petrusha O., Pawlos M., Kowalczyk M., Buniowska-Olejnik M., Szajnar K. (2023), The effect of psyllium and chokeberry fiber addition on the quality of probiotic fremented milk, *Zywnosc-nauka Technologia Jakosc,* 30, pp. 103-122, https://doi.org/10.15193/zntj/2023/136/457

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Vegetable snacks added with probiotic microorganisms

Gabriela Jackeline Acosta Prócel[1,2], María Monserrath Morales Padilla[1]

1 – Technical University of Cotopaxí, Latacunga, Ecuador
2 – Pelileo Higher Technological Institute, Patate, Ecuador

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Corresponding author:

Gabriela Jackeline Acosta Prócel
E-mail: clib2432025@gmail.com

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Abstract

Introduction. The incorporation of probiotics into vegetable-based matrices demonstrated promising potential for developing functional snacks with significant health benefits.

Materials and methods. A 3×3×4 factorial experimental design was used, which results in 36 experimental combinations (3 microorganisms × 3 plant matrices × 4 process steps). Three replicates were carried out for each experimental combination, generating 108 observations.

Results and discussion. The incorporation of probiotics in plant matrices showed promising potential for developing functional snacks with significant health benefits. The factorial design revealed that the plant matrix (carrot, sweet potato and chayote) and processing stage significantly influenced probiotic viability, with carrot showing the highest colony forming unit (CFU) levels and better sensory acceptability. While lactic acid bacteria *Lactobacillus acidophilus*, *L. bulgaricus* and yeasts *Saccharomyces cerevisiae* exhibited viability throughout processing, matrix properties and dehydration-storage stages influenced CFU counts. For example, in carrot, average *L. acidophilus* counts of 8.0×10^6 CFU/100 g were observed after dehydration, while in sweet potato counts were 5.5×10^6 CFU/100 g at the same stage. During storage, the viability of *S. cerevisiae* in carrot remained relatively high, with an average of 7.5×10^6 CFU/100 g at the end of the period evaluated, demonstrating good stability. Texture was the most influential attribute in the sensory analysis, with significant differences between samples. The results demonstrate the technical and scientific potential of developing functional vegetable snacks through the successful incorporation of viable probiotic microorganisms without compromising consumer sensory acceptability. The carrot matrix was found to be particularly effective in maintaining probiotic viability, especially in combination with S. cerevisiae during storage, reaching average levels of 8.33×10^6 CFU/100 g. Although dehydration and storage generated a reduction in viability and affected texture, the results support the viability of these products as an innovative alternative within the functional food sector.

Conclusions. This study establishes a sound scientific basis for the production of innovative functional snacks that combine significant microbiological viability with favourable consumer sensory acceptability and promotes the use of agro-industrial raw materials, contributing to a healthier and more sustainable diet.

Introduction

The development of functional food products has experienced remarkable growth in recent years, driven by a convergence between advances in food biotechnology and a growing consumer awareness of the relationship between diet and health. This phenomenon has been particularly visible in the area of functional foods, defined as those that not only provide essential nutrients, but also provide additional benefits that improve quality of life and contribute to the prevention of chronic diseases (Essa et al., 2023; Cencic et al., 2010; Ivanov et al., 2021; Tsykhanovska et al., 2023). In this context, probiotics have emerged as a central pillar in the formulation of such products, due to their ability to modulate the intestinal microbiota and strengthen the gastrointestinal barrier, which has direct implications on immunological, metabolic and neurological health (Cui et al., 2018).

Among the most studied probiotics, *Lactobacillus acidophilus* stands out for its ability to effectively colonize the intestinal tract and contribute to microbial balance. Several studies have shown that its regular consumption can reduce the incidence of gastrointestinal infections, improve lactose digestion and modulate inflammatory responses at the systemic level. These mechanisms are explained, in part, by the production of antimicrobial compounds such as bacteriocins and organic acids, which inhibit intestinal pathogens, in addition to their interaction with immune cells through molecular patterns associated with beneficial microorganisms (Nuñez et al., 2024).

On the other hand, the formulation of vegetable-based snacks has gained significant relevance due to their exceptional nutritional profile and their potential to act as ideal matrices for the incorporation of probiotics. Vegetables such as carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*) and chayote (*Sechium edule*) are not only rich in dietary fibre, vitamins and antioxidants, but also present a matrix structure suitable for protecting probiotic microorganisms during processing and storage. Dietary fibre, in particular, can act as a natural prebiotic, favouring the survival of beneficial bacteria and increasing their efficacy after consumption (Rajagukguk et al., 2022).

The development of products that integrate *Lactobacillus acidophilus* in vegetable matrices poses important technical and scientific challenges. One of the critical aspects is the viability of the probiotic during the thermal processing and dehydration stages, processes necessary for snacking. Recent research has explored strategies such as microencapsulation and the use of natural cryoprotectants, which have shown promising results in protecting probiotics from adverse conditions. In addition, it is essential to evaluate probiotic stability during storage, as factors such as water activity (Aw), acidity and temperature play a crucial role in microbial survival (Taco and García-Godos, 2021).

From the consumer's point of view, the success of functional snacks depends not only on their nutritional value and health benefits, but also on their sensory properties, such as texture, flavour and aroma. Acceptance studies have shown that the incorporation of probiotics can slightly modify these characteristics, which makes rigorous sensory testing essential to optimize formulations and ensure high market acceptability. In this sense, the design of functional products must balance the consumer's sensory perception with the functional benefits offered by probiotics (Payne et al., 2024).

Therefore, the incorporation of *Lactobacillus acidophilus* in vegetable snacks represents an innovative opportunity to respond to current market demands for functional foods. However, the success of these products requires a comprehensive approach ranging from the selection of suitable plant matrices and processing techniques to the scientific validation of their functional and sensory benefits. This approach will not only contribute to

expanding the possibilities in the field of functional foods, but will also reinforce the role of agribusiness in promoting global health and wellness (Zhan et al., 2023).

The present research aims to incorporate *Lactobacillus acidophilus* in snacks made from vegetable matrices such as carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*) and chayote (*Sechium edule*), in order to evaluate the viability and stability of the probiotic during the processing and storage stages (Álvarez and Lamas, 2021). It is proposed that these matrices, due to their composition rich in dietary fiber, antioxidants and carbohydrates, may act as natural protective substrates that favour the survival of the microorganism under adverse conditions, such as heat treatment or dehydration.

The central hypothesis of the study was established that it is possible to maintain the viability of *Lactobacillus acidophilus* above the minimum recommended limits (10^6-10^7 CFU/100 g) in final products, thus guaranteeing its functional properties and health benefits. In turn, the incorporation process was not expected to significantly alter keys sensory characteristics of the product, such as taste, texture and appearance, key parameters for consumer acceptance. To validate this hypothesis, the microbial stability, the behaviour of the probiotic during storage and its influence on the sensory attributes of the final snack were analysed in detail, which was allow determining its market potential as an innovative functional food (Latif et al., 2023).

The approach of the present research is experimental, based on a quantitative and descriptive research design that allows the controlled manipulation of independent variables to analyse their effect on the dependent variables. The experimental methodology facilitated the identification and quantification of cause-effect relationships between the selected factors - microorganism, plant matrix and process stage - and the responses of the evaluated system, such as probiotic viability, sensory properties and consumer acceptance (Oniszczuk et al., 2021). This approach ensures methodological rigor through the application of standardized procedures, the repetition of experiments and the use of robust statistical analysis to validate the results obtained. The quantitative nature of the study allowed objective and measurable data to be obtained, while its descriptive nature made it possible to interpret and detail the characteristics and behaviors observed in the different stages of the experimental process. Likewise, this design constituted an ideal tool to generate scientific evidence to support the formulation of functional products, contributing to the development of innovative alternatives in the agro-industrial and nutritional sector (Ilango and Antony, 2021).

The research was applied and experimental, focused on the development and evaluation of a functional food product that integrates probiotic microorganisms in vegetable matrices. Its purpose was to analyse, in a systematic and rigorous manner, the behavior of the product in terms of microbiological viability during the different stages of the production and storage process, as well as to determine the sensory properties - flavor, texture, appearance and aroma- and their impact on consumer acceptance and preference. This approach combined the generation of practical knowledge with the scientific validation of the factors involved, ensuring reproducible and applicable results in the functional food industry (Kruk et al., 2024).

Thus, the specific objectives of this study focused on evaluating the stability and viability of *Lactobacillus acidophilus* in vegetable snacks during different storage stages, analysing keys sensory properties – flavor, texture, appearance and aroma - using standardized sensory evaluation methods, and determining consumer acceptance and preference using hedonic and preference tests.

Materials and methods

Materials

Vegetable ingredients from local carrot (*Daucus carota*), sweet potato (*Ipomoea batatas*) and chayote (*Sechium edule*) crops available in the region were selected for their nutritional value and availability.

The sample included three vegetable matrices (carrot, sweet potato and chayote) that were subjected to different stages of the snack production process: liquid impregnation, impregnated matrix, dehydrated and stored. Three probiotic microorganisms were evaluated: Lactobacillus acidophilus, Lactobacillus bulgaricus and Saccharomyces cerevisiae, applied in factorial combinations. The selection of these factors responded to their relevance in obtaining functional foods with high microbial viability and optimal sensory properties (Li et al., 2020).

Experimental design

A 3x3x4 factorial experimental design was used, which results in a total 36 experimental combinations (3 microorganisms × 3 plant matrices × 4 process steps). Three replicates were carried out for each experimental combination, generating 108 observations. The factors evaluated were microorganisms, plant matrices and process steps, while the dependent variables were microbial viability, sensory properties and consumer acceptance (Bernal-Castro et al., 2019), where experimental factors used were; probiotic microorganisms: *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Saccharomyces cerevisiae*; vegetable matrices: carrot, sweet potato and chayote and process stages: from initial impregnation to storage.

Variables evaluated

The research adopted an empirical approach, based on the observation and systematic measurement of the dependent variables by means of a 3×3×4 factorial experimental design. This design allowed simultaneous evaluation of the effect of three independent factors - type of probiotic microorganism (*Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Saccharomyces cerevisiae*), vegetable matrix (carrot, sweet potato and chayote) and stage of processing (liquid impregnation, impregnated, dehydrated and stored matrix) - on the dependent variables, which included microbiological viability, sensory properties and consumer acceptance of the product (Managa et al., 2021).

To determine microbiological viability, colony forming unit (CFU) count tests were performed using standard microbiological methods on specific selective means, ensuring accuracy and reproducibility of results. Sensory evaluation was included taste, texture, appearance and aroma tests by a panel of trained judges, using validated hedonic scales to measure the perceived organoleptic quality of the snacks. Finally, acceptance and preference surveys were conducted on a representative sample of consumers, applying descriptive and inferential statistical techniques to identify acceptance trends and correlate results with sensory and microbiological attributes (de Souza et al., 2024).

The combination of these empirical and analytical methods ensured a comprehensive evaluation of product performance at different stages, providing robust and reliable information for the development of functional snacks with stable probiotics and high market acceptance (Muñoz-Pabon et al., 2024).

Microbiological analysis

Measurement of colony forming units for the average probiotic concentration expressed in CFU/100 g.

The viability of *Lactobacillus acidophilus* was evaluated at each critical stage of the snack production process (impregnation, dehydration and storage) by counting colony-forming units. The plate dilution technique was applied using specific culture means such as MRS agar, which favour the selective growth of probiotic bacteria. Plates was incubated at optimum temperature 37 °C for a period of 24 to 48 hours to ensure accurate recovery and quantification of the probiotic, in order to determine its viability and stability in the different plant matrices (Tixicuro et al., 2021).

Sensory evaluation

A sensory analysis of the final product was carried out by a panel of trained tasters, selected according to criteria of sensitivity and previous experience in organoleptic testing. Critical attributes such as flavor, texture, appearance and aroma were evaluated using a hedonic scale of 1 to 5 (where 1 is "very unpleasant" and 5 is "very pleasant"). This analysis was allowing the identification of possible sensory variations derived from the probiotic incorporation process and production stages, ensuring a final product that meets sensory acceptability standards (Cosme et al., 2022).

Acceptance surveys

Product preference and acceptance were measured through structured surveys applied to a representative sample of potential consumers. A Likert scale of 1 to 5 was used to evaluate parameters of overall liking, willingness to purchase, and perception of the product in terms of quality and health benefits. The surveys were provided quantitative data to identify acceptance trends and correlate them with sensory and microbiological characteristics, facilitating decision making in the process of improving and positioning the product in the market (Drake et al., 2023). These techniques, combined with a rigorous statistical analysis, were guarantee reliable and scientifically supported results, contributing to the development of a functional snack with high nutritional and probiotic value that meets the expectations of the modern consumer (Zhu et al., 2024).

Statistical analysis

The data collected were analysed by ANOVA (analysis of variance) to determine the impact of the experimental factors on the dependent responses. Post hoc tests, such as Tukey, were applied to compare means and detect significant differences between treatments. In addition, scatter plots and boxplots were used to visually interpret the trends and variability of the data, using Minitab 21 and Phyton statistical software for data analysis.

Results and discussion

Analysis of Variance for general factorial regression

Microorganisms. (1: *Lactobacillus acidophilus*, 2: *Lactobacillus bulgaricus*, 3: *Sacchromyces cerevisiae*); Matrix; Stage, by means of analysis of variance (ANOVA), in which different sources of variation were evaluated in a model with three factors (microorganisms, matrix and stage) and their interactions.

Model global. The F-value of 14.34 with a p-value of 0.000 suggests that the model as a whole is significantly different from zero, i.e., at least one of the sources of variation has a significant effect.

Main effects. The microorganisms did not have a significant effect (p-value = 0.180), since the F-value (1.76) is not high enough to reject the null hypothesis. While in the matrix, they had a very significant effect (p-value = 0.000) on the dependent variable, with an F-value of 202.16, indicating a significant difference between the levels of the matrix. On stage, they also had a significant effect (p-value = 0.000), with an F-value of 18.27, indicating that the different stages affect the dependent variable (Boev et al., 2024).

Interactions of two terms. The interaction Microorganisms × Matrix, was not significant (p-value = 0.992), indicating that there is no significant interaction between these two factors. The following interaction. Microorganisms × Stage, is also not significant (p-value = 1.000), suggesting that there is no relevant interaction between microorganisms and stage. Finally, the interaction Matrix × Stage, is significant (p-value = 0.000), with an F-value of 6.38, suggesting that the interaction between matrix and stage significantly affects the dependent variable (Gutiérrez-Tlahque et al., 2024).

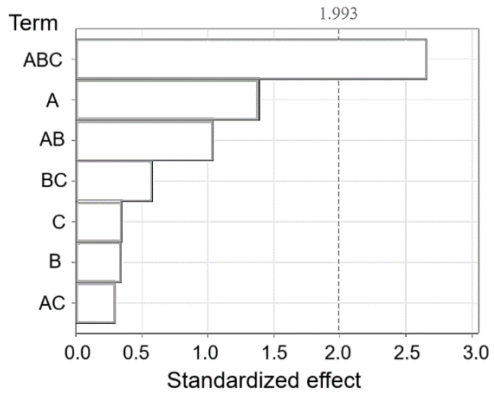
Three-term interactions. The interaction Microorganisms × Matrix × Stage is not significant (p-value = 1.000), which implies that the combination of the three factors does not have a significant effect on the dependent variable. With respect to Error, the variability explained by this is small, with a sum of squares of 20.012 and a mean squares value of 0.2780. Therefore, the model is significant, but the most important effects come from the matrix, the stage and the interaction between matrix and stage. Microorganisms have no relevant impact by themselves or in interaction with other factors (Table 1).

Table 1

Analysis of Variance for general factorial regression, Average probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage

Source	GL	SC Ajust.	MC Ajust.	Valor F	Valor p
Model	35	139.498	3.986	14.34	0.000
Linear	7	128.593	18.371	66.09	0.000
Microorganisms	2	0.976	0.488	1.76	0.180
Matrix	2	112.380	56.190	202.16	0.000
Stage	3	15.238	5.079	18.27	0.000
2-term interactions	16	10.759	0.672	2.42	0.006
Microorganisms×Matrix	4	0.075	0.019	0.07	0.992
Microorganisms×Stage	6	0.041	0.007	0.02	1.000
Matrix×Stage	6	10.643	1.774	6.38	0.000
3-term interactions	12	0.146	0.012	0.04	1.000
Microorganisms×Matrix×Stage	12	0.146	0.012	0.04	1.000
Error	72	20.012	0.278		
Total	107	159.511			

Using a Pareto diagram, significant effects on the average probiotic concentration expressed in CFU/100 g were identified ($\alpha = 0.05$). Factor B (Matrix) had the greatest impact, exceeding the critical value (1.993) with the most significant effect. Factor C (Stage) and the BC interaction were also significant, indicating their relevant influence on the response. In contrast, Factor A (Microorganisms) and the interactions AB, AC and ABC did not exceed the critical value, so they are not considered significant. These results highlight the importance of prioritizing the study and control of factors B, C and their interaction BC to optimize the response (Figure 1).



Factor	Name
A	Microorganisms
B	Matrix
C	Stage

Figure 1. Pareto plot of standardized effects for the general factorial regression, average probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage

The residual plots assessed the validity of the model assumptions and the quality of the fit. The normal probability plot showed that the residuals follow a reasonably normal pattern, with slight deviations in the tails. The residuals vs. fits show no clear patterns, suggesting that the model does not omit important terms or have fit problems. The histogram indicates that the residuals have a symmetric distribution centered at zero, supporting normality. The residuals vs. order of observation show no significant trends, although a dip is observed at the end, possibly related to outliers. In general, the assumptions of normality and random distribution are acceptable, with minor deviations (Figure 2).

Main effects

The main effects graph evaluated the impact of the factors on the response (average in CFU/100 g). The factor Microorganisms (A) did not show a significant effect, since its line is practically horizontal. Matrix (B) showed a notable difference between levels: level 1 had a higher mean (~8), while level 2 was lower (~6), confirming its significant impact. Stage (C) showed a clear downward trend, with a decrease in the mean response from level 1 to 4, indicating a significant effect. In summary, the most relevant factors are Matrix (B) and Stage (C), where level 1 in both factors generates the highest response. In contrast, the factor Microorganisms (A) has no significant impact (Figure 3).

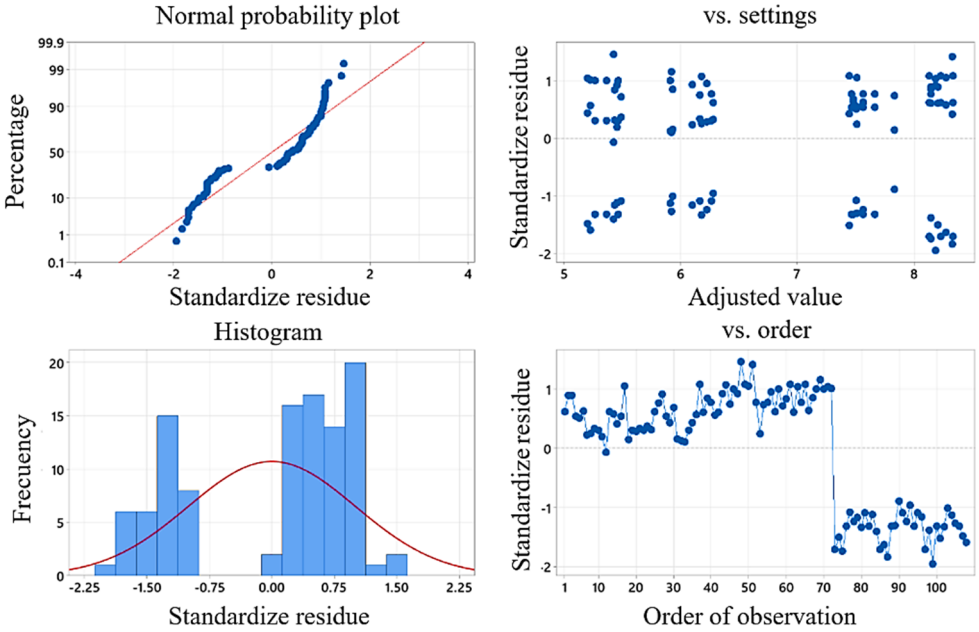


Figure 2. Graphs of average residues of probiotic concentration expressed in CFU/100 g for vegetable snacks (carrot, sweet potato, chayote), with addition of probiotic microorganisms

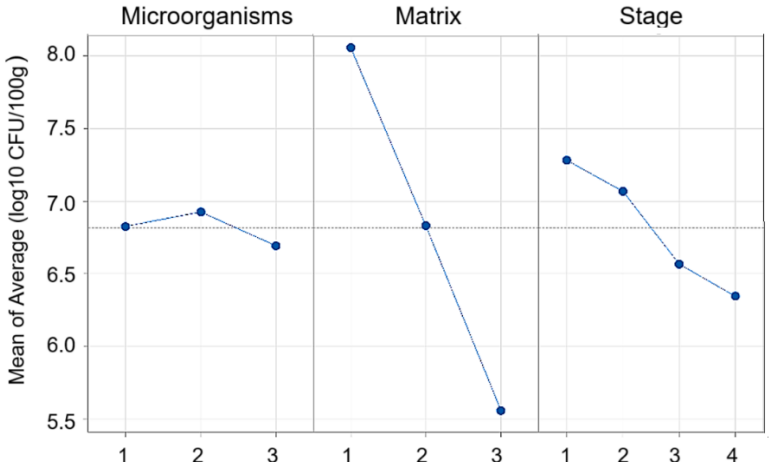


Figure 3. Main effect plot for average probiotic concentration expressed in CFU/100 g

Figure 4 shows the interactions between factors and their effect on the response (log10 CFU/100 g). No significant interactions were observed for combinations including Microorganisms (A), as the lines are parallel in the Matrix × Microorganisms, Stage × Microorganisms and Microorganisms × Stage panels. In the case of Matrix × Stage, the lines are not parallel: in Matrix 1, the response decreases markedly as one progresses through Stages (1 to 4), whereas in Matrix 2, the decrease is less pronounced. This confirms a significant interaction between Matrix and Stage, in line with previous results of the Pareto analysis. In summary, the most relevant interaction is between Matrix and Stage, while no significant interactions are detected for combinations with Microorganisms.

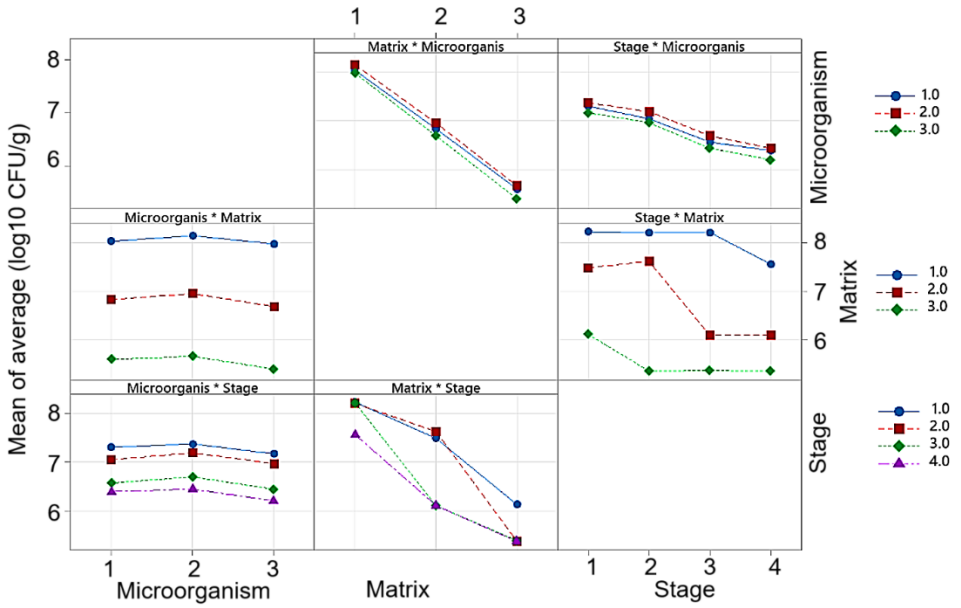


Figure 4. Interaction for average probiotic concentration expressed in CFU/100 g

The surface graph shows how the Matrix and the Microorganisms influence the average probiotic concentration expressed in CFU/100 g. Level 1 of the Matrix generates a higher response, while level 2 reduces it considerably (Figure 5). As for the Microorganisms, their impact is limited, which is in agreement with previous analyses where they were not significant. Although Microorganisms do not have a significant direct effect, specific combinations with the Matrix generate peaks in the response, possibly due to non-linear effects. In summary, the Matrix is the most influential factor in the response, while the Microorganisms have a minor impact and are limited to particular combinations.

Figure 6, a contour plot, shows the interaction between Matrix and Microorganisms on the average probiotic concentration expressed in CFU/100 g. Matrix 1 is associated with the highest response areas (CFU/100 g between 7 and 8.5), regardless of the level of Microorganisms. In contrast, Matrix 2 generates lower responses (CFU/100 g between 5 and 6), with little variation between Microorganism levels. This confirms that Matrix 1 has a greater influence on the response, while Microorganisms have a lesser impact.

Using a response optimization graph, Figure 7, in relation to the identified optimum, the graph shows the optimum conditions to maximize the response (Average CFU/100 g concentration). The maximum value reached (y) with 8.3333. The Desirability (D) with 0.8622, indicating that the solution is highly desirable but not perfect, and the optimum factors such as Microorganisms-Level 3, Matrix-Level 1 and Stage-Level 4 are conditions that optimize the average CFU/100 g concentration, reaching a high value. The lowest level of the Matrix and the highest level of Microorganisms and Stage contribute significantly to maximize the response.

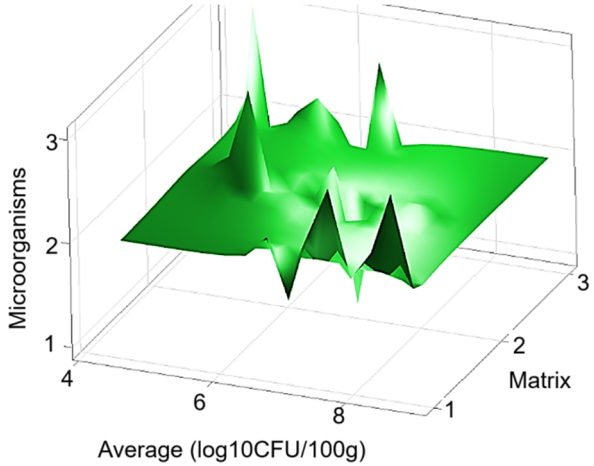


Figure 5. Surface plot Microorganisms vs. Matrix; average probiotic concentration expressed in CFU/g

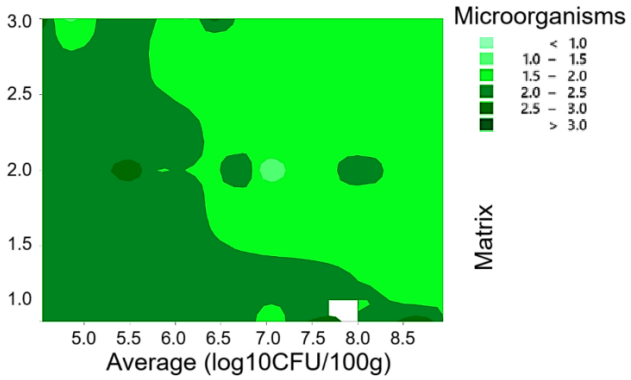


Figure 6. Contour plot of Microorganisms vs. Matrix; Average probiotic concentration expressed in CFU/100 g

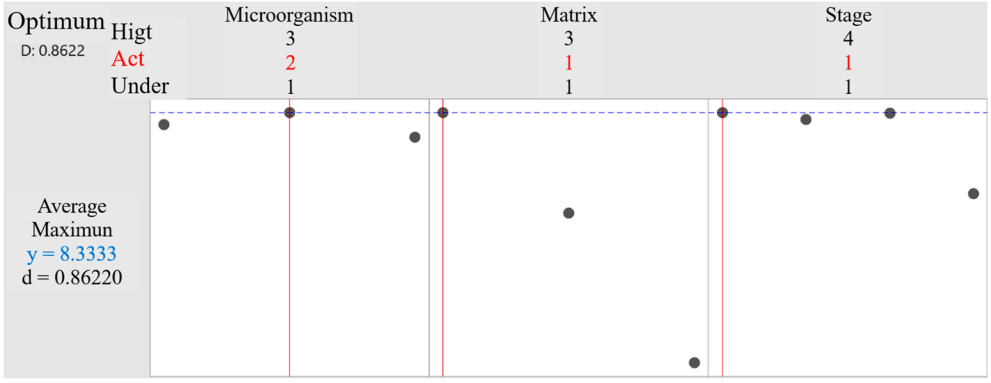


Figure 7. Response optimization graph for Average probiotic concentration expressed in CFU/100 g

Sensory evaluation

In this sense, the analysis of variance (ANOVA), with respect to Flavor, the value of p = 0.2068 (> 0.05), did not find significant differences between the samples with respect to the flavor attribute, since the changes in the samples do not significantly affect this attribute. Regarding Texture, the value of p = 0.0399 (< 0.05), indicates significant differences between samples with respect to texture, being the only sensory attribute where the samples present statistically significant differences, which could be relevant for the evaluation of the product. As for appearance, the p-value = 0.3945 (> 0.05), reveals no significant differences between the samples in relation to appearance, i.e., the samples are similar in appearance. Finally, the Aroma, presents a value of p = 0.3935 (> 0.05), where there were also no significant differences in terms of aroma, the samples do not differ significantly in terms of aroma (Table 2).

Table 2

Analysis of Variance sensory attributes of the average probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage

Sensory contribution	*p-value*	Result
Flavor	0.2068	Not significant
Texture	0.0399	Significant
Appearance	0.3945	Not significant
Aroma	0.3935	Not significant

The interpretation of Tukey's test for flavor reveals that no significant differences were found between the combinations of samples (Sweet Potato, Chayote and Carrot), therefore, all samples have a similar flavor. Regarding texture, a significant difference was identified between sweet potato and carrot (p = 0.0327), where carrot presented a different texture compared to sweet potato. There were no significant differences between the combinations Sweet Potato and Chayote or Chayote and Carrot, since Carrot differed significantly from Sweet Potato in texture, but not from Chayote. Regarding Appearance, no significant differences were found between any of the sample combinations, which have a similar appearance. On Aroma, there were also no significant differences between sample combinations, all samples are similar in aroma. From the Tukey tests, only significant differences were detected in texture between sweet potato and carrot. In the attributes of flavor, appearance and aroma, no significant differences were observed between samples. This reinforces that texture is the main sensory attribute that discriminates between samples (Table 3).

Observations by sensitive property, for carrot, Flavor (blue), has fairly high ratings (medians close to 4-5), with little dispersion. This indicates consistent acceptability. On Texture (orange), ratings vary widely, and there are low outliers, which could reflect a greater polarization in the perception of texture. On Appearance (green), similar to flavor, but with slightly lower acceptance (median around 3). Aroma (red), low overall acceptance (median close to 2), with a more moderate dispersion.

As for sweet potato, Flavor (blue) has moderate acceptance (median between 3-4), with less dispersion. Texture (orange), with high scores (median above 4), indicating that it was the most highly valued aspect of the sweet potato. In Appearance (green), there is lower acceptance compared to flavor and texture, with some dispersion. On Aroma (red), moderate median (~3), but more dispersion than in the other properties.

Table 3

Tukey's test results for Mean probiotic concentration expressed in CFU/100 g vs. microorganisms; Matrix; Stage.

Contribution	Comparison	Mean Difference	*p-value*	Significant
Flavor	Sweet Potato vs. Chayote	-1.75	0.1981	No
	Sweet Potato vs. Carrot	-0.50	0.8546	No
	Chayote vs. Carrot	1.25	0.4065	No
Texture	Sweet Potato vs. Chayote	-1.25	0.3844	No
	Sweet Potato vs. Carrot	-2.75	0.0327	Si
	Chayote vs. Carrot	-1.50	0.2675	No
Appearance	Sweet Potato vs. Chayote	-0.25	0.9729	No
	Sweet Potato vs. Carrot	1.25	0.5275	No
	Chayote vs. Carrot	1.50	0.4091	No
Aroma	Sweet Potato vs. Chayote	0.75	0.6784	No
	Sweet Potato vs. Carrot	1.25	0.3669	No
	Chayote vs. Carrot	0.50	0.8380	No

The distribution of ratings by sample and sensory property (Flavor, Texture, Appearance and Aroma) for three samples: Carrot, Sweet Potato and Chayote (Figure 8). The bars in the graph represent the median and interquartile range (IQR) for each sensory property per sample. Points outside the bars represent outliers, indicating individual responses that were significantly different from the others. Each color represents a different sensory property.

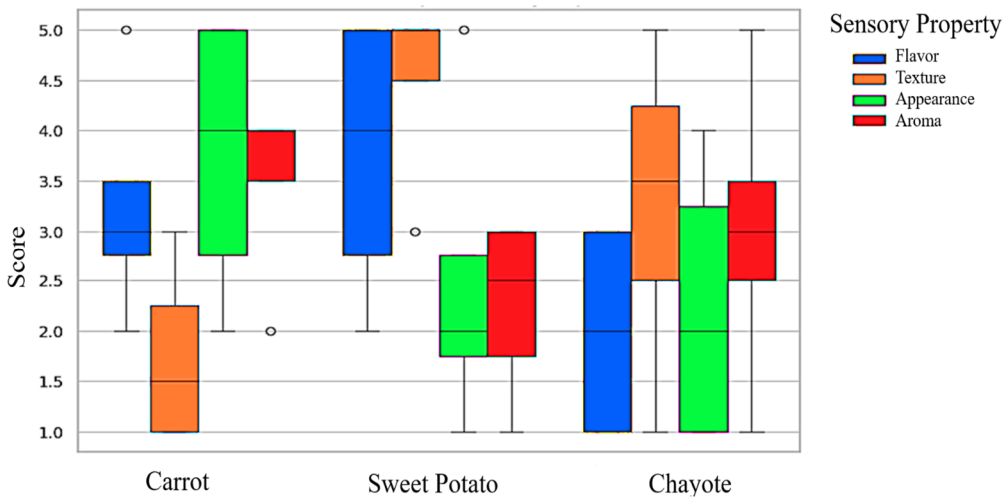


Figure 8. Graph of grade distribution by sample and sensory property such as flavor, texture, appearance

In the case of Chayote, Flavor (blue), presented ratings similar to sweet potato (median ~3.5), although more dispersed. For Texture (orange), intermediate acceptance (~3.5), but less dispersion than in flavor. Appearance (green), similar to flavor, but with slightly lower values and greater dispersion. Finally, in Aroma (red), consistent acceptance (median ~3), with less dispersion than in other properties.

Comparing the samples, we can infer that, in Flavor, Carrot obtained the highest scores, followed by Sweet Potato and then Chayote. In Texture, Sweet Potato stands out as the best evaluated, while Carrot has high variability (and outliers). In Appearance, the ratings are similar among the three samples, but somewhat lower compared to flavor and texture. On Aroma, all samples have a moderate to low rating (medians close to 2-3), being the least outstanding sensory property overall. Summarizing, Carrot stands out for flavor, but has a split perception in texture. Sweet Potato is highly valued for its texture, while its flavor and aroma are moderate. Chayote has a consistent evaluation in all properties, but does not excel in any, and aroma is the least accepted attribute overall for all three samples, with consistently low ratings (Boubezari et al., 2022).

Acceptance surveys

An analysis of variance (ANOVA) was applied to determine if there are significant differences between the samples based on the attribute ratings. Table 4. Where it is observed that the sum of squares (sum_sq) indicates the variability in the ratings explained by the "Sample" factor (52.92) versus the residual variability (9.0). Degrees of freedom (df) are 11 degrees for the "Sample" factor and 36 for the residual error. The F-statistic value is 19.24, suggesting a significant difference between the groups. The PR (>F), i.e. the p-value is extremely low ($6.90 \times 10\text{-}^{12}$), much less than 0.05. This indicates that the differences between the samples are statistically significant. In sum, the average scores differ significantly between the different samples evaluated. This result reinforces the idea that the sensory properties and the specific characteristics of each sample have an important impact on the perception of the evaluators (Sipos et al., 2021).

Table 4

ANOVA test results for acceptance surveys of probiotic-impregnated samples

ANOVA	sum_sq	df	F	PR(>F)
C (Sample)	52.916667	11.0	19.242424	6.895967^{-12}
Residual	9.000000	36.0	NaN	NaN

The results of ANOVA for this case, because of the length of the results, summarize the main findings clearly and concisely presented below. Therefore, dehydrated and stored samples (regardless of species) tend to differ significantly compared to liquid and impregnated samples. This could be related to the impact of treatments on key sensory properties such as texture, aroma and appearance. Consequently, these results show that the treatments applied to the different samples (liquid, impregnated, dehydrated and stored) significantly affect the sensory perception of the evaluators. In particular, the stored and dehydrated treatments seem to be the most influential in generating differences. The impregnation technique appears to be less disruptive compared to the dehydrated and stored treatments (Kręcisz et al., 2021).

Distribution of attributes by sample

Figure 9 shows the distribution of the values of the sensory attributes evaluated for each sample according to the treatment applied (liquid, impregnated, dehydrated, and stored). The key points are highlighted below.

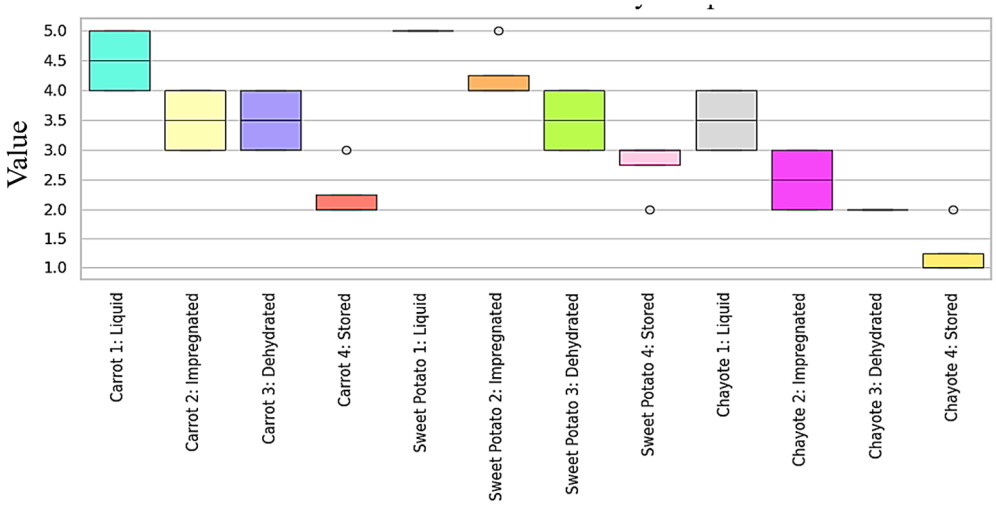


Figure 9. Boxplot for the distribution of the values of the sensory attributes evaluated for each sample as a function of the treatment applied (liquid, impregnated, dehydrated, stored)

The visual graph reinforces the statistical findings; the liquid treatments perform significantly better in sensory terms than the dehydrated or stored samples, which is consistent with the p-values obtained in the tests. The graph therefore supports the conclusion that liquid treatments are the most favored sensorially, while stored and dehydrated treatments have a negative impact on sensory ratings, regardless of the species evaluated. In addition, the lower dispersion in liquid samples could be related to a greater uniformity in sensory perception among evaluators.

The liquid samples (Carrot 1, Sweet Potato 1, Chayote 1) tend to have higher values in the sensory evaluation, with medians close to 4 or higher. This suggests that this treatment was more sensorially acceptable. Dehydrated and stored samples show lower values, with more dispersed medians and, in some cases, less than 3. This indicates that these treatments had a negative impact on sensory properties.

The stored samples show greater variability (longer box lengths), indicating more pronounced differences in raters' perceptions. Liquid and impregnated samples show lower dispersion, reflecting a more consistent evaluation among participants. There are some points outside the boxes (outliers), which could represent individual opinions significantly different from the majority. This suggests that some evaluators perceived certain samples very differently, particularly in treatments such as the stored simples.

Within the same species (carrot, sweet potato or chayote), dehydration and storage treatments tend to generate a greater drop in sensory scores. For example, Sweet Potato 4 (Stored) and Chayote 4 (Stored) have considerably lower values compared to the liquid versions of the same species.

Conclusions

1. On microbiological viability, the plant matrix and processing stage were critical factors that significantly influenced the microbiological viability of the probiotics. Carrot stood out as the best matrix for retaining high levels of probiotic viability, reaching an average of 8,333 CFU/100 g at the storage stage. This finding reinforces the importance of selecting matrices with protective properties for probiotics. As for the impact of the processing steps, these dehydration and storage steps generated a significant reduction in microbiological viability and sensory properties, particularly in texture and aroma. This underlines the need to optimize these processes to minimize probiotic viability losses and preserve consumer acceptance.
2. The sensory properties, among the attributes evaluated (flavor, texture, appearance and aroma), texture was the only one that showed significant differences among the samples. Carrot and sweet potato stood out for their overall sensory acceptability, while chayote showed a consistent but less outstanding evaluation. Consumer acceptance, in relation to the samples treated in the initial stages (impregnation liquid and impregnation) presented the highest values of sensory acceptance. However, acceptance decreased in the final stages (dehydration and storage), indicating a negative effect of the thermal and preservation processes on the organoleptic characteristics of the product.
3. Regarding industrial relevance, this study demonstrated that vegetable snacks can be effective matrices for probiotic vehicles, provided that processing steps are optimized to maximize microbiological viability and sensory properties. This has a positive impact on the development of innovative functional products that meet both market needs and consumer expectations. The results obtained support the development of functional vegetable snacks with high probiotic potential, offering an innovative and healthy nutritional solution. The application of these findings could significantly contribute to the advancement of the agri-food industry, promoting consumer health and innovation in functional products.

References

Álvarez S.T., Lamas D. (2021), Enrichment of a vegetable drink based on Macadamia nut (*Macadamia tetraphylla*) with omega-3 and probiotics, *Reportes Científicos de La FACEN*, 12(2), pp. 63–73, https://doi.org/10.18004/rcfacen.2021.12.2.63

Bernal-Castro C.A., Díaz-Moreno C., Gutiérrez-Cortés C. (2019), Inclusion of prebiotics on the viability of a commercial *Lactobacillus casei* subsp. *rhamnosus* culture in a tropical fruit beverage, *Journal of Food Science and Technology*, 56(2), pp. 987–994, https://doi.org/10.1007/s13197-018-03565-w

Boev M., Stănescu C., Turturică M., Cotârleț M., Batîr-Marin D., Maftei N., Chițescu C., Grigore-Gurgu L., Barbu V., Enachi E., Lisă E.L. (2024), Bioactive potential of carrot-based products enriched with *Lactobacillus plantarum*, *Molecules*, 29(4), 917, https://doi.org/10.3390/molecules29040917

Boubezari M.T., Bouchefra A., Djemai S. (2022), Changes in physico-chemical parameters, bioactive compounds and survival of *Lactiplantibacillus plantarum* J12 in fermented carrot juice, *Carpathian Journal of Food Science and Technology*, 14(4), pp. 17–27, https://doi.org/10.34302/crpjfst/2022.14.4.2

Cencic A., Chingwaru W. (2010), The role of functional foods, nutraceuticals, and food supplements in intestinal health, *Nutrients*, 2(6), pp. 611-625, https://doi.org/10.3390/nu2060611

Cosme F., Inês A., Vilela A. (2022), Consumer's acceptability and health consciousness of probiotic and prebiotic of non-dairy products, *Food Research International,* 151, 110842, https://doi.org/10.1016/j.foodres.2021.110842

Cui L., Niu L.Y., Li D.J., Liu C.Q., Liu Y.P., Liu C.J., Song J.F. (2018), Effects of different drying methods on quality, bacterial viability and storage stability of probiotic enriched apple snacks, *Journal of Integrative Agriculture*, 17(1), pp. 247–255, https://doi.org/10.1016/s2095-3119(17)61742-8

de Souza H.F., Monteiro G.F., Di Próspero Gonçalves V.D., dos Santos J.V., de Oliveira A.C.D., Pereira K.N., Carosia M.F., de Carvalho M.V., Brandi I.V., Kamimura E.S. (2024), Evaluation of sensory acceptance, purchase intention and color parameters of potentially probiotic mead with *Saccharomyces boulardii*, *Food Science and Biotechnology*, 33(7), 1651–1659, https://doi.org/10.1007/s10068-023-01459-y

Drake M.A., Watson M.E., Liu Y. (2023), Sensory analysis and consumer preference: Best practices, *Annual Review of Food Science and Technology*, 14(1), pp. 427–448, https://doi.org/10.1146/annurev-food-060721-023619

Essa M.M., Bishir M., Bhat A., Chidambaram S.B., Al-Balushi B., Hamdan H., Govindarajan N., Freidland R.P., Qoronfleh M.W. (2023), Functional foods and their impact on health, *Journal of Food Science and Technology*, 60(3), pp. 820-834, https://doi.org/10.1007/s13197-021-05193-3

Gutiérrez-Tlahque J., Altamirano-Romo S.E., Garrido-Torres M., Vargas-Torres A., Muñoz-Sánchez G. (2024), Effect of starch from chayotextle (*Sechium edule* (Jacq.) Sw.) and erythritol on the physicochemical and sensorial quality of a yogurt enriched with inulin, *AgEcon Search*, 17(8), pp. 117–126, https://doi.org/10.22004/AG.ECON.348528

Ilango S., Antony U. (2021), Probiotic microorganisms from non-dairy traditional fermented foods, *Trends in Food Science & Technology*, 118, pp. 617–638, https://doi.org/10.1016/j.tifs.2021.05.034

Ivanov V., Shevchenko O., Marynin A., Stabnikov V., Gubenia O., Stabnikova O., Shevchenko A., Gavva O., Saliuk A. (2021), Trends and expected benefits of the breaking edge food technologies in 2021–2030, *Ukrainian Food Journal*, 10(1), pp. 7-36, https://doi.org/10.24263/2304-974X-2021-10-1-3

Kręcisz M., Kolniak-Ostek J., Stępień B., Łyczko J., Pasławska M., Musiałowska J. (2021), Influence of drying methods and vacuum impregnation on selected quality factors of dried sweet potato, *Agriculture*, 11(9), 858, https://doi.org/10.3390/agriculture11090858

Kruk M., Lalowski P., Hoffmann M., Trząskowska M., Jaworska D. (2024), Probiotic bacteria survival and shelf life of high fibre plant snack - model study, *Plant Foods for Human Nutrition*, 79(3), pp. 586–593, https://doi.org/10.1007/s11130-024-01196-5

Latif A., Shehzad A., Niazi S., Zahid A., Ashraf W., Iqbal M.W., Rehman A., Riaz T., Aadil R.M., Khan I.M., Özogul F., Rocha J. ., Esatbeyoglu T., Korma S.A. (2023), Probiotics: Mechanism of action, health benefits and their application in food industries, *Frontiers in Microbiology*, 14, 1216674, https://doi.org/10.3389/fmicb.2023.1216674

Li J., Bai J., Li S., Zhu Z., Yi Y., Wang H., Lamikanra O. (2020), Effect of lactic acid bacteria on the postharvest properties of fresh lotus root, *Postharvest Biology and Technology*, 160(110983), 110983, https://doi.org/10.1016/j.postharvbio.2019.110983

Managa M.G., Akinola, S.A., Remize F., Garcia C., Sivakumar D. (2021), Physicochemical parameters and bioaccessibility of lactic acid bacteria fermented chayote leaf (*Sechium edule*) and pineapple (*Ananas comosus*) smoothies, *Frontiers in Nutrition*, 8, 649189, https://doi.org/10.3389/fnut.2021.649189

Muñoz-Pabon K.S., Roa-Acosta D.F., Bravo-Gómez J.E. (2024). Second-generation snacks prepared from quinoa with probiotic, Physicochemical properties, *in vitro* digestibility, antioxidant activity and consumer acceptability, *Heliyon*, 10(16), e36525, https://doi.org/10.1016/j.heliyon.2024.e36525

Nuñez H., Jaques A., Belmonte K., Elitin J., Valdenegro M., Ramírez C., Córdova A. (2024), Development of an apple snack enriched with probiotic *Lacticaseibacillus rhamnosus*: Evaluation of the Refractance Window drying process on cell viability, *Foods*, 13(11), 1756, https://doi.org/10.3390/foods13111756

Oniszczuk A., Oniszczuk T., Gancarz M., Szymańska J. (2021), Role of gut microbiota, probiotics and prebiotics in the cardiovascular diseases, *Molecules (Basel, Switzerland)*, 26(4), 1172, https://doi.org/10.3390/molecules26041172

Payne J., Bellmer D., Jadeja R., Muriana P. (2024), The potential of *Bacillus* species as probiotics in the food industry: A review, *Foods*, 13(15), 2444, https://doi.org/10.3390/foods13152444

Rajagukguk Y.V., Arnold M., Sidor A., Kulczyński B., Brzozowska A., Schmidt M., Gramza-Michałowska A. (2022), Antioxidant activity, probiotic survivability, and sensory properties of a phenolic-rich pulse snack bar enriched with *Lactiplantibacillus plantarum*, *Foods*, 11(3), 309, https://doi.org/10.3390/foods11030309

Shang Z., Ye Z., Li M., Ren H., Cai S., Hu X., Yi J. (2022), Dynamics of microbial communities, flavor, and physicochemical properties of pickled chayote during an industrial-scale natural fermentation: Correlation between microorganisms and metabolites, *Food Chemistry*, 377, 132004, https://doi.org/10.1016/j.foodchem.2021.132004

Sipos L., Nyitrai Á., Hitka G., Friedrich L.F., Kókai Z. (2021), Sensory panel performance evaluation—comprehensive review of practical approaches, *Applied Sciences*, 11(24), 11977, https://doi.org/10.3390/app112411977

Taco K.R., García-Godos P. (2021), Optimización de parámetros para la elaboración de leche ácida con *Lactobacillus acidophilus*, *CIT Informacion Tecnologica*, 32(1), pp. 179–186, https://doi.org/10.4067/s0718-07642021000100179

Tixicuro J.M.F., Chanfrau J.M.P., Sánchez de Céspedes I.S., Fiallos M.V. L., Pérez J.N. (2021), Optimización estadística de un bioproceso de ácido láctico a partir de lactosuero, *Ciencia Latina Revista Científica Multidisciplinar*, 5(3), pp. 3259–3274, https://doi.org/10.37811/cl_rcm.v5i3.530

Tsykhanovska I., Lazarieva T., Stabnikova O., Kupriyanov O., Litvin O., Yevlash V. (2023), Potential benefits of functional antianemic energy bars, *Ukrainian Food Journal*, 12(4), pp. 578-598, https://doi.org/10.24263/2304-974X-2023-12-4-7

Zhang S., Shang Z., Liu Z., Hu X., Yi J. (2023), Flavor production in fermented chayote inoculated with lactic acid bacteria strains: Genomics and metabolomics based analysis, *Food Research International*, 163, 112224, https://doi.org/10.1016/j.foodres.2022.112224

Zhu L., Mu T., Ma M., Sun H., Zhao G. (2022), Nutritional composition, antioxidant activity, volatile compounds, and stability properties of sweet potato residues fermented with selected lactic acid bacteria and bifidobacteria, *Food Chemistry*, 374, 131500, https://doi.org/10.1016/j.foodchem.2021.131500

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Technological modes of pressing for cherry kernel oil production

Yevhenii Kotliar, Bogdan Iegorov

Odesa National University of Technology, Odesa, Ukraine

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Corresponding author:

Yevhenii Kotliar
E-mail:
yevhenii11@ukr.net

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Abstract

Introduction. The aim of the research was to study the influence of temperature and duration of wet-heat treatment and pressing on the process of oil extraction from cherry pits.

Materials and methods. The raw material for making oil was the kernels of the following cherry cultivars: English Early, Lotivka, Lyubska, Griotte d'Ostheim, Podbyelska, and their mixture (20% of each sample). Treating cherry pits with NaCl solution was used. Modes of wet-heat treatment and pressing were studied.

Results and discussion. It was proposed to treat pits with NaCl. The mode selected as the most suitable for pits of different cherry varieties involves treating them with NaCl solution (one part rock salt to one part water) for 5–10 min. This treatment ensures that 90–100% of the pits crack. Thus, this is the optimum solution ratio to make this operation effective, which gives reasons for the proposed improvement of the technology.

Process conditions of cold pressing (extra virgin) have been determined for kernels of various cherry cultivars. The most practical temperatures of wet-heat treatment of crushed cherry kernels were as follows: 50–60°C and duration of 5–10 min for Podbyelska, English Early, Lotivka, Lyubska, and the mixture; 40–50°C and duration of 10–15 min for Griotte d'Ostheim.

It has been found that the pressing of cherry kernels was effective at temperature 60–70°C and duration of 6–15 min for Podbyelska, English Early, Lotivka, Lyubska, and the mixture, and at temperature 50–60°C and duration of 4–10 min for Griotte d'Ostheim. The parameters of the pressing extraction stage most effective for the kernels of all the cherry cultivars were: residual oil content in the oilcake 5.0—6.0%; oil yield 94.0%; oilcake thickness 1.8 mm (English Early), 2.0 mm (Lotivka), 1.7 mm (Lyubska), 2.2 mm (Griotte d'Ostheim), 2.0 mm (Podbyelska), and 1.8 mm (the mixture); load resistance time 6 min (English Early), 8 min (Lotivka), 10 min (Lyubska), 4 min (Griotte d'Ostheim), 10 min (Podbyelska), and 15 min (the mixture); and, for all samples, compressive strength 10.0 kN and rate of load application 5.0 kN/cm.

Conclusion. The results showed the practical value of preliminary processing of cherry pits before removing kernels from them, substantiated the choice of temperature and duration of their wet-heat treatment and pressing. The results were confirmed when these methods were introduced into the technology of oil production from cherry kernels of different varieties.

Introduction

Cherry (*Prunus avium*) is classified as a type of fruit called a drupe. Drupes consist of a thin skin, a fleshy body, a pit (or stone), which is composed from a hard outer shell and internal kernel (or seed). Cherry fruits contain a number of valuable substances necessary for the human body and are an integral part of a balanced diet. It is widely used in Europe, North America, and Asia. The global output of cherry fruit has increased over the last few years from 14.1 million to 38.1 million metric tons (Chatzimitakos et al., 2024).

Cherry fruit contains organic acid, sugars, phenolic compounds, soluble dietary fibre, and vitamin C (Sezer et al., 2021). One of the main components of a cherry kernel is oil (17–36%), which is a rich source of polyunsaturated and monounsaturated fatty acids. Amino acids such as lysine (essential amino acid) and glutamic acid (dominant amino acid), minerals such as calcium and potassium, and vitamins thiamine (B1), niacin (B3), pantothenic acid (B5), and pyridoxine (B6), are valuable compounds found in cherry kernels (Górnaś et al., 2016).

Adding natural additives, including fruit processed materials, to recipes of traditional food products is a modern trend that allows increasing their nutritional value and sensory properties (Stabnikova et al., 2021). Dried pomace, a by-product of cherry processing, can be crushed and immediately used in food recipes. For example, destoned cherry pomace was added to muffins as a source of phenolic antioxidants (Bajerska et al., 2016). Gumul et al. (2020) proposed adding extruded cherry pomace to gluten-free bread.

Extracts of pitted cherry pomace contain significant amounts of phenolic compounds. Encapsulation of such extracts allows obtaining preparations with a high content of phenolic compounds, which can be used in the manufacturing of functional foods (Pavlović et al., 2023; Toprakçı et al., 2023). Sweet cherry pomace is rich in pectin substances and can serve as a source of it (Zhang et al., 2023). The possibility of simultaneous extraction of pectin and phenols from cherry pomace was shown (Hosseini et al., 2020).

Cherry kernels are characterised by a high content of protein and fat and can be viewed as a raw material for oil production (Kazempour-Samak et al., 2021a; Maryam et al., 2022). Oil from cherry kernels is a powerful anti-inflammatory agent and antioxidant (Maragheh et al., 2019). The potential use of cherry kernels as a raw material to extract lipids was shown, but this process was restricted by high moisture content (Kniepkamp et al., 2024). Oil from *Prunus* kernels is high in unsaturated fatty acids and bioactive compounds (Natić et al., 2020).

It was reported that various techniques, such as solvent extraction, microwave, ultrasound, and extraction using supercritical fluid, enzyme-assisted methods, and cold pressing could be used to extract oil from drupes (Azmir et al., 2013). Kazempour-Samak et al. (2021b) studied oil production from fresh cherry kernels by cold pressing. The oil and moisture contents were found to be 31.89% and 4%, respectively. The sensory evaluation of the oil was acceptable, and the free fatty acid value was 1.36 mg KOH/g oil. Besides, the peroxide value and anisidine index of the cherry kernel oil were obtained as 0.99 mg O_2/kg oil and 0.15 mg O_2/kg oil, respectively.

The physicochemical parameters, fatty acid composition, and yield of cherry kernel oil depend on the cherry cultivar (Canbay and Doğantürk, 2019). Başyiğit et al. (2020, 2021) extracted oil from sour cherry kernels using cold pressing and characterised its physicochemical properties. The prevailing carotenoid in extracted oil was zeaxanthin (13.15 mg/kg) and it was rich in essential to human unsaturated fatty acids. High-quality oils was obtained at low temperatures by cold pressing containing of ω6 (linoleic, C18:2ω-6) and ω3

(alpha-linolenic, C18:3ω-3) polyunsaturated fatty acids with ω6/ω3 ratio 5:1 (Kmiecik et al., 2022), close to their accepted optimal value for healthy nutrition 4:1 (Stabnikova and Paredes-Lopez, 2024). However, the effects of wet-heat treatment prior to pressing remain unstudied.

Despite the great progress in oil production technology and techniques (including modern high-performance continuous screw presses), the process of obtaining oil by mechanical pressing has a significant drawback: the cake contains too much residual oil, which is lost along with it (Kotlyar and Gladkikh, 2023).

From the above, it is evident that a very valuable and promising raw material for oil production is cherry kernels because they contain beneficial nutritional and biological substances (Dimić et al., 2023; Stryjecka et al., 2019, 2022). Meanwhile, by-products and wastes generated in the agro-industrial sector and the food processing are not fully utilized (Protzman, 2023; Salo et al., 2024). Therefore, it makes sense to study pressing modes that include pre-treatment of cherry pits before kernel extraction, as well as to analyse the effect of temperature and duration of wet-heat treatment and pressing.

Material and methods

Raw materials

The following cherry varieties (harvested in 2021, 2022 and 2023) were selected as sources of cherry pits kernels that were used as raw materials for oil extraction (Figure 1a–f).

Figure 1a. English Early: fruit, pits, and kernels

Figure 1b. Lotivka: fruit, pits, and kernels

Figure 1c. Lyubska: fruit, pits, and kernels

Figure 1d. Griotte d'Ostheim: fruit, pits, and kernels

Figure 1e. Podbyelska: fruit, pits, and kernels

Figure 1f. Mixture: pits (left) and kernels (right) (20% of each sample)

Characteristics of the raw materials

Moisture content was measured by the rapid method involving the single-time keeping of the kernels and the hard shells of cherry pits in a drying chamber at a certain temperature and duration, according to (Canbay and Doğantürk, 2019; Górnaś et al., 2016).

The content of impurities and oil admixtures, the volumetric mass of the cherry pits, the length of a cherry pit, and the weight of 1000 cherry pits were determined according to (Canbay and Doğantürk, 2019).

Operating modes of pressing in laboratory conditions to produce oil from cherry kernels

Firstly, cherry kernels were cleaned from impurities. Next, the pits were treated in a 2% sodium chloride solution at a ratio of 1:1 for 3–5 minutes to soften the shell. The treated pits were then split.

Determination of the parameters of moisture-heat treatment and their influence on oil yield

100 g of cherry kernels were ground on the LZMK-1 mill as fine as 90–95% of the undersize from a 1 mm sieve. The crushed kernels were subjected to wet-heat treatment at the certain temperature and duration of saturation of the crushed kernels with steam. The temperature of the crushed kernels during the wet-heat treatment was regulated from 30 to 50°C, and the process duration from 10 to 20 min. The oil was obtained by pressing on a laboratory manual press PROM-1U. The oil yield was determined as a percentage of the maximum oil content in cherry kernels.

Determination of the parameters of pressing crushed kernels and the influence of these parameters on the oil yield

The pulp obtained because of the wet-heat treatment of crushed kernels was pressed using a laboratory hydraulic press of the U1 EPM type. 100 g of the pulp was poured into the press container, closed with a plunger. Using a hand pump, the pressure was increased to the maximum indicated on the manometer P = 0.072 mPa. During the pressing process, the temperature was adjusted to 40–60°C, the rate of load application to 5–15 kN/cm, the compression strength to 10–20 kN, and the duration of the loading was 3–8 min. The thickness of the pressed pulp and the yield of oil were determined, respectively, in mm and in % after they flowed into a pre-weighed flask.

Physicochemical study

The content of moisture in the crushed material was measured by the rapid drying method or using an electrical moisture meter (Latif et al., 2011).

The thickness of a cherry oilcake piece was measured according to (Latif et al., 2011). This measurement is necessary in order to monitor daily the operation of the press, and when testing new makes of presses.

The oil content in the cherry pit kernels, oilcake, and pores of the crushed material was determined by exhaustive extraction in a Soxhlet extractor according to (Başyiğit et al., 2021).

The acid value was determined according to (Topchij et al., 2016). The oil obtained before pressing (in the Soxhlet extractor) and after pressing the kernels of different cherry cultivars by the above methods was placed in a 250 ml conical flask. Three to five grams of the oil analysed were weighed with accuracies down to 0.01 g and heated on a water bath, then 50 ml of neutralised alcohol/hexane mixture was poured into the vessel to fill it up, and the sample was stirred. The resulting solution, continuously stirred, was quickly titrated with a potassium or sodium hydroxide solution (C (KOH) = 0.1 mol/l) until it became distinctly pink and could keep the colour for 30 s.

During titration with a potassium or sodium hydroxide solution, the quantity of alcohol in the composition of the alcohol/hexane mixture should be 5 times as big as the volume of the potassium or sodium hydroxide solution, to avoid hydrolysis of the soap formed. The acid value (AV) expressed in mg KOH/g was calculated according to the formula:

$$AV = \frac{V \cdot k \cdot c(KOH) \cdot M(KOH)_{eq}}{m},$$

where V is the volume of the KOH or NaOH solution spent on titration, ml;

k is the correction coefficient for the alkali solution to be expressed in terms of exact 0.1 mol/l;

m is the weight of the oil under study, g;

$c\ (KOH)$ is the molar concentration of the alkali, 0.1 mol/l;

$M\ (KOH)_{eq}$ is the molar equivalent mass equal to 56.11 g/mol.

The lipid content in the cherry kernels was measured by the hexane extraction method and by pressing, and that in the oilcake and pores of the crushed material – by exhaustive extraction in a Soxhlet extractor according to (Dominik et al., 2022).

Statistical analysis

All the numerical data obtained were processed with the Excel program from the service software package Microsoft Office 2007. The numerical data were presented as mean and the standard deviation.

Results and discussion

Characteristics of raw materials

Globally, there are quite few studies considering the quality parameters of cherry pits depending on their varietal features and year of harvest. After the raw material (cherry kernels) enters oil enterprises, the very first test is the determination of impurities, as they affect the output and general quality of the final product. The quality indicators of kernels and pits of different cherry cultivars are presented in Table 1.

According to the research results shown in Table 1, Griotte d'Ostheim is the variety with the highest average content of impurities found in the kernels after processing the fruit from which they are obtained (1.41±0.3 %). The mixture is the lowest in impurities (0.51±0.1 %). One can regard all the samples as complying with the general quality standards. No sample was infected or treated with pesticides. In the studies of Górnaś et al. (2016) and Canbay and Doğantürk (2019), a significant level of contamination in cherry pits (1.50%) was shown, but the obtained values did not affect their further processing.

The increased moisture in raw materials during their storage can activate unwanted processes in them (oxidative, hydrolytic), intensify their respiration, and promote the activity of microbiota that leads to the quality degradation (Górnaś et al., 2016). That is why, prior to directing the material for storage or processing, its moisture content is determined.

When determining this parameter, the moisture contents of the shell and the kernel were measured individually, and their average percentage was calculated. The moisture content of English Early was 7.9±0.3 %, Lotivka – 7.8±0.3 %, Lyubska – 7.0±0.3 %, Griotte d'Ostheim – 5.2±0.2 %, Podbyelska – 6.7±0.3 %, and the mixture – 7.0±0.4 %.

Table 1

Quality indicators of kernels and pits of different cherry varieties

Indicators	English Early			Lotivka			Lyubska			Griotte d'Ostheim			Podbyelska			Mixture (20% of each sample)		
	Years																	
	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023
Impurities, %	1.20 ±0.3	0.34 ±0.6	0.67 ±0.1	1.03 ±0.5	1.00 ±0.8	1.12 ±0.2	1.25 ±0.3	0.70 ±0.4	0.97 ±0.2	2.18 ±0.1	1.41 ±0.2	0.64 ±0.1	0.95 ±0.7	0.90 ±0.9	1.36 ±0.3	0.25 ±0.1	0.70 ±0.2	0.57 ±0.1
Moisture, %	7.00 ±0.3	8.00 ±0.4	8.70 ±0.5	8.50 ±0.4	7.00 ±0.2	8.00 ±0.3	7.00 ±0.4	6.00 ±0.2	8.0 ±0.4	7.10 ±0.3	4.50 ±0.2	4.10 ±0.2	7.20 ±0.3	6.50 ±0.3	6.50 ±0.3	7.00 ±0.4	6.00 ±0.3	8.0 ±0.4
Kernel content in the pits, mm	0.90 ±0.2	0.85 ±0.1	0.80 ±0.1	0.85 ±0.2	0.80 ±0.1	0.75 ±0.1	0.75 ±0.1	0.70 ±0.1	0.60 ±0.1	0.90 ±0.3	0.85 ±0.2	0.80 ±0.2	0.80 ±0.1	0.85 ±0.1	0.80 ±0.1	0.85 ±0.2	0.80 ±0.1	0.75 ±0.1
Weight of 1000 pits, kg	210 ±0.3	210 ±0.2	215 ±0.2	245 ±0.4	240 ±0.4	245 ±0.3	170 ±0.3	170 ±0.2	165 ±0.3	210 ±0.5	205 ±0.4	210 ±0.5	240 ±0.2	235 ±0.3	240 ±0.2	190 ±0.1	190 ±0.2	190 ±0.1
Oil content, %	24.75 ±0.3	24.70 ±0.6	24.70 ±0.1	20.03 ±0.5	19.00 ±0.8	21.10 ±0.2	29.10 ±0.3	28.60 ±0.4	28.50 ±0.4	22.70 ±0.1	23.41 ±0.2	23.02 ±0.1	26.95 ±0.7	24.90 ±0.9	25.54 ±0.3	24.80 ±0.1	23.70 ±0.2	22.50 ±0.1
Acid value, mg KOH/g	1.20 ±0.1	1.30 ±0.3	1.20 ±0.2	0.80 ±0.1	0.90 ±0.1	0.70 ±0.1	1.40 ±0.3	1.10 ±0.1	1.20 ±0.2	0.50 ±0.1	0.40 ±0.1	0.20 ±0.1	1.30 ±0.3	1.40 ±0.4	1.50 ±0.3	0.60 ±0.1	0.50 ±0.1	0.40 ±0.1

The kernel size determines the price, the saleability of the product, and the yield of oil. Normally, bigger kernels are spent in bigger quantities, and vice versa, and this should be taken into account when processing them. The kernels were measured with vernier callipers. The results obtained correlate with the findings of Górnaś et al. (2016) and Canbay and Doğantürk (2019), which did not exceed 8.20%. Based on the findings, it can be concluded that every variety ofcherry has its own average size of a pit. Thus, size of English Early pit was 0.9±0.2 mm; of Lotivka – 0.85±0.2 mm; of the mixture – 0.85±0.2 mm. Górnaś et al. (2016) and Canbay and Doğantürk (2019) obtained almost the same results (0.9 mm), but without taking into account the varietal features of cherries, though this is highly important when setting up the equipment and technological process.

Besides, studies conducted in enterprises include weighing 1,000 cherry pits. The weight of 1,000 cherry pits averages, depending on the cultivar: English Early – 210±0.3 g, Lotivka – 245±0.4 g, Lyubska – 170±0.3 g, Griotte d'Ostheim – 210±0.5 g, Podbyelska – 240±0.2 g, and the mixture – 190±0.1 g. Górnaś et al. (2016) and Canbay and Doğantürk (2019) obtained 220 g for cherry pits.

Oilseed lipids contain various groups of substances with different physicochemical properties and biological significance (Canbay and Doğantürk, 2019).

Based on the average data presented in Table 1, the content of oil varies with the variety of cherry pit kernels. The average oil percentage is the highest in Lyubska (28.7±0.4 %), and the lowest is that of the Lotivka kernels (20.0±0.3 %). Górnaś et al. (2016) researched the kernels of six cherry cultivars for their oil content. The average of this parameter was 31.8%.

When oils are in storage, hydrolysis takes place in them due to the action of enzymes, microorganisms, high temperatures, moisture, light, and other factors (Dominik et al., 2022).

High temperatures and exposure to light activate ester bonds in acyl glycerol, and the presence of moisture causes the development of microorganisms (Topchij et al., 2016). Hydrolysis results in the release of free fatty acids, and their content characterises the acid value (Latif et al., 2011). The acid value determines the freshness of oil and is the number of milligrams of potassium or sodium hydroxide necessary to neutralise free fatty acids contained in 1 g of oils or fat (Başyiğit et al., 2020, 2021).

The average results presented in Table 1 prove that the samples under study meet the general requirements, and the acid value in the samples never exceeds 3.0±0.3 mg KOH/g. Başyiğit et al. (2021) state that the acid value depends on the crop year and varietal features.

Operating modes

Two modes of processing cherry pits with a salt solution were considered to ensure their most complete cracking (Table 2).

Table 2

Selection of the pre-treatment mode for cracking the pits

Cherry varieties	%, cracking	
	33% NaCl, 5 min	50% NaCl, 10 min
English Early	65 ± 5	95 ± 5
Lotivka	73 ± 5	95 ± 5
Lyubska	69 ± 3	95 ± 5
Griotte d'Ostheim	73 ± 7	95 ± 5
Podbyelska	68 ±7	95 ± 5
Mixture	67 ± 8	95 ± 5

The first mode included short-term treatment for 5 minutes with a 33% NaCl solution, and the second treatment used a 50% salt solution and duration of treatment was 10 minutes. The use 50% solution of sodium chloride (one part rock salt to one part water), with the treatment duration 10 min resulted in 90–100% cracking of the pits. This indicates the recommended ratio of the solution components for this technological operation and gives reasons for the proposed improvement of the technology by the method described in the section Materials and methods.

The wet-heat treatment of the crushed cherry kernels was provided in three steps differing in temperature (t) and processing time (τ): I – 40°C, 3 min; II – 50°C, 5 min; III – 60°C, 10 min. For all the studied cherry varieties, as well as for their mixture, the same pattern was established. The degree of filling the pores in crushed kernels with oil depends on the parameters of the wet-heat treatment (its temperature and duration) and was as follows: 80% after step I; 90% after step II, and 100% after step III. Thus, the moisturising was the most effective at the second and third stages, as compared with the first ones. The crushed mass of cherry kernels of the cultivars Podbyelska, English Early, Lotivka, Lyubska, and their mixture has the treatment temperature 50–60°C, τ 5–10 min; Griotte d'Ostheim – 40–50°C, τ 10–15 min. In addition, in all the cultivars and in their mixture, the pores in the crushed kernels are completely open and filled with oil: by 90% at the second stage and by 100% at the third stage (see the method described in the section Materials and methods). All this creates favourable conditions for the further stage of processing. The temperature conditions, duration, and oil yield are given in Table 3.

The processed mash enters the press for final removal of oil (Kazempour-Samak et al., 2021b; Yılmaz et al., 2020). This is an element of the manufacturing line where oil is obtained by the cold pressing of different varieties of cherry kernels. It has been established that the pressing of cherry kernels is effective at t = 60–70°C, τ 6–15 min for Podbyelska, English Early, Lotivka, Lyubska, and their mixture and at t = 50–60°C, τ 4–10 min for Griotte d'Ostheim (Table 3).

Table 3

Pressing modes

Parameters	**English Early**			**Lotivka**			**Lyubska**			**Griotte d'Ostheim**			**Podbyelska**			**Mixture (20% of each sample)**		
	Stages																	
	I	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
Temperature, °C	50	60	70	50	60	70	50	60	70	40	50	60	50	60	70	50	60	70
Time, min	3	6	15	3	6	15	3	6	15	2	4	10	3	6	15	3	6	15
Oil yield, %	94	92	89	94	93	88	94	91	86	94	93	90	94	92	88	94	92	88
Thickness of oilcake piece, mm	1.8±0.01			2.0±0.02			1.7±0.03			2.20±0.04			2.0±0.02			1.8±0.03		

Oil pressed out of various cherry cultivars contains many suspended particles, including the mineral ones (Kniepkamp et al., 2024). The oil is purified from mechanical impurities by settling. After this, it is storable or ready for further use (Maragheh et al., 2019). Oilcake is good as animal feed. It can also be used as a fertiliser or as a food additive in the human diet (Azmir et al., 2013).

The thickness of an oilcake piece is a major indicator of the operation of a press (Natić et al., 2020). The determination of this parameter allows monitoring daily the operation of a press, and is necessary when testing new makes of presses (Canbay and Doğantürk, 2019). The thickness of the oilcake pieces is as follows: English Early – 1.8±0.01 mm, Lotivka – 2.0±0.02 mm, Lyubska – 1.7±0.03 mm, Griotte d'Ostheim – 2.20±0.04 mm, Podbyelska – 2.0±0.02 mm, and the mixture – 1.8±0.03 mm.

According to the data presented in Table 3, the temperature and duration of treating the kernels to obtain the mash were as follows: for the cultivars Podbyelska, English Early, Lotivka, Lyubskaya, and their mixture – 50–60°C, τ 5–10 min; for Griotte d'Ostheim – 40–50°C, τ 10–15 min.

This is an element of the production line where oil is obtained by cold pressing the pits of different varieties of sour cherries. The data in Table 4 show that the pressing of cherry pits of different varieties is effective with the following parameters (see the method described in the section Materials and methods): Podbielska, Early English, Lotivka, Lyubska and their mixture – t = 60–70°C, τ 6–15 min; Griotta d'Ostheim – t = 50–60°C, τ 4–10 min. The oil obtained passes the primary stage of refining and is directed for bottling, labelling, packaging, and storage. The press cake is portioned, labelled, and put into storage.

Conclusions

1. A number of cherry cultivars were analysed as sources of raw materials for the manufacture of oil from kernels of cherry pits. As a result, it was found that the following cultivars were the most promising for studying the kernels of their pits: English Early, Lotivka, Lyubska, Griotte d'Ostheim, Podbyelska, and their mixture (20% of each sample). These cultivars are the ones most often used in the fruit-processing and canning industries.

2. As for the contamination level, the research results show that the cultivar with the highest average content of impurities is Griotte d'Ostheim (1.41%), and the mixture is the lowest in impurities (0.51%). One can regard all the samples as complying with the general quality standards. No sample was infected or treated with pesticides. The average mass fraction of moisture in the samples of cherry pits under study (harvested in 2021–2023) is as follows: English Early – 7.9%, Lotivka – 7.8%, Lyubska – 7.0%, Griotte d'Ostheim – 5.2%, Podbyelska – 6.7%, and the mixture – 7.0%. One can conclude that all the samples meet the requirements. The average size of a cherry pit depends on the variety: English Early – 0.9 mm, Lotivka – 0.85 mm, Lyubska – 0.75 mm, Griotte d'Ostheim – 0.85 mm, Podbyelska – 0.9 mm, and the mixture – 0.85 mm. The weight of 1,000 cherry pits depends on the cultivar and averages: English Early – 210 g, Lotivka – 245 g, Lyubska – 170 g, Griotte d'Ostheim – 210 g, Podbyelska – 240 g, and the mixture – 190 g. The average mass fraction of oil varies with the variety of cherry kernels. The average oil percentage is the highest in Lyubska (28.7%), and the lowest is that of the Lotivka kernels (20%). The average acid value in the oils prepared from the samples of cherry kernels under study never exceeds 3.0 mg KOH/g, which meets the general requirements.

3. The mode selected as the most suitable for pits of different cherry cultivars involves treating them with 1:1 NaCl solution (one part rock salt to one part water) for 5–10 min. This treatment ensures the cracking of 90–100% of pits. Thus, this is the optimum solution ratio

to make this operation effective, which gives reasons for the proposed improvement of the technology.

4. It has been found that the most practical temperatures of wet-heat treatment of crushed cherry kernels are as follows: for Podbyelska, English Early, Lotivka, Lyubska, and the mixture – 50–60°C, τ 5–10 min; for Griotte d'Ostheim – 40–50°C, τ 10–15 min.

5. The pressing of cherry kernels of different cultivars is effective at t = 60–70°C, τ 6–15 min for Podbyelska, English Early, Lotivka, Lyubska, and their mixture, and at t = 50–60°C, τ 4–10 min for Griotte d'Ostheim.

6. The parameters of the pressing extraction stage found to be the most effective for the kernels of all the cherry cultivars are: residual oil content in the oilcake 5.0—6.0%; oil yield 94.0%; thickness of the oilcake: 1.8 mm (English Early), 2.0 mm (Lotivka), 1.7 mm (Lyubska), 2.2 mm (Griotte d'Ostheim), 2.0 mm (Podbyelska), and 1.8 mm (the mixture); load resistance time: 6 min (English Early), 8 min (Lotivka), 10 min (Lyubska), 4 min (Griotte d'Ostheim), 10 min (Podbyelska), and 15 min (the mixture); and, for all samples, compressive strength 10.0 kN and rate of load application 5.0 kN/cm.

References

Azmir J., Zaidul I.S.M., Rahman M.M., Sharif K.M., Mohamed A., Sahena F., Jahurul M.H.A., Ghafoor K., Norulaini N.A.N., Omar A.K.M. (2013), Techniques for extraction of bioactive compounds from plant materials: A review, *Journal of Food Engineering,* 117(4), pp. 426–436, https://doi.org/10.1016/j.jfoodeng.2013.01.014

Bajerska J., Mildner-Szkudlarz S., Górnaś P., Seglina D. (2016), The effects of muffins enriched with cherry pomace on acceptability, glycemic response, satiety and energy intake: A randomized crossover trial, *Journal of the Science of Food and Agriculture*, 96(7), pp. 2486–2493, https://doi.org/10.1002/jsfa.7369

Başyiğit B., Sağlam H., Kandemir S., Karaaslan A., Karaaslan M. (2020), Microencapsulation of cherry oil by spray drying: Evaluation of physical morphology, thermal properties, storage stability, and antimicrobial activity, *Powder Technology*, 364, pp. 654–663, https://doi.org/10.1016/j.powtec.2020.02.035

Başyiğit B., Sağlam H., Hayoğlu I., Karaaslan M. (2021), Spectroscopic (LC-ESI-MS/MS, FT-IR, NMR) and functional characterization of fruit seed oils extracted with green technology: A comparative study with *Prunus cerasus* and *Punica granatum* oils, *Journal of Food Processing and Preservation,* 45(5), 15451, https://doi.org/10.1111/jfpp.15451

Canbay H.S., Doğantürk M. (2019), Oil ratio and fatty acid composition of cherry seed oil, *Turkish Journal of Health Science and Life,* 2(1), pp. 21–24.

Dimić I., Pavlić B., Rakita S., Kljakić A.C., Žeković Z., Teslić N. (2023), Isolation of cherry seed oil using conventional techniques and supercritical fluid extraction, *Foods*, 12(11), pp. 1–18, https://doi.org/10.3390/foods12010011

Gumul D., Korus A., Ziobro R. (2020), Extruded preparations with cherry pomace influence quality and increase the level of bioactive components in gluten-free breads, *International Journal of Food Science,* 7, pp.1–11, https://doi.org/10.1155/2020/8024398

Górnaś P., Rudzińska M., Raczyk M., Mišina I., Soliven A., Segliņa D. (2016), Composition of bioactive compounds in kernel oils recovered from cherry (*Prunus cerasus* L.) by-products: Impact of the cultivar on potential applications, *Industrial Crops and Products*, 82, pp. 44–50, https://doi.org/10.1016/j.indcrop.2015.12.010

Hosseini S., Parastouei K., Khodaiyan F. (2020), Simultaneous extraction optimization and characterization of pectin and phenolics from cherry pomace, *International Journal of Biological Macromolecules*, 158, pp. 911–921, https://doi.org/10.1016/j.ijbiomac.2020.04.241

Kazempour-Samak M., Rashidi L., Ghavami M., Sharifan A., Hosseini F. (2021a), Antibacterial and antioxidant activity of cherry kernel oil (*Cerasus vulgaris* Miller)

against some food-borne microorganisms. *Journal of Food Measurement and Characterization,* 15(4), pp. 1–10, https://doi.org/10.1007/s11694-021-01035-0

Kazempour-Samak M., Rashidi L., Ghavami M., Sharifan A., Hosseini F. (2021b), Cherry (*Cerasus vulgaris* Miller) kernel oil as the novel functional edible oil: Sensory evaluation and antioxidant and physicochemical properties, *Journal of Food Quality*, 4, pp. 1–9, https://doi.org/10.1155/2021/5529613

Kniepkamp K., Errico M., Yu M., Roda-Serrat M.C., Eilers J.G., Wark M., Haren R. (2024), Lipid extraction of high-moisture cherry stones by supercritical carbon dioxide, *Journal of Chemical Technology & Biotechnology*, 99(4), pp. 810–819, https://doi.org/10.1002/jctb.7581

Kmiecik D., Monika Fedko M., Siger A., Kowalczewski P.L. (2022), Nutritional quality and oxidative stability during thermal processing of cold-pressed oil blends with 5:1 ratio of ω6/ω3 fatty acids, *Foods*, 11(8), 1081, https://doi.org/10.3390/foods11081081

Kotliar Y., Gladkikh R. (2023), Prospects for obtaining oil from the kernels of the seeds of different varieties of peaches, *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies*, 25(99), pp. 75-79, https://doi.org/10.32718/nvlvet-f9913

Latif S., Anwar F., Hussain A.I., Shahid M. (2011), Aqueous enzymatic process for oil and protein extraction from *Moringa oleifera* seed, *European Journal of Lipid Science and Technology*, 113, pp. 1012–1018, https://doi.org/10.1002/ejlt.201000525

Maragheh A.D., Masoud M.H., Karimi E., Seyedi S.M.R., Khatamian N. (2019), Producing the cherry pit oil nanoemulsion and evaluation of its anti-cancer effects on both breast cancer murine model and MCF-7 cell line, *Journal of Microencapsulation*, 36(4), pp. 399–409, https://doi.org/10.1080/02652048.2019.1638460

Maryam K.S., Ladan R., Mehrdad G., Anoosheh S., Fakhrisadat H. (2022), Correction to: Antibacterial and antioxidant activity of cherry kernel oil (*Cerasus vulgaris* Miller) against some food-borne microorganisms, *Journal of Food Measurement & Characterization,* 16(1), 804, https://doi.org/10.1007/s11694-021-01197-x

Natić M., Zagorac D.D., Ćirić I., Meland M., Rabrenović B., Akšić M.F. (2020), Cold pressed oils from genus Prunus, *Journal of Science Direct,* 56, pp. 637–658, https://doi.org/10.1016/B978-0-12-818188-1.00056-6

Pavlović R.Ž., Blagojević B., Kukrić T., Blažek K.M., Lazzara G., Četojević-Simin D., Popović B.M. (2023), Phenolic compounds from cherry pomace: microencapsulation, in vitro digestion, and cell growth activities, *Plant Foods for Human Nutrition,* 78, pp. 221–227, https://doi.org/10.1007/s11130-023-01046-w

Protzman E. (2023), Stone Fruit: World Markets and Trade, 19, pp. 1–8, https://www.fas.usda.gov/report-release-announcement/stone-fruit-world-markets-and-trade-3

Salo I., Zavalniuk O., Skakun V. (2024), Stone fruit crops market development in Ukraine and throughout the world, *AIC Economics and Management,* 1, pp. 85–94, https://doi.org/10.33245/2310-9262-2024-189-1-85-94

Sezer D.B., Ahmed J., Sumnu G., Sahin S. (2021), Green processing of cherry (*Prunus cerasus* L.) pomace: Process optimization for the modification of dietary fibers and property measurements, *Journal of Food Measurement and Characterization,* 15, pp. 3015–3025, https://doi.org/10.1007/s11694-021-00883-0

Stabnikova O., Marinin A., Stabnikov V. (2021), Main trends in application of novel natural additives for food production, *Ukrainian Food Journal*, 10(3), 524–551, https://doi.org/10.24263/2304-974X-2021-10-3-8

Stabnikova O., Paredes-Lopez O. (2024), Plant materials for the production of functional foods for weight management and obesity prevention, *Current Nutrition & Food Science*, 20(4), pp. 401–422, https://doi.org/10.2174/1573401319666230705110854

Stryjecka M., Kieltyka-Dadasiewicz A., Michalak M. (2019), Chemical composition and antioxidant properties of oils from the seeds of five apricot (*Prunus armeniaca* L.) cultivars (*Prunus armeniaca* L.), *Journal of Oleo Science*, 68(8), pp. 729–738, https://doi: 10.5650/jos.ess19121

Stryjecka M., Michalak M., Cymerman J., Kiełtyka-Dadasiewicz A. (2022), Comparative assessment of phytochemical compounds and antioxidant properties of kernel oil from

eight cherry (*Prunus cerasus* L.) cultivars, *Molecules*, 27(3), 696, https://doi.org/10.3390/molecules27030696

Topchij O.A., Kotljar J.O., Tkachenko N.A., Sevast'janov O.V., Makovs'ka T.V. (2016), Method for determining acid number, Patent Ukraine 12837.

Toprakçı İ., Torun M., Şahin S. (2023), Development of an encapsulation method for trapping the active materials from cherry biowaste in alginate microcapsules, *Foods*, 12, 130, https://doi.org/10.3390/foods12010130

Yılmaz E., Keskin O., Ok S. (2020), Valorization of cherry and cherry seeds: cold press oil production and characterization, *Journal of Agroalimentary Processes and Technologies,* 26(3), pp. 28–240.

Zhang S., Waterhouse G.I.N., Du Y., Fu Q., Sun Y., Wu P., Ai S., Sun-Waterhouse D. (2023), Structural, rheological and emulsifying properties of RG-I enriched pectins from sweet and cherry pomaces, *Food Hydrocolloids*, 139, 108442, https://doi.org/10.1016/j.foodhyd.2022.108442

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Changes in the content of biologically active substances during germination of cereal and legume grains

Irma Berulava[1], Maria Silagadze[1], Volodymyr Kovbasa[2], George Pkhakadze[3]

1 – Akaki Tsereteli State University, Kutaisi, Georgia
2 – National University of Food Technologies, Kiev, Ukraine
3 – Agricultural University of Georgia, Tbilisi, Georgia

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Corresponding author:

Maria Silagadze
E-mail: mariasilagadze@yahoo.com

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Abstract

Introduction. The research aims to study the changes in the content of bioactive compounds accumulated during the swelling and subsequent germination of cereal and legume grains, as well as to provide their qualitative and quantitative assessment.

Materials and methods. Wheat, green lentil, pea, and soybean grains swollen in drinking or in a weak radonic mineral water and germinated. The content of amino acids was determined by chromatography; fats by Soxhlet method; total phenols by Folin-Ciocalteu; total flavonoids by spectral and antioxidant activity by DPPH methods.

Results and discussion. The amount of amino acids during the grain germination process increased. The obtained results indicate the positive effect of mineral water on the accumulation of amino acids. Particularly good results were observed for soybean and pea samples. The change in the total amount of fats (%) during the process of grain swelling and germination was determined. An increase in the amount of fat was observed in all samples, especially in samples swollen in mineral water and subsequently germinated. The best results were observed for pea (increased by 1.47 times) and lentil (increased by 1.23 times) grains. Based on the study of phenolic compounds, it was found that the amount of total phenols in germinated grains increased compared to the original ones, except pea grains, where a slight decrease was observed. The amounts of total phenols changed significantly in grains germinated after swelling in weak radonic mineral water, compared to grains germinated after swelling in drinking water. The best results were shown by wheat, soybean, and green lentil grains. As for the content of flavonoids, their increase was found in the all test samples, especially in grains germinated after swelling in mineral water. Based on the comparative analysis of the flavonoid content, soybean (1.80 mg/g) and green lentil (1.29 mg/g) grains were considered the best, followed by peas (1.02 mg/g). A similar result was observed for the content of phenolic acids, mg/g: green lentils, 1.61; soybeans, 1.52; peas, 0.85. It was established that when using mineral water for swelling, the antioxidant activity of the test samples increased? %: soybeans by 45.8; peas by 43.6; green lentils by 40.87%. It was also shown that the grains germinated after swelling in mineral water possess high antioxidant activity, %: soybeans, 64.51; peas, 46.63; green lentils, 42.5.

Conclusions. The use of weak radonic mineral water has a positive effect on activation of the grain swelling and germination, as well as accumulation of biologically active substances in them.

Introduction

The grains of traditional cereals, legumes, and pseudocereals (quinoa, amaranth, buckwheat) significantly contribute to a balanced human diet worldwide (Perri et al., 2020). Large-scale studies conducted in recent years have established that the use of these raw materials provides enrichment of the body with a full set of biologically active substances (Alvarez-Jubete et al., 2010), and the efficiency of absorption is significantly increased by using germinated grains (Ali and Elozeiri, 2017; Khatun et al., 2023) and extracts derived from them (Jeong et al., 2019).

It is known enzymes produced during the germination process break down complex reserve substances into simpler ones. At this time, the amount of biologically active compounds synthesized by the germ increases. Germinated grains contain biologically active protein complexes, peptides, free amino acids, soluble sugars, soluble dietary fiber, biogenic macro- and microelements, vitamins, phytohormones, and other useful food components (Nkhata et al., 2019; Rodrigues, 2010; Stabnikova et al., 2019; 2022).

Due to the significant changes in nutritional and physicochemical properties of grains after the activation of dormant enzymes, germination has been recognized as a method for improving the nutritional quality of whole grains (Donkor et al., 2012). In addition, it has also been proven that the germination process even leads to changes in the structure of the microbiota in several types of cereals (Landry et al., 2018, Owolabi et al., 2020).

Germination is a simple, inexpensive, and environmentally friendly method for producing plant-based foods with functional properties. It enhances the nutritional and medicinal properties of plant-based foods by reducing antinutrients and increasing the accumulation of antioxidants, flavonoids, phenolic acids, and vitamins, thereby increasing the value of the grains (Carrera-Castaño et al., 2020; Onopriichuk et al., 2022; Onopriichuk et al., 2024; Rahman et al., 2023).

Various studies have shown that germination leads to increased bioavailability of minerals in the seeds (Oghbaei and Prakash, 2020; Pjak et al., 2019), and besides, germination is considered a desirable process because it has the function of reducing the amount of antinutrients in the grains (e.g., phytic acid, which combines with various minerals to form phytates) (Atudorei et.al., 2020; Chinma et al., 2020; Ghavidel and Prakash, 2007).

Based on numerous studies of the germination process of cereal and legume grains, germinated grain semi-finished products are widely used in various sectors of the food industry (bakery, confectionery, dairy, and meat products) (Atudorei et al., 2021; Eker et al., 2020), especially in the form of powders and extracts, to improve their nutritional profile or sensory and quality characteristics. Germinated grains (malt) have been used as the main raw materials in beer production for many years. Grains of various cereals and legumes, as well as many non-traditional raw materials, have been proposed for the preparation of malt. It was established that the germination process improves the bioactive properties of grains, which are important for use as raw materials in the food industry.

Analysis of the materials searched for germination methods and intensification of the mentioned processes revealed that the processes of grain swelling and germination are mostly accelerated by means of chemical compounds (Dufková, 2019), enzyme preparations (Kurmanbayeva et al., 2023), ultrasonic and thermo-alkaline hydrolysis (Luo et al., 2022; Xia et al., 2017), acoustic effects (Guimarães et al., 2020), and weak radonic mineral water (Berulava et al., 2024).

Although numerous studies demonstrated a certain increase in the content of biologically active substances during grain germination. It is noteworthy that the processes of grain swelling and germination still remain quite long, which poses a threat to the

microbiological safety (contamination) of the intermediate and target final product, which is unacceptable for food production. The duration of this technological process has been reduced on average by 5-20% compared to classical methods.

The present research aims to study the dynamics of changes in the content of bioactive compounds accumulated during the pre-processing (swelling) and subsequent germination process of some cereal and legume grains using Georgia's unique, weak radonic chloride-hydrocarbonate-sulfate mineral water, as well as to provide their qualitative and quantitative assessment.

Material and methods

Materials

The objects of the research were cereals grown in western Georgia – wheat and legumes – green lentils, peas and soybeans. Grains swollen in drinking water; grains swollen in weak radonic chloride–hydrocarbonate–sulfate mineral water sustradone (3–7.5 Mache units; or 40–100 BAC), chlorine–hydrocarbonate–sulfate, sodium–magnesium–calcium mineral water (with total mineralization of 0.7–0.8 g/l), as well as grains swollen after germination in drinking water, grain germinated after swelling in mineral water.

Swelling and germination of cereal and legume grains

The raw materials for germination were selected according to the following principle: the raw materials had to be local (Georgia), low–glycemic, agglutinative, or low–gluten, with a high protein content. Initially, foreign impurities and damaged grains were removed from the selected grains, washed well with running water, and only then was soaked in water for germination. During the experiments, each grain was taken in an amount of 20 g, and 100 ml of water was added. Swelling was carried out both in drinking water and in the weak radonic chloride–hydrocarbonate–sulfate mineral water of the Tskaltubo resort (instead of ordinary drinking water). The samples were placed in a thermostat and kept for 10 hours at a temperature of 22–40ºC. During the swelling process, changes were determined in the reaction medium pH and grain mass (swelling ability). It should be noted that no significant changes in pH and grain mass were observed because of germination in drinking water. It was determined that the use of mineral water significantly accelerated this process and reduced its time by 50–60% (4–5 hours) compared to traditional, drinking water germination. At the next stage, those grains were germinated that were swollen in both drinking and mineral water, for which an automated germinator was used. Germination was carried out at different temperatures in the range of 20–38ºC. The duration of germination was 48 hours. The high temperature regime was not used in order to preserve the natural properties of the main useful substances. Based on the conducted studies, the optimal regimes were established: for the swelling process in weak radonic mineral water, the duration was 3–6 hours at the temperature 22–40 ºC, and for the germination process the duration for different grains ranged from 8 to18 hours, at the temperature 28–33ºC (Berulava et al., 2024).

Methods

Determination of dry matter content. The amount of dry matter was determined using a RADWAG MA 50r. (Finland) thermometer, where drying was carried out under the influence of infrared rays (Berulava et al., 2024).

Determination of the amount of amino acids. Amino acids were studied by high-performance liquid chromatography HPLC-UV, RI; UPLC-PDA, MS method (Waters, UPLC Acquity, QDa Detectore). For the separation of compounds, a chromatographic column Acquity UPLC BEN C18, 1.7 m, solvent system: 0.3% formic acid (solvent A) and acetonitrile (solvent B) was used. Gradient-solvent B: 0 – 20 min, 5–16%; 20–28 min, 16–40%; 28–32 min, 40–47%; 32–36 min, 70–99%; 36–45 min, 99% and 45–46 min, 99–5%. Injection: 10 μL. Prior to chromatography, samples and eluents were filtered through 0.45 μm pore filters.

Determination of the amount of fats. The Soxhlet method was used to obtain fat. ISO 14156:2001 | IDF 172:2001 (Short Note No. 348/2019).

Determination of total phenols by the Folin-Ciocalteu method (in terms of gallic acid). The identification of phenolic compounds was carried out by ultra-high-performance (pressure) liquid chromatography UPLC-PDA, MS method (Waters, UPLC Acquity, QDa Detectore). For the separation of compounds, a chromatographic column Acquity UPLC BEN C18, 1.7 m, solvent system: 0.3% formic acid (solvent A) and acetonitrile (solvent B) were used. Gradient-solvent B: 0 – 20 min, 5–16%; 20–28 min, 16–40%; 28–32 min, 40–47%; 32–36 min, 70–99%; 36–45 min, 99% and 45–46 min, 99–5%. Injection: 10 μL. Prior to chromatography, samples and eluents were filtered through a 0.45 μm pore filter. The sample taken for analysis was extracted with 80%–ethyl alcohol. 1 ml of the total extract volume was placed in a 25–ml volumetric flask, then 5 ml of distilled water and 1 ml of Folin–Ciocalteu reagent were added, and the solution was kept for 8 minutes at room temperature, then 10 ml of 7% sodium carbonate solution was added and made up to volume with distilled water, mixed well, and kept in the dark at room temperature for 60 minutes to stabilize the reaction. The determination was performed at 750 nm using a 1 cm thick cuvette using 1 ml of 80%–ethyl alcohol as a control and the analysis was performed in the same sequence, recalculating the data obtained because of the determination on the calibration curve of gallic acid.

The total phenol content is calculated according to the formula:

$$X = (D \cdot K \cdot V \cdot F) \cdot 1000 / m, \quad (1)$$

where X is the total phenol content, mg/kg; D is an optical density; K is a gallic acid conversion factor; F is a dilution factor; V is the total extract volume, ml; m is the mass of raw material taken for extraction, g.

Quantitative determination of total flavonoids by spectral method using the $AlCl_3$ reagent. The sample taken for analysis was extracted with 80%-ethyl alcohol at a temperature of 70–75 °C. 1 ml of the total extract volume was placed in a 10-ml test tube, then 5 ml of H_2O was added, 0.3 ml of 5 % $NaNO_2$ was left for 5 minutes, then 0.3 ml of 10% $AlCl_3$ was added and left for 6 minutes, then 2 ml of 1N NaOH was added, and the determination was carried out at 510 nm. 1 ml of the corresponding extractant was taken as a control and undergone the same process. The data obtained as a result of the determination were recalculated on a rutin calibration curve. The total flavonoid content was calculated by the formula:

$$X = (D \cdot K \cdot V \cdot F) \cdot 1000 / m, \quad (2)$$

where D is an optical density; K is a rutin conversion factor; F is a dilution factor; V is the total extract volume, ml; m is the mass of raw material taken for extraction, g.

Determination of total antioxidant activity by the DPPH method. Antioxidant activity was determined (using the stable radical of 2,2-diphenyl-1-picryl hydrazyl) by the

DPPH method. For the determination of total antioxidant activity, reactions occurring by the radical mechanism between a specific, colored radical and an extract with antioxidant activity were used, where the change in the optical density of the solution was determined spectrophotometrically, and the total antioxidant activity of both a specific substance and compounds was evaluated. DPPH – ($C_{18}H_{12}N_5O_6$ M=394.33) is a stable free radical with a maximum absorption at 515 – 517 nm, the purple-violet coloration of the methanol extract changes to light yellow upon reduction. To determine antioxidant activity – radical bonding ability, 3 ml of DPPH reagent (0.1 mM DPPH – 0.004 g/100 ml of ethyl alcohol) was added to 1 ml of the analyzed extract, and 30 minutes later, the optical density of the test sample was determined spectrophotometrically at 515 nm. The reference solution was a DPPH reagent added to 96%-ethyl alcohol. Antioxidant activity was calculated by 50% inhibition of stable free radicals (DPPH) using the following formula:

$$In\ \% = AC - AS/AC \cdot 100, \quad (3)$$

where In % is the inhibition of 0.1 mM DPPH (within 40-60%); AC is the absorption of a 0.1 mM a DPPH alcoholic solution, and AS is the absorption of the test extract. The antioxidant activity of 1 mg of sample was calculated using the following formula:

$$C = m/V \cdot F \cdot 50/In, \quad (4)$$

where C is a sample's mg, which inhibits 0.1 mM DPPH by 50%; m is a mass of the sample taken in milligrams; V is a volume of the test extract (ml); F is the dilution factor; inhibition of In % – 0.1 mM DPPH (within 40-60%); 50 % is a calculated inhibition.

Statistical analysis

All measurements were conducted in triplicate. Values were presented as the mean ± standard deviation.

Results and discussion

Determination of dry matter content in the grains

In order to germinate the grain, at the initial stage of the research, the dry matter content of wheat, soybean, pea, and green lentil grains was determined in the original grain and at different stages of the swelling process – in the grains swollen in drinking water, in grains germinated after swelling in drinking water, in grains swollen in mineral water, and in grains germinated after swelling in mineral water. Figure 1 illustrates the results obtained.

As the diagrams illustrate, the amount of dry matter in the selected original grains is almost the same and varies within 90.22–91.67%. During the swelling process, the amount of dry matter in grains swollen in drinking water decreased as follows: for wheat by 31.14%; for soybeans by 49.27%; for peas by 43.01%; for green lentils by 52.01%; and in grains swollen in mineral water, the results were as follows: for wheat by 27.47%; for soybeans by 52.57%; for peas by 39.69%; for green lentils by 47.79%. During the germination, the dry matter content in the grains germinated after swelling in drinking water decreased as follows: for wheat by 43.79%; for soybean by 51.12%; for chickpea by 48.39%; for green lentils by 57.54%; as for the grains germinated after swelling in mineral water, the results were as follows: for wheat by 41.68%; for soybean by 53.43%; for pea by 53.8%; for green lentils by 56.81%. The best results were observed for soybeans and green lentils.

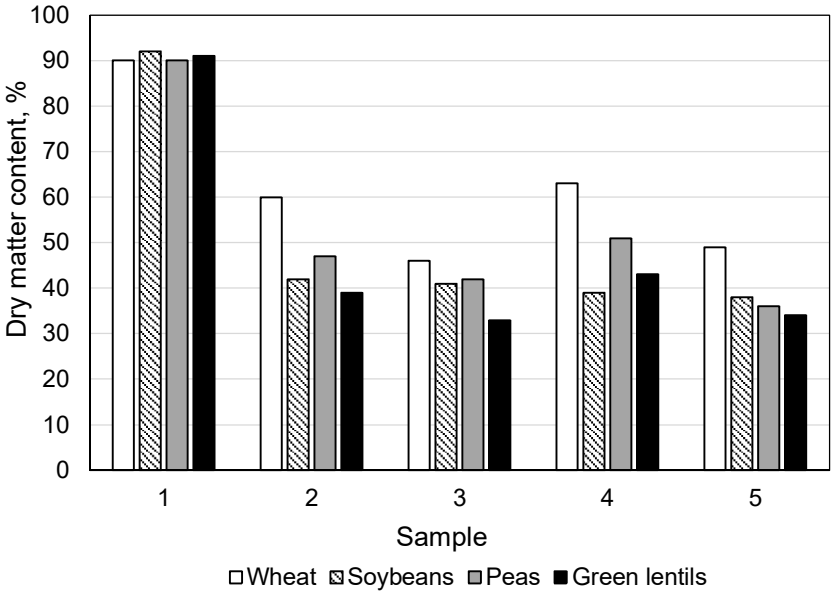

Figure 1. Dry matter content in the grains, %:
1 – Original grain;
2 – Swollen in drinking water;
3 – Germinated after swelling in drinking water;
4 – Swollen in mineral water;
5 – Germinated after swelling in mineral water.

Determination of the amino acid and fat content in the grains

The amino acid composition of proteins was determined in the test samples on a mg/g glycine basis, and the change in the total amount of fats (%) during the process of grain swelling and germination was also determined. The results are presented in Table 1 and 2.

Results from the analysis indicate that at all stages of the grain germination process, there is an increase in the number of amino acids compared to the original grain. The number of amino acids in wheat grains germinated after swelling in drinking water increased by 1.69 times, in soybean grains by 2.8 times, in pea grains by 4.26 times, and in green lentils by 1.75 times. In grains germinated after swelling in mineral water, the figures are as follows: in wheat by 2.8 times, in soybeans by 2.77 times, in peas by 5.06 times, in green lentils by 1.69 times. The results obtained indicate the positive effect of mineral water on the accumulation of amino acids. Particularly good effects were observed in soybean and pea samples. As for the change in fat, an increase was observed in all samples, especially in samples germinated after swelling in mineral water. The best results were achieved with peas (increased by 1.47 times) and lentils (increased by 1.23 times).

Table 1

Amino acid content in the grains

Grains	Amino acids, on a mg/g glycine basis
Wheat	
Original grain	0.75±0.01
Swollen in drinking water	1.18±0.01
Germinated after swelling in drinking water	1.27±0.02
Swollen in mineral water	0.96±0.03
Germinated after swelling in mineral water	2.11±0.02
Soybeans	
Original grain	6.66±0.04
Swollen in drinking water	10.75±0.02
Germinated after swelling in drinking water	18.66±0.03
Swollen in mineral water	12.42±0.02
Germinated after swelling in mineral water	18.48±0.01
Peas	
Original grain	3.64±0.01
Swollen in drinking water	14.92±0.03
Germinated after swelling in drinking water	15.52±0.01
Swollen in mineral water	17.76±0.04
Germinated after swelling in mineral water	18.41±0.01
Green lentils	
Original grain	12.87±0.02
Swollen in drinking water	14.00±0.01
Germinated after swelling in drinking water	22.52±0.01
Swollen in mineral water	16.73±0.03
Germinated after swelling in mineral water	21.82±0.02

Table 2

Fat content in the grains,%

Treatment	Cereals			
	Soybean	Wheat	Green lentils	Peas
Germinated after swelling in mineral water	18.03	2.26	2.30	2.03
Swollen in mineral water	18.66	2.08	2.90	1.61
Germinated after swelling in drinking water	17.11	2.27	2.17	1.87
Swollen in drinking water	15.03	2.02	1.48	1.56
Original grain	16.53	2.25	1.86	1.37

Changes of the total phenolic contents during the germination of the grains

The swelling and germination processes had a significant impact on the amount of total phenols in all grains (Table 3).

Table 3

Total phenolic content in the grains

Grains	TPC, mg/g*	PCA, mg/g**	TFC, mg/g***
Wheat			
Original grain	1.25±0.01	0.41±0.02	0.82±0.02
Swollen in drinking water	3.27±0.03	1.82±0.01	1.23±0.01
Germinated after swelling in drinking water	3.61±0.04	1.57±0.03	1.83±0.03
Swollen in mineral water	2.86±0.02	1.43±0.04	1.24±0.05
Germinated after swelling in mineral water	4.47±0.01	1.94±0.05	1.67±0.06
Soybeans			
Original grain	1.30±0.02	0.71±0.01	0.53±0.01
Swollen in drinking water	3.78±0.03	1.60±0.03	1.28±0.04
Germinated after swelling in drinking water	3.20±0.04	1.51±0.02	1.50±0.02
Swollen in mineral water	4.00±0.01	2.10±0.04	1.84±0.02
Germinated after swelling in mineral water	3.55±0.05	1.52±0.02	1.80±0.06
Peas			
Original grain	1.47±0.02	0.82±0.02	0.46±0.01
Swollen in drinking water	1.43±0.03	0.67±0.04	0.75±0.05
Germinated after swelling in drinking water	1.49±0.02	0.79±0.02	0.67±0.04
Swollen in mineral water	1.71±0.05	0.69±0.06	0.94±0.07
Germinated after swelling in mineral water	2.01±0.07	0.85±0.05	1.02± 0.06
Green lentils			
Original grain	1.19±0.02	0.70±0.04	0.31±0.06
Swollen in drinking water	2.73±0.04	0.98±0.03	1.64±0.03
Germinated after swelling in drinking water	2.89±0.01	1.49±0.01	1.20±0.01
Swollen in mineral water	3.15±0.03	1.67±0.02	1.33±0.02
Germinated after swelling in mineral water	3.08±0.01	1.61±0.02	1.29±0.04

Note: *TPC, total phenols, mg/g, in terms of chlorogenic acid; **PCA, phenol-carbonic acids, mg/g, in terms of gallic acid; TFC, total flavonoid content, mg/g, on a quercetin basis.

The total phenolic content of germinated grains generally increased compared to the original grain, except for pea grains, where a slight decrease was observed. The total phenolic content of grains germinated after swelling in weak radonic mineral water was significantly changed compared to that of grains germinated after swelling in drinking water. The best results were shown by wheat, soybean, and green lentil grains. Peas showed the lowest content. It is noteworthy that these indicators changed insignificantly within 10 hours after germination, decreasing in pea samples. The prolonged germination time significantly increased the total phenolic content. Kruma et al. (2016) and Kim et al. (2016) studied the germination process of wheat, oats, barley, and rye grains using drinking water. The content of total phenols during the germination process was studied. It was shown that an increase in total phenols was observed in oat samples and a decrease in rye samples. According to (Tiana et al., 2011), lentils have the highest amount of total phenols compared to soybeans, beans, and peas. Compared to the results of the research of these scientists, in our studies, soybeans are considered the best in terms of phenolic content, followed by green lentils and finally peas.

As for the content of flavonoids, their increase was observed in all the test samples, especially in the grains germinated after swelling in mineral water. Based on the comparative analysis, the best were soybean (1.80 mg/g) and green lentil (1.29 mg/g) grains, followed by peas (1.02 mg/g). A similar result was observed in the content of phenolic acids: green lentils, 1.61 mg/g; soybeans, 1.52 mg/g; peas, 0.85 mg/g.

Antioxidant activity of the grains

It is known that antioxidant activity is closely related to the content of phenols (Khang et al., 2016; Xu et al., 2009). All the studied samples showed a significant amount of total phenols and more or less effective antioxidant activity. Antioxidant activity was assessed by the DPPH method, and the results are illustrated in Figure 2.

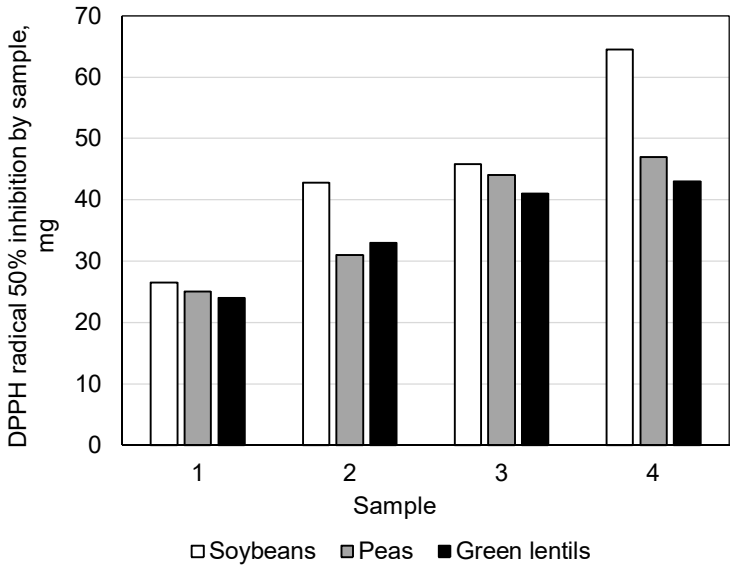

Figure 2. Antioxidant activity of the grains:
1 – Original grain;
2 – Swollen in drinking water;
3 – Germinated after swelling in drinking water;
4 – Swollen in mineral water

It was determined that the antioxidant activity of the studied samples increased when swelling using mineral water (soybean 45.8%, peas 43.6%, green lentils 40.87%). It was also shown, according to the samples, that the grains germinated after swelling in mineral water are distinguished by high antioxidant activity (soybean 64.51%, peas 46.63%, green lentils 42.5%) compared to the grains germinated after swelling in drinking water. The high antioxidant activity of swollen and germinated grains and legumes has been confirmed by studies by other scientists research works (Khang et al., 2016; Şenlik et al., 2023).

Our studies have confirmed the high efficiency of weak radonic mineral water used at the swelling stage in the germination process of grains and legumes, a significant optimization of the technological process of germination, which contributed to reducing the

time of the overall germination process of various grains taken for analysis by 16-24 hours instead of 36-48 hours compared to the traditional method. It should also be noted that the optimization process contributed to achieving better results in the accumulation of biologically active substances in the conditions of a short germination duration compared to the traditional process.

Conclusions

1. The dry matter content of wheat, soybean, pea and green lentil grains was determined in the original grain and at different stages of the germination process – in grains swollen in drinking water, in grains germinated after swelling in drinking water, swollen in weak radonic mineral water, and in grains germinated after swelling in mineral water. It was shown that the amount of dry matter decreases at different stages of the germination process. The best results were obtained for soybean and green lentil grains.
2. The amino acid composition of proteins was determined in the test samples on a mg/g glycine basis. It was established that at all stages of the grain germination process, there is an increase in the amount of amino acids compared to the original grain. After swelling in drinking water, the amount of amino acids in wheat grains germinated increased by 1.69 times, in soybean grains by 2.8 times, in pea grains – by 4.26 times, and in green lentils by 1.75 times. In grains germinated after swelling in mineral water, the figures are as follows: in wheat by 2.8 times, in soybeans by 2.77 times, in peas by 5.06 times, and in green lentils by 1.69 times. The results obtained indicate the positive effect of mineral water on the accumulation of amino acids. Particularly good effects were observed in soybean and pea samples.
3. The change in the total amount of fats (%) during the process of swelling and germination of grains was determined. An increase in the amount of fat was observed in all samples, especially in samples germinated after swelling in mineral water. The best results were observed in peas (increased by 1.47 times) and lentils (increased by 1.23 times).
4. Based on the study of phenolic compounds, it was determined that the amount of total phenols in germinated grains increased compared to the original grain, except for pea grains, where a slight decrease was observed. The amount of total phenols changed significantly in grains germinated after swelling in weak radonic mineral water, compared to grains germinated after swelling in drinking water. The best results were shown by wheat, soybean and green lentil grains. As for the content of flavonoids, their increase was found in all the test samples, especially in the grains germinated after swelling in mineral water. Based on the comparative analysis, soybean (1.80 mg/g) and green lentil (1.29 mg/g) grains were considered the best, followed by peas (1.02 mg/g). A similar result was observed in the content of phenolic acids: green lentils, 1.61 mg/g, soybeans, 1.52 mg/g; peas, 0.85 mg/g.
5. It was established that the antioxidant activity of the test samples increases when swelling in mineral water (soybeans 45.8%, peas 43.6%, green lentils 40.87%). Also, according to the samples, it was shown that grains germinated after swelling in mineral water are distinguished by high antioxidant activity (soybean 64.51%, peas 46.63%, green lentils 42.5%), compared to grains germinated after swelling in drinking water.
6. Based on the analysis of the above results, the use of weak radonic mineral water was considered a priority in terms of optimizing the processes of grain swelling and germination and the accumulation of biologically active substances in these processes.

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References

Ali A., Elozeiri A. (2017), Metabolic processes during seed germination. In: J. C. Jimenez-Lopez (Ed.), *Advances in Seed Biology,* pp. 141-166, IntechOpen, https://doi.org/10.5772/intechopen.70653

Alvarez-Jubete L., Wijngaard H., Arendt E., Gallagher E. (2010), Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking, *Food Chemistry*, 119(2), pp. 770-778, https://doi.org/10.1016/j.foodchem.2009.07.032

Atudorei D., Stroe S., Codină G. (2020), Physical, physiological and minerals changes of different legumes types during the germination process, *Ukrainian Food Journal,* 9(40), pp. 844-863, https://doi.org/10.24263/2304-974X-2020-9-4-10

Atudorei D., Stroe S., Codina G. (2021), Impact of germination on the microstructural and physicochemical properties of different legume types, *Plants*, 10(3), pp. 1-19, https://doi.org/10.3390/plants10030592

Berulava I., Pkhakadze G., Silagadze M., Khetsuriani G., Gachechiladze S., Burjaliani N. (2024), The influence of various factors of the germination process of grains and legume crops, *European Science Review*, 11–12, pp. 3-13, https://doi.org/10.29013/ESR-24-11.12-3-13

Berulava I., Silagadze M., Kovbasa V., Pkhakadze G., Khetsuriani G., Rukhadze M. (2024), Fermented drink based on secondary raw milk materials, *Ukrainian Food Journal*, 13(4), pp. 708-722, https://doi.org/10.24263/2304-974X-2024-13-4-6

Carrera-Castaño G., Calleja-Cabrera J., Pernas M., Gómez L., Oñate-Sánchez L. (2020), An updated overview on the regulation of seed germination, *Plants*, 9, pp. 1-41, https://doi.org/10.3390/plants9060703

Chinma C., Adedeji O., Etim I., Aniaka G., Mathew E., Ekeh U., Anumba N. (2020), Physicochemical, nutritional, and sensory properties of chips produced from germinated African yam bean (*Sphenostylis stenocarpa*), *LWT – Food Science and Technology*, 136(1), 110330, https://doi.org/10.1016/j.lwt.2020.110330

Donkor O., Stojanovska L., Ginn P., Ashton J., Vasiljevic T. (2012), Germinated grains – Sources of bioactive compounds, *Food Chemistry*, 135(3), pp. 950-959, https://doi.org/10.1016/j.foodchem.2012.05.058

Dufková H. (2019), Seed germination promoting chemical compounds and their potential use in the malting industry, *Kvasny prumysl,* 66, pp. 201–207, https://doi.org/10.18832/kp2020.66.201

Eker M., Karakaza S**.** (2020), Influence of the addition of chia seeds and germinated seeds and sprouts on the nutritional and beneficial properties of yogurt, *International Journal of Gastronomy and Food Science,* 22, 100276, https://doi.org/10.1016/j.ijgfs.2020.100276

Ghavidel R., Prakash J. (2007), The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds, *LWT – Food Science and Technology*, 40, pp. 1292–1299, https://doi.org/10.1016/j.lwt.2006.08.002

Guimarães B., Polachini C.T., Augusto P.E.D. (2020), Ultrasound-assisted hydration of wheat grains at different temperatures and power applied: Effect on acoustic field, water

absorption and germination, *Chemical Engineering and Processing – Process Intensification*, 155, 108045, https://doi.org/10.1016/j.cep.2020.108045

Jeong H.S., Lee Y.R., Lee S.H., Jang G.Y., Lee Y.J., Kim M.Y., Kim Y.B., Lee J. (2019), Antioxidative and antidiabetic effects of germinated rough rice extract in 3T3-L1 adipocytes and C57BLKS/J-db/db mice, *Food & Nutrition Research*, 63(11), 3603, https://doi.org/10.29219/fnr.v63.3603

Khang D.T., Dung T.N., Elzaawely A.A., Xuan T.D. (2016), Phenolic profiles and antioxidant activity of germinated legumes, *Foods*, 5(2), 27, https://doi.org/10.3390/foods5020027

Khatun L., Brahma R., Ray S. (2023), Nutritional and functional potentials of germinated rice: A systematic review, *Food Science and Applied Biotechnology,* 6(2), pp. 263-281, https://doi.org/10.30721/fsab2023.v6.i2.286

Kim M.Y., Jang G.Y., Lee Y., Li M., Ji Y.M., Yoon N., Lee S. H., Kim K.M., Lee J., Jeong H.S. (2016), Free and bound form bioactive compound profiles in germinated black soybean (*Glycine max* L.), *Food Science and Biotechnology,* 25(6), pp. 1551–1559, https://doi.org/10.1007/s10068-016-0240-2

Kruma Z., Tomsone L., Ķince T., Galoburda R., Senhofa S., Sabovics M., Straumite E., Sturite I. (2016), Effects of germination on total phenolic compounds and radical scavenging activity in hull-less spring cereals and triticale, *Agronomy Research,* 14(S2), pp. 1372-1383.

Kurmanbayeva I., Nabiyeva Z., Zheldybayeva A. (2023), Enzyme preparations and their role in grain processing, *The Journal of Almaty Technological University,* 4, pp. 70-76, https://doi.org/10.48184/2304-568X-2023-4-70-76

Landry K., Sela D., Mclandsborough L. (2017), Influence of sprouting environment on the microbiota of sprouts, *Journal of Food Safety,* 38(1), e12380, https://doi.org/10.1111/jfs.12380

Luo X., Li D., Tao Y., Wang P., Yang R., Han Y. (2022), Effect of static magnetic field treatment on the germination of brown rice: changes in α-amylase activity and structural and functional properties in starch, *Food Chemistry*, 383(7), 132392, https://doi.org/10.1016/j.foodchem.2022.132392

Nkhata S.G., Ayua E., Kamau E.H., Shingiro J.B. (2018), Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes, *Food Science and Nutrition*, 6(8), pp. 2446-2458, https://doi.org/10.1002/fsn3.846

Oghbaei M., Prakash J. (2020), Effect of dehulling and cooking on nutritional quality of chickpea (*Cicer arietinum* L.) germinated in mineral fortified soak water, *Journal of Food Composition and Analysis,* 94, 103619, https://doi.org/10.1016/j.jfca.2020.103635

Onopriichuk O., Grek O., Tymchuk A. (2022). Influence of malt properties on the indicators of milk-protein concentrates. In: O. Paredes-López, O. Shevchenko, V. Stabnikov, V. Ivanov, (Eds.), *Bioenhancement and Fortification of Foods for a Healthy Diet,* pp. 179-202, CRC Press, Boca Raton, https://doi.org/10.1201/9781003225287-12

Onopriichuk O., Skuibida V. (2024), Improvement of fermented dairy-plant concentrate technology, *Ukrainian Journal of Food Science*, 12(2), pp. 117–130, https://doi.org/10.24263/2310-1008-2024-12-2-4

Owolabi I., Datarun P., Yupanqui C., Wichienchot S. (2020), Gut microbiota metabolism of functional carbohydrates and phenolic compounds from soaked and germinated purple rice, *Journal of Functional Foods*, 66(3), 103787, https://doi.org/10.1016/j.jff.2020.103787

Perri G., Calabrese F., Giuseppe R., Angelis M., Gobbetti M., Calasso M. (2020), Sprouting process affects the lactic acid bacteria and yeasts of cereal, pseudocereal and legume flours, *LWT*, 126, 109314, https://doi.org/10.1016/j.lwt.2020.109314

Pjak P., Socha R., Broniek J., Królikowska K., Fortuna T. (2019), Antioxidant properties, phenolic and mineral composition of germinated chia, golden flax, evening primrose, phacelia and fenugreek, *Food Chemistry,* 275, pp. 69–76, https://doi.org/10.1016/j.foodchem.2018.09.081

Rahman S., Mohammed S., Dubey P., Kumar S. (2023), A comprehensive review on the effect of germination on the physiochemical properties of wheat, millet, and legumes, *Journal of Food Chemistry & Nanotechnology*, 9(S1), pp. 323-334, https://doi.org/10.17756/jfcn.2023-s1-042

Rodriguez M., Barrero J., Corbineau F., Gubler F., Benech-Arnold R. (2015), Dormancy in cereals (not too much, not so little): About the mechanisms behind this trait, *Seed Science Research*, 25(2), pp. 99-119, https://doi.org/10.1017/S0960258515000021

Şenlik A., Alkan D. (2023), Improving the nutritional quality of cereals and legumes by germination, *Czech Journal of Food Sciences* 41(5), pp. 348-357, https://doi.org/10.17221/44/2023-CJFS

Stabnikova O., Antonuk M., Stabnikov V., Arsen'eva L. (2019), Ukrainian dietary bread with selenium-enriched malt, *Plant Foods for Human Nutrition*, 74(2), pp. 157–163, https://doi.org/10.1007/s11130-019-00731-z

Stabnikova O., Stabnikov V., Antoniuk M., Arsenieva L., Ivanov V. (2022), Bakery products enriched with organoselenium compounds, In: O. Paredes-Lopez, V. Stabnikov, O. Shevchenko, V. Ivanov (Eds.), *Bioenhancement and Fortification of Foods for a Healthy Diet*, pp. 89-111, CRC Press, Boca Raton, https://doi.org/10.1201/9781003225287

Tijana M., Slavica S., Suzana I. (2011), Antioxidant activity and total phenolic content in some cereals and legumes, *International Journal of Food Properties*, 14, pp.175–18, https://doi.org/10.1080/10942910903160364

Xia Q., Wang L., Xu C., Mei J., Li Y. (2017), Effects of germination and high hydrostatic pressure processing on mineral elements, amino acids and antioxidants in vitro bioaccessibility, as well as starch digestibility in brown rice (*Oryza sativa* L.), *Food Chemistry*, 214, pp. 533-542, https://doi.org/10.1016/j.foodchem.2016.07.114

Xu J., Tian C., Hu, Q., Luo J., Wang X., Tian X. (2009), Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination, *Journal of Agricultural and Food Chemistry*, 57(21), pp. 10392–10398, https://doi.org/10.1021/jf902778j

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Determination of ejection coefficient of liquid-gas ejector based on theory of attached mass

Vitalii Ponomarenko, Roman Yakobchuk, Serhii Yastreba

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Roman Yakobchuk
E-mail: yakroman@ukr.net

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Abstract

Introduction. The aim of the research was to develop a theory of ejection processes of gas-liquid ejectors with a dispersed liquid jet, based on the application of the principles of the theory of attached mass.

Materials and methods. Analytical methods for determining the gas phase flow rate were used. To calculate the main characteristics of water-gas ejectors with a dispersed liquid jet, it was proposed to use the modified Butakov-Hemeon theory.

Results and discussion. The basic ejection equation with an energy transfer coefficient not equal to unity is obtained according to the attached mass theory.

The change in the supply pressure of the working medium, which constantly occurs in production conditions, leads to a change in the speed regime of the liquid flow. Accordingly, changes in hydraulic resistances and redistribution of energy losses occur. The energy transfer coefficient for this reason is also variable and the calculation of its numerical value is recommended to be carried out for the nominal operating mode of the existing pumping equipment.

The design of the receiving chamber affects the amount of losses, since a mixed flow is formed in it. The method for determining energy losses in ejectors consists of finding the numerical values of component losses considering different designs and fluid flow rates. The obtained value of the energy transfer coefficient for an ejector with a cylindrical mixing chamber and a dispersed liquid jet is 1.075, which corresponds to the numerical value of the ejection coefficient of 3.918 (the actual ejection coefficient is 3.47). For an ejector with a combined mixing chamber, the energy transfer coefficient is 1.727, and the ejection coefficient is 6.72 (the actual ejection coefficient is 4.71).

Conclusions. The calculation of flow characteristics considers both the flow rate regime in the ejector elements and the design features of the mixing chamber. The accuracy of the determination the ejection coefficient is 12–14%.

Introduction

Ejection devices combine high intensity of the process flow with reliability in operation, which is explained by the simplicity of the design and the absence of moving elements (He et al., 2020). They apply to carry out various technological processes in the food industry, in particular, sulfitation of liquids in the production of sugar (Ponomarenko et al., 2017). In addition to the food industry, they are indispensable in gas production, in the aerospace industry as the main element of engines. The range of applications of liquid-gas ejectors is expanding every year, designs are being improved, theoretical, simulation, and experimental research of their operation is being conducted.

However, with a sufficiently simple design of ejectors, it is impossible to theoretically considered the processes and phenomena occurring in the ejector mixing chamber (resistance to the entry of the active flow into the mixing chamber during sudden expansion of the channel, degassing of the liquid in the zone of reduced pressure, vortex cavitation, which contributes to the dispersion of liquid droplets, the formation of vortex zones with local pulsations of velocity and pressure, the phenomenon of reverse circulation flows, evaporation of water into the gas phase to a saturation state). In addition, the length of the zone of formation of the dispersed composition of the spray jet droplets is sometimes proportional to the length of the mixing chamber. The transfer of energy from the liquid to the gas is accompanied by friction, and therefore irreversible losses. This is only a part of the phenomena that cannot be considered in the theoretical description of the ejector hydrodynamics.

Some of these phenomena significantly affect the ejection processes, the influence of other phenomena within the error range. When the ejection conditions change, the influence of the parameters changes due to the change in the speed regime.

Theoretical works describing ejection processes are based on a number of classical physical laws: the law of conservation of mass, the law of conservation of energy, the law of conservation of momentum (Ponomarenko et al., 2022; Zhang et al., 2019). This approach is traditional, however, this theory does not consider the state of the active flow of the ejector: whether a dispersed or compact liquid jet is used as the active flow. However, depending on this, the ejection coefficients may differ by several times. It is known that the ejection coefficients of devices with a compact liquid jet are within 0.5–1.5 (Zhao et al., 2022), and for ejectors with a dispersed jet they reach values of 1–5 (Ponomarenko et al., 2017). The classical theory of the description of ejection processes cannot explain this difference (the results are corrected by introducing experimental constants). In addition, the mathematical description of some processes, for example, the process of liquid jet disintegration (Villermaux et al., 2004) and droplet formation (Tang et al., 2023), which is important in describing ejection processes, is at the stage of formation.

In connection with the above, performing a mathematical description of ejection processes and theoretically obtaining their operating characteristics considering the noted phenomena in the ejector elements and in its mixing chamber is a rather complex, relevant and unresolved problem to date.

One of the approaches to describing ejection processes is based on the principle of the theory of the attached mass effect, which considers the particle dispersion and was first used to describe the ejection of a gas phase by a flow of bulk material (the Butakov-Hemeon theory). This approach was proposed to be used for the theoretical description of the ejection processes of a water-gas ejector (Ponomarenko et al., 2022). Under the assumptions made (liquid droplets are of equal size, quasi-stationary, and the resistance to the droplets is directly proportional to the velocity) and with an energy transfer coefficient equal to unity, equations

(Ponomarenko et al., 2022) were obtained that describe ejection processes similar to the equations of the Butakov-Hemeon theory.

Analysis of this theory, at first, analysis of the magnitudes of energy losses in the ejector elements showed that the energy transfer coefficient is not equal to unity. The development of the theory is to find an expression for the main equation of the ejector considering losses, which is considered by the energy transfer coefficient. According to this method, determining its numerical value is the main difficulty in calculating the ejection coefficient, since it depends on the speed regime of the liquid phase flow and the design of the ejectors, that is, it is variable. In addition, it requires clarification of the definition of the quantities describing the flow of a mixed flow, based on the basic provisions of the theory of homogeneous flow.

Materials and methods

Objects

The ambiguity of the results of studies of water-gas jet devices with a dispersed liquid jet at low pressures of the active medium prompted the conduct of full-scale experiments to establish the laws of hydrodynamics of a two-phase flow.

The object of the study was ejectors with a cylindrical and conical-cylindrical mixing chamber nozzles. The design of an ejector with a receiving chamber and a cylindrical mixing chamber is presented in Figure 1.

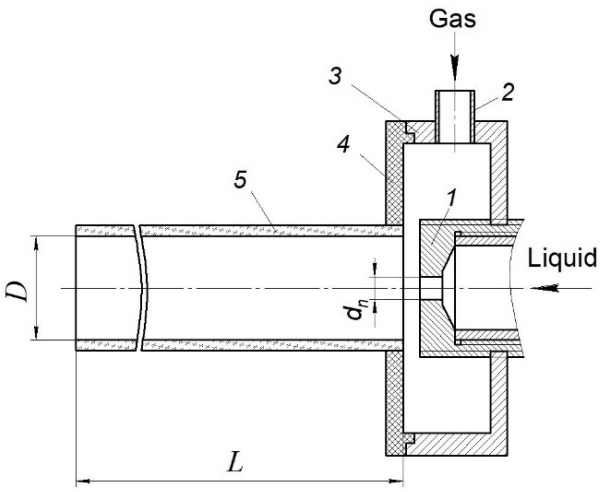

Figure 1. Design of ejector with receiving chamber and cylindrical mixing chamber: 1 – nozzle; 2 – inlet pipe; 3 – housing; 4 – cover; 5 – cylindrical mixing chamber

Main dimensions: diameter of the nozzle of the centrifugal jet nozzle with inclined channels 4 mm, diameter of the ejector mixing chamber 19 mm, which corresponds to the geometric characteristic of the ejector (ratio of the area of the mixing chamber to the area of the nozzle) m = 22.56. Length of the mixing chamber – 0.2 m, values of physical properties of the phases are taken at a temperature of 20 °C.

A study was also conducted on an ejector with a conical-cylindrical (combined) mixing chamber (Figure 2), which has an ejection coefficient (ratio of active flow to passive flow) 15–20% higher than that of a classical ejector with a cylindrical mixing chamber of the same geometric characteristics (Ponomarenko et al., 2023). The angle of the conical part of the ejector is 25°, the length of the conical part is 0.014 m, the length of the cylindrical mixing chamber is L = 0.2 m, the diameter of the base of the truncated cone is 225 mm, and the diameter of the cut is 50 mm.

The studies were conducted in the water-air system at a phase temperature of 20°C and a relative humidity of 85% in order to minimize the influence of heat exchange processes on ejection processes. The circulation of the liquid in a closed circuit (measuring capacity–ejector–measuring capacity) ensures the constancy of its physical properties for all experiments. The physical properties of the liquid depending on the temperature were determined on the basis of tables of the thermophysical state of water and water vapor.

For hydraulic tests of ejectors, a pouring instalation was made, shown in Figure 3. The stand is equipped with a volumetric meter PREMA G 1.6 for measuring gas flow. The liquid flow was measured with a rotary flow meter of the KV-1.5 type, accuracy class 1.5. The liquid pressure in the nozzle was controlled with a manometer OBM1-160, accuracy class 1.5. The rarefaction in the mixing chamber was recorded with a differential manometer in mm of water.

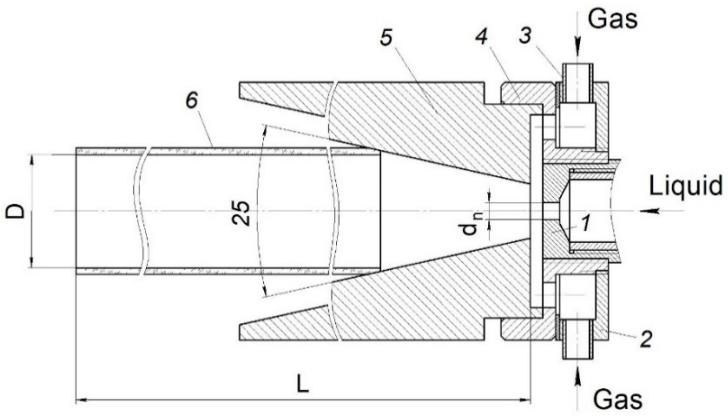

Figure 2. Water-gas ejector with a combined mixing chamber:
1 – working nozzle; 2, 4 – receiving chamber assembly; 3 – branch pipe;
5 – conical adapter; 6 – cylindrical mixing chamber

The studies were conducted according to the plan of a randomized two-factor experiment in the pressure range of 0.08–0.25 MPa. Changing the ejector operating modes was carried out by adjusting the pressure of the liquid supplied to the working nozzle of the ejector using regulating valves and controlled by a pressure gauge. At least three experiments were conducted. Each study lasted two minutes. The readings of the liquid and gas flow meters at the beginning and end of the experiments, as well as the liquid pressure, were recorded.

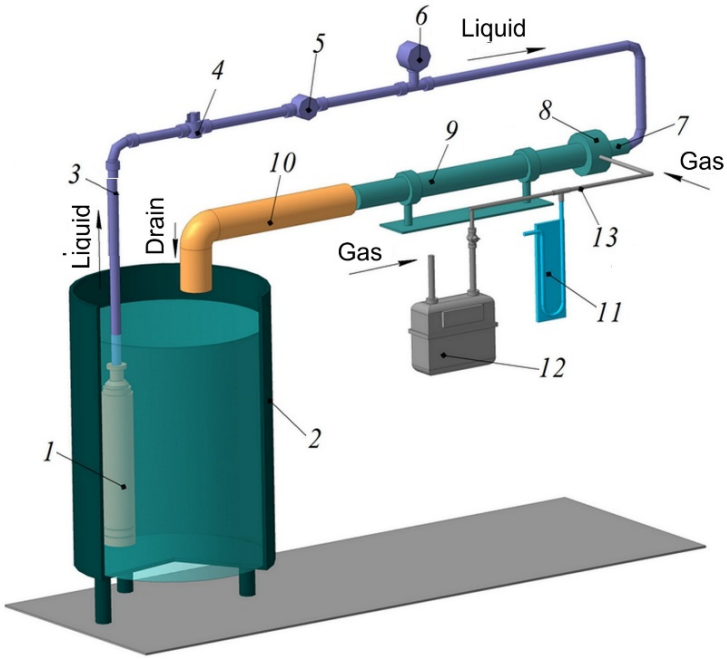

Figure 3. Hydraulic pouring instalation:
1 – pump; 2 – measuring tank; 3 – pipeline; 4 – regulating valve;
5 – liquid flow meter; 6 – pressure gauge; 7 – nozzle; 8 – receiving chamber;
9 – mixing chamber; 10 – drain pipeline; 11 – differential pressure gauge;
12 – gas flow meter; 13 – gas pipeline

Analytical methods for researching ejection processes

To describe the ejection processes in a jet apparatus with a dispersed liquid jet, the Butakov-Hemeon theory (for the flow of bulk materials) was used, modified to describe the ejection processes of liquid-gas ejectors (Ponomarenko et al., 2022). According to the model, part of the kinetic energy of liquid droplets is lost to overcome the resistance of the medium. The magnitude of the losses is determined by the force of the aerodynamic resistance of these particles. An expression for the basic ejection equation with an energy transfer coefficient equal to unity was found.

Results and discussion

Basic equation of ejection with energy transfer coefficient not equal to unity

The main thesis of the Butakov-Hemeon theory (Ponomarenko et al., 2022) to describe the process of air ejection by loose material was the proposition that the lost kinetic energy of the movement of droplets per unit time is converted into the kinetic energy of the gas phase flow, which moves together with the droplets (attached mass effect):

$$dE_g = Q_g dp \quad (1)$$

where Q_g – the volumetric air flow rate (attached mass) m^3/s, dp – the driving force of the air movement (pressure difference along the length of the mixing chamber), Pa.

For n liquid droplets, the lost energy can be found by the expression:

$$dE_g = nR_0 dx \quad (2)$$

where R_0 – the aerodynamic drag force, dx – the coordinate of the path traveled by the particle.

Or:

$$nR_0 dx = Q_g dp \quad (3)$$

Integration of the last expression within the length of the mixing chamber considering the expression for pressure losses in the ejector mixing chamber

$$\Delta p = \sum \varsigma \frac{v^2}{2} \rho \quad (4)$$

where ς – the total resistance coefficient (sum of local resistance and along the length), v – the velocity of the mixture, ρ – the density of the mixture in the mixing chamber and by introducing the energy transfer coefficient ξ_e we obtain the expression:

$$Q_z \sum \varsigma \frac{v_g^{\ 2}}{2} \rho_g = \xi_e \int_0^l nR_0 dx \quad (5)$$

The solution of this equation was obtained (Ponomarenko et al., 2022) for the simplest case, when the energy transfer coefficient $\xi_e = 1$ and they are similar to the equations of the Butakov-Hemeon theory for solid particles, however, the determination of the quantities used in determining the state of the gas-liquid phase has its own characteristics. This allowed us to conclude that the method is universal and that it can be used in calculating the flow characteristics of water-gas ejectors with a dispersed liquid jet. This was sufficient at the stage of auditing the theory to describe the ejection processes.

Let us turn to the correctness of the assumption of the equality of the energy transfer coefficient to unity. As has already been partially noted, quite complex physical phenomena occur in the mixing chamber, the main ones being:

- the movement of liquid droplets in a cylindrical mixing chamber under the action of gravity leads to a change in their trajectory (*Sedano* et al., 2020), and when they hit a solid wall, liquid droplets either form a film that moves along the walls, or are partially reflected from it and move along a new trajectory *(*Alizadeh Kaklar et al., 2018*)*. When the liquid film moves along the walls of the mixing chamber, a tangential stress or friction force arises, which is accompanied by energy losses *(Li* et al., 2023*)*. In both cases, a part of the energy is lost;
- the flight of droplets in a gas environment occurs with resistance (Dadash-Zade et al., 2024; Kong et al., 2021), which leads to a decrease in kinetic energy, and when droplets are formed near the injector nozzles, when the gas phase has almost zero velocity, the resistance to the movement of droplets is the highest. At the outlet of the mixing chamber, the velocities of the liquid and gas are equalized, the resistance decreases (Kong et al., 2020);
- the formation of a drop at the outlet of the nozzle is accompanied by a change in its shape and size *(*Chen et al., 2025; Liu et al., 2023*)*, i.e. transient processes occur that occupy almost a third of the time the liquid droplets spend in the ejector mixing chamber, which is accompanied by a redistribution of energy, its partial loss;

– due to the imbalance of phases and the appearance of rarefaction zones in the ejector receiving chamber (Rosenberger et al., 2018), liquid degassing processes occur, which affect the formation and formation of droplets. At the same time (Yan et al., 2023), the process of saturation of the gas phase with water vapor occurs, which reduces the energy of the flow of the liquid-gas mixture. All processes are intensified by cavitation phenomena (Zhang et al., 2022), which occur when a liquid jet leaks from the nozzle of the injector (Zhou et al., 2024; Salim et al., 2023). These processes are the most complex and practically cannot be explained mathematically.

As follows from this analysis, as a result of energy exchanges between phases, a part of the energy is lost to the accompanying processes, and the equality of the energy transfer coefficient to unity can be said only in the context of the motion of an ideal fluid in a vacuum without friction.

Let us find the general expression of the main ejection equation for an energy transfer coefficient not equal to unity.

Let us assume that the aerodynamic drag force of liquid droplets R_0 is proportional to the cross-sectional area of the droplet d and the square of the relative velocity (Dadash-Zade et al., 2024):

$$R_0 = f_0 \frac{\pi d^2}{4} \frac{(v_l - v_g)^2}{2} \rho_g , \tag{6}$$

where f_0 – the drag coefficient of the droplet, v_l – the velocity of the liquid flowing out of the nozzle, v_g – the velocity of the gas entrained by the liquid droplets.

We transform expression (5) by multiplying it by $\frac{F}{F}$ and denote R_g (resistance to the gas phase per unit area of the mixing chamber) by:

$$R_g = \sum \varsigma \frac{\rho}{2} \frac{1}{F} . \tag{7}$$

Integrating expression (5) within the length of the mixing chamber and considering relations (6) and (7), we obtain the expression:

$$(Q_g R_g F - \zeta_e n f_0 \rho_g l \frac{\pi d^2}{8}) v_g^2 - \zeta_e n f_0 \rho_g l \frac{\pi d^2}{8} v_l^2 + 2\zeta_e n f_0 \rho_g l \frac{\pi d^2}{8} v_l v_g = 0 \tag{8}$$

After a series of simple transformations, the basic equation of ejection with an energy transfer coefficient not equal to unity takes the form:

$$Q_g^3 - Q_g^2 \zeta_e (n f_0 \rho_g l \frac{\pi d^2}{8} \frac{1}{F}) \frac{1}{R_g} + Q_g \zeta_e (2 n f_0 \rho_g l \frac{\pi d^2}{8} v_l) \frac{1}{R_g} - \zeta_e n f_0 \rho_g l \frac{\pi d^2}{8} v_l^2 \frac{F}{R_g} = 0 \tag{9}$$

Let us introduce the notation:

$$a = -n f_0 \rho_g l \frac{\pi d^2}{8} \frac{1}{F} \frac{1}{R_g} ; \tag{10}$$

$$b = 2 n f_0 \rho_g l \frac{\pi d^2}{8} v_l \frac{1}{R_g} ; \tag{11}$$

$$c = -n f_0 \rho_g l \frac{\pi d^2}{8} v_l^2 \frac{F}{R_g} ; \tag{12}$$

$$A = n f_0 \rho_g l \frac{\pi d^2}{8} \frac{1}{R_g} . \tag{13}$$

With the introduced assumptions and accepted notations, the basic ejection equation (9) is reduced to the form:

$$Q_g^3 + Q_g^2 \zeta_e a + Q_g \zeta_e b + \zeta_e c = 0 . \quad (14)$$

The solution of this equation allows us to obtain the amount of gas phase ejected by the liquid in the ejector mixing chamber, considering energy losses.

It should be noted that when the pressure of the liquid in the nozzle changes, and such changes during the sulfation of the liquid in production occur constantly, almost all the main calculated values change, which leads to:

- the liquid velocity in the nozzle changes, which leads to a change in one of the main calculated parameters–the droplet size (Tsai et al., 2019);
- a change in the liquid velocity when the pressure changes leads to a change in the Reynolds criterion *Re*, which is equivalent to a change in the droplet resistance in the mixing chamber;
- a change in the mixture velocity in the cylindrical part of the mixing chamber is similar to a change in pressure losses along its length (Joshi et al., 2019).

Such preliminary analysis leads to the opinion that the first estimated calculation of the ejection coefficients and the energy transfer coefficient is advisable to be carried out for a fixed value of the liquid pressure in the injector nozzle considering the nominal mode of the installed pumping equipment.

If the calculated value of the ejection coefficient for a given ejector is satisfactory, calculations can be performed for other values of the liquid pressure in the nozzle (in general, it is advisable to perform such calculations for the limit values of the liquid pressure, which will allow obtaining the range of operating ejection coefficients). The final stage is the construction of the operating characteristics of the ejector for the entire operating pressure range.

Ejection coefficient for an ejector with a cylindrical mixing chamber

We present the data for calculating the energy transfer coefficient and gas phase flow rate for an ejector with a cylindrical mixing chamber (Figure 1). The calculation is performed at a liquid pressure in the nozzle opening of 0.2 MPa, which corresponds to a liquid flow rate through the nozzle of 1.835×10^{-4} m^3/s and is the nominal tick of the pumping equipment during liquid sulfation.

An important parameter for the calculation is the droplet diameter, which we will take as d_{32} = 724 μm. This diameter was obtained experimentally when studying a centrifugal jet nozzle with a nozzle-opening diameter of 4 mm (Fang et al., 2024).

The fluid loss coefficient in the mixing chamber was determined at the value of *Re*:

$$\mathrm{Re} = \frac{v_l D \rho_l}{\mu_l} = 2.747\times10^5 ,$$

where v_l – the velocity of the liquid along the wall of the mixing chamber, taken equal to the velocity of the liquid in the nozzle opening, ρ_l, μ_l – are the density and dynamic viscosity of the liquid, respectively, D – the diameter of the mixing chamber.

With this value of *Re*, we apply the Altshull formula, which is recommended for determining the loss coefficient in the transition region (tabular value of the coefficient $k = 7\times10^5$):

$$\lambda = 0.11\left(\frac{k}{D} + \frac{68}{\mathrm{Re}}\right)^{0.25} = 0.028$$

From where the pressure losses of the liquid due to its friction on the wall of the mixing chamber will be equal to:

$$\Delta P_1 = \varsigma \rho_l \frac{v_l^2}{2} = 3.09 \times 10^4 \text{, Pa.}$$

The second component of the losses is the pressure loss due to the movement of liquid droplets in the gas that have flown out of the nozzle. For simplicity, let us assume the droplet drag coefficient (Dafsari et al., 2021) to be constant and equal to 0.38, then the pressure loss will be:

$$\Delta P_2 = \varsigma_d \rho_l \frac{v_l^2}{2} = 4.05 \times 10^4$$

In addition to these losses, according to the theory of homogeneous flow, it is possible to calculate energy losses that were not considered when determining the ejected gas flow rate. These are losses from the action of gravity, which changes the trajectory of the drops:

$$\Delta P_g = \rho g = 2.667 \times 10^3$$

where ρ – the density of the mixture, and from the acceleration of the gas flow due to energy transfer from the liquid phase:

$$\Delta P_a = \frac{Q_g \rho_g}{F} a_v = 3.117 \times 10^3 .$$

Calculating energy losses allows you to find the numerical value of the energy transfer coefficient, as the ratio:

$$\psi = 1 + \frac{\Delta P_g + \Delta P_a}{\Delta P + \Delta P_g + \Delta P_a} = 1.075$$

Further calculation of the ejected gas phase flow rate according to equation (14) gives the value $Q_g = 7.188 \times 10^{-4}$ m^3/s, or the calculated ejection coefficient $k_e = 3.918$.

Experimental studies of the ejector with a cylindrical mixing chamber (MC) allowed us to establish the actual ejection coefficient (Figure 4) and at a liquid pressure in the nozzle of 0.2 MPa it was 3.47. The calculation of k_e according to the modified attached mass theory gives slightly overestimated results, since not all losses were considered.

The second reason for the error lies in the fact that at present there is no reliable theoretical description of the process of dispersing liquid droplets. The described mechanisms of droplet formation show that significant fluctuations in their shape occur: from films, cylinders to drops (Khan et al., 2023). According to the ideas of the theory of attached mass, such fluctuations in shape change the amount of attached mass of the gas phase. For example, the value of the attached mass of gas for a liquid in the form of a film differs from the attached mass of gas to a liquid drop of the same mass by almost 22 times. In the ejector mixing chamber, such non-stationary processes of droplet formation take place, which occupy up to 1/3 of the residence time of the phases in the ejector mixing chamber, but at present it is not possible to take such transformations into account.

In addition, some data (for example, the diameter of the drops) according to different primary sources may differ significantly. There is no reliable method for calculating the diameter of the drops or such experimental data for nozzles, especially those that have been developed recently.

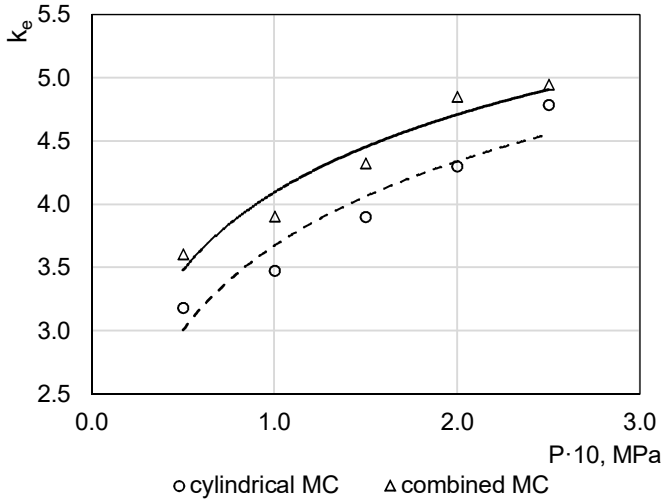

Figure 4. Dependence of ejection coefficient on liquid pressure in the nozzle

Ejection coefficient for ejector with a combined mixing chamber

We present the data for calculating the flow rate of gas phase according to the proposed method for an ejector with a combined mixing chamber, shown in Figure 2 (the initial part of the mixing chamber is conical, which turns into a cylindrical one) at a nominal operating mode of 0.2 MPa. The initial data for the calculation are similar to the calculation of k_e for an ejector with a cylindrical mixing chamber.

With the specified dimensions of the ejector and flow parameters, we calculate the energy losses in the ejector from the acceleration of the flow, due to friction on the walls of the ejector, from the action of gravity. The results are as follows:

- Losses along the length in the cylindrical part of the mixing (Ugli et al., 2023) chamber are 2.56×10^4 Pa.
- Pressure losses due to gravity at the level of 2.462×10^3 Pa.
- Losses due to acceleration of the gas phase, to which energy is transferred from the liquid – 4.3781×10^3 Pa.

The main energy losses are along the length of the cylindrical part of the mixing chamber, and they are considered when finding the ejection coefficient. Unaccounted losses are from acceleration and the action of gravity.

With total pressure losses in the ejector of 9.403×10^4 Pa, the unaccounted losses from acceleration and gravity are 6.84×10^3 Pa, which allows us to find the numerical value of the energy transfer coefficient of 1.727 to compensate for the unaccounted losses.

The calculation of the ejection coefficient at a given energy transfer coefficient allowed us to find the ejection coefficient k_e = 6.72 (the actual ejection coefficient according to experimental data for the ejector with these dimensions is 4.71).

Features of determining ejection coefficient according to attached mass theory

As previously noted, one of the main calculation parameters in determining the energy transfer coefficient is the diameter of the droplets formed during the dispersion of the liquid by the nozzle. There is no unambiguous information regarding the diameter of the liquid droplets. Different authors, based on their own research, give different values of the diameters, which can be partially explained by the different designs of the nozzles studied. So, when studying a centrifugal nozzle (Bezrodny et al., 2013) with a nozzle diameter of 0.94 mm, a swirl chamber diameter of 6 mm, a nozzle length of 0.4 mm at a liquid pressure in the nozzle of up to 0.6 MPa. The diameter d_{32} of the liquid droplets was 130 μm.

In numerical simulation of the spraying process of a two-nozzle centrifugal nozzle (Shulhin et al., 2022), the average droplet diameter was 0.43 μm. These data were confirmed by other studies (Shulhin et al., 2022) of liquid leakage from a nozzle with a diameter of 0.2 mm. The average droplet size was 300 μm, which is confirmed with sufficient accuracy by their CFD studies.

When determining the diameters of liquid droplets (Martins et al., 2021) of various hydraulic sprayers, it was found that the droplet sizes at a liquid pressure of 207 kPa lie in the range of 285–388 μm.

It was found (Zhu, 2023) that the droplet diameter depends on the nozzle diameter and the liquid pressure. Thus, at a liquid pressure of 207 kPa and a nozzle diameter of 3.8 mm, the droplet diameter is 1.00 mm.

The mentioned works on determination of droplet diameter show that there is no unambiguous information about droplet size. However, the correct choice of numerical value of droplet diameter allows to determine ejection coefficient quite accurately theoretically. That is, when choosing droplet diameter when determining ejection coefficients according to the theory of attached mass, it is necessary, if necessary, to conduct additional studies of the dispersed composition, or to use known data, however, the nozzle design should be as close as possible to the design of the sprayer, which is used in the ejector under study.

With insufficiently justified choice, the error in determining k_e can reach several times.

Conclusions

1. By mathematical modeling of the ejection processes of liquid-gas devices with a dispersed liquid jet based on the modified Butakov-Hemeon theory, an expression of the basic ejection equation with an energy transfer coefficient not equal to unity was obtained.
2. It is shown that the energy transfer coefficient is a variable value that depends on the ejector operating mode and its design features. An algorithm for determining the components of energy losses in ejector elements of different designs at nominal operating pressure is given, which allows finding a discrete numerical value of the energy transfer coefficient.
3. According to the given dependencies, for an ejector with a cylindrical mixing chamber, its numerical value of 1.075 at a liquid supply pressure of 0.2 MPa was found, which allows theoretically determining the ejection coefficient with satisfactory accuracy.
4. For an ejector with a combined (conical initial part and subsequent cylindrical) energy transfer coefficient was 1.727, which allowed theoretically determine the gas phase flow rate and the ejection coefficient 6.72. The error in determining k_e is 12–14%.
5. Further research will be aimed at refining the mathematical model of water-gas ejectors with a dispersed liquid jet.

References

Alizadeh Kaklar Z., Ansari M. (2018), Numerical analysis of the internal flow and the mixing chamber length effects on the liquid film thickness exiting from the effervescent atomizer, *Journal of Thermal Analysis and Calorimetry*, 135(1), pp. 1-10, http://doi.org/10.1007/s10973-018-7485-3

Bezrodny M., Goliad N., Rachynskyi A., Barabash P., Holubiev A. (2013), The influence of input water parameters on the spray fineness of centrifugal nozzles, *Energy: Economics, Technologies, Ecology: Scientific Journal*, 2, pp. 23-30. https://ela.kpi.ua/handle/123456789/10788

Chen H., Chen B., Xu Z., Ge J., Chen H., Zhong Z. (2025), A thermodynamic model for performance prediction of an ejector with an adjustable nozzle exit position, *Processes*, 13(3), 879, https://doi.org/10.3390/pr13030879

Dadash-Zade M., Cao R. (2024), Fluid Mechanics of Gas-Liquid Systems, *Academic Journal of Science and Technology*, 12(2), pp. 286–287, https://doi.org/10.54097/wpx4z528

Dafsari R., Yu S., Yong C., Lee J. (2021), Effect of geometrical parameters of air-induction nozzles on droplet characteristics and behaviour, *Biosystems Engineering*, 209, pp. 14-29, https://doi.org/10.1016/j.biosystemseng.2021.06.013.

Fang Z., Wentong Q., Caoxing M., Jingxuan L., Lijun Y., Qingfei F. (2024), Experimental study of Cryogenic jet injection using centrifugal nozzles at supercritical pressure, *Acta Astronautica*, 221, pp. 240-254, https://doi.org/10.1016/j.actaastro.2024.05.025

He L., Su Y., Jie T., Liang J., Tang Z., Yang B., Zhang S. (2020), Structural design and performance analysis of gas-liquid ejector, *Chemical Processes and Equipment*, 39(4), pp 1245-1251, https://doi.org/10.16085/j.issn.1000-6613.2019-1235

Joshi T., Parkash O., Krishan G. (2022), Numerical investigation of slurry pressure drop at different pipe roughness in a straight ppe using CFD, *Arabian Journal for Science and Engineering,* 47, pp. 15391-15414, https://doi.org/10.1007/s13369-022-06583-1

Khan M., Kulkarni A. (2023), Dynamics of drop formation in the presence of interfacial mass transfer, *Langmuir*, 39(5), pp. 12627–12639, https://doi.org/10.1021/acs.langmuir.3c01309

Kong L., Jiaqing C., Tian L., Huan S., Kuisheng W. (2021), Spray and mixing characteristics of liquid jet in a tubular gas–liquid atomization mixer, *Chinese Journal of Chemical Engineering*, 34, pp. 1-11, https://doi.org/10.1016/j.cjche.2020.08.016

Li A., Chen J., Xi G., Huang Z. (2023), Numerical investigation of the effect of primary nozzle geometries on flow structure and ejector performance for optimal design, *Journal of Mechanical Science and Technology*, 37(5), pp. 2139–2148, https://doi.org/10.1007/s12206-023-2101-2

Liu J., Wan J., Li H., Jiang Y. (2023), Water droplet distribution for non-circular sprinkler nozzles. *Advances in Modern Agriculture*, 4(1), 2057, https://doi.org/10.54517/ama.v4i1.2057

Martins R., Moraes H., Freitas M., Lima A., Furtado Júnior, M. (2021), Effect of nozzle type and pressure on spray droplet characteristics, *Idesia*, 39(1), pp. 101-107, https://doi.org/10.4067/S0718-34292021000100101

Ponomarenko V., Pushanko N., Khitriy Y., Liulka D., Babko E. (2017), Research of operation of liquid-gas ejectors with compact and dispersed jets of liquid, *Technology Audit and Production Reserves,* 4(1(36), pp. 4-10, https://doi.org/10.15587/2312-8372.2017.109048

Ponomarenko V., Sliusenko A., Liulka D., Lementar S., Forostiuk I., Yakymchuk M. (2022), Process of sugar solutions sulfitation in terms of hygienic requirements for equipment operation, *Journal of Hygienic Engineering and Design*, 40, pp. 3-12, https://keypublishing.org/jhed/jhed-volumes/volume-40/

Ponomarenko V., Sliusenko A., Liulka D., Yakobchuk R. (2023), Determination of the ejection coefficient of a liquid-gas ejector with a combined mixing chamber, *Ukrainian Food Journal*, 12(3), pp. 419-432, https://doi.org/10.24263/2304-974X-2023-12-3-8

Rosenberger T., Kiesler D., Kruis F., Münzer A., Wiggers H. (2018), Ejector-based sampling from low-pressure aerosol reactors, *Journal of Aerosol Science*, 123, pp. 105-115, https://doi.org/10.1016/j.jaerosci.2018.06.003

Salim A., Sugati D. (2023), The effects of diffuser profile on the performance of the liquid-gas ejector, *Applied Research and Smart Technology*, 4(2), pp. 64-71, https://doi.org/10.23917/arstech.v4i2.1456

Sedano C., Aguirre C., Rondan G., Brizuela A. (2020), Numerical simulation of spray ejection from a nozzle for herbicide application: Evaporation and binary collision models, *Computers and Electronics in Agriculture*, 175, 105551, https://doi.org/10.1016/j.compag.2020.105551

Shulhin A., Samuleev V., Chemerys Ye., Trehub O. A. (2022), The model adaptation of the two-nozzle swirl atomizer for the design-experimental research of the working process in the main combustion chambers of turbojet engines, *Collection of Scientific Works of State Research Institute of Aviation*, 18(25), 117–122, https://doi.org/10.54858/dndia.2022-18-18

Tang S., Wang F., Qin Z., Yin M., Li C., Cai Y., Wei X., You H. (2023), Experimental investigation of dynamics of primary and satellite droplet formation, *Journal of the Brazilian Society of Mechanical Sciences and Engineering*, 45(9), 501, https://doi.org/10.1007/s40430-023-04367-8

Tsai P.H., Wang A.B. (2019), Classification and prediction of dripping drop size for wide range of nozzles by wetting diameter, *Langmuir*, 35(13), pp. 4763–4775, https://doi.org/10.1021/acs.langmuir.8b04228

Ugli M., Mamatkulovich M., Alikulovich F., Muhiddinovich H. (2024), Numerical investigation of laminar-turbulent flow in a suddenly expanding channel, *Internet of Things, Smart Spaces, and Next Generation Networks and Systems. Lecture Notes in Computer Science*, *Springer, Cham*, 14543, https://doi.org/10.1007/978-3-031-60997-8_8

Villermaux E., Marmottant P., Duplat J. (2004), Ligament-mediated spray formation, *Physical Review Letters*, 92(7), 074501, https://doi.org/10.1103/PhysRevLett.92.074501

Yan J., Shu Y., Jiang J., Wen H. (2023), Optimization of two-phase ejector mixing chamber length under varied liquid volume fraction, *Entropy*, 25(1), 7, https://doi.org/10.3390/e25010007

Zhang G., Zhang X., Wang D., Jin Z., Qin X. (2019), Performance evaluation and operation optimization of the steam ejector based on modified model, *Applied Thermal Engineering*, 163, pp. 114388, https://doi.org/10.1016/j.applthermaleng.2019.114388

Zhang Y., He C., Sun L. (2022), Optimization of an ejector to mitigate cavitation phenomena with coupled CFD/BP neural network and particle swarm optimization algorithm, *Progress in Nuclear Energy*, 153, 104412, https://doi.org/10.1016/j.pnucene.2022.104412

Zhao J., Wei X., Zou J., Zhang Y., Sun J., Liu Z. (2022), Research on performance optimization of gas–liquid ejector in multiphase mixed transportation device, *Journal of Mechanics*, 38, pp. 22-31, https://doi.org/10.1093/jom/ufac001

Zhou Z., Han Y., Li Z., Sun N., He L. (2024), Effect of mixing chamber structure on the performance of adjustable ejector, *Thermal Science,* Online-first (00), pp. 229-229, https://doi.org/10.2298/TSCI240714229Z

Zhu X. (2023), Modelling of water drop movement and distribution in no wind and windy conditions for different nozzle sizes, *Dynamic Fluidic Sprinkler and Intelligent Sprinkler Irrigation Technologies*, 3, pp. 109-124, https://doi.org/10.1007/978-981-19-8319-1_6

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Dependence of biological activity of surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 on physiological state of yeast inducer

Anastasiia Okhmakevych[1], Tetiana Pirog[1,2], Liliia Kliuchka[1]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine

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Corresponding author:

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

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Abstract

Introduction. Surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 can be used as antibiofilm agents due to their antimicrobial activity. The antimicrobial activity of surfactants can be increased by adding inducers to the *R. erythropolis* cultivation medium.

Materials and methods. *R. erythropolis* IMV Ac-5017 was cultivated in ethanol-containing medium with *Saccharomyces cerevisiae* BTM-1 as an inducer. Surfactant concentrations were determined gravimetrically. The minimum inhibitory concentration was used to assess the antimicrobial activity of surfactants, and their ability to disrupt biofilms was determined by the spectrophotometric method.

Results and discussion. The antimicrobial activity of surfactants synthesized by *R. erythropolis* IMB Ac-5017 and their ability to destroy biofilms can be significantly increased by introduction of *S. cerevisiae* BTM-1 cells being at different physiological states into the medium for cultivation. The surfactants synthesized by *R. erythropolis* IMV Ac-5017 in the presence of live *S. cerevisiae* BTM-1 cells, as well as the corresponded supernatant, exhibited the highest biological activity. Under these conditions, surfactants synthesized demonstrated the minimum inhibitory concentrations, which were 3.6–240 times lower compared to the values reported for surfactants produced in the medium without an inducer.

Surfactants synthesized in the presence of inducers destroyed single- and dual-species biofilms by 9–59% and 1–29% more effectively, respectively, than those obtained in the medium without them.

Surfactants of *R. erythropolis* IMV Ac-5017 obtained with inactivated inducer cells were less active, probably because proteins of inducer cells could be denatured during autoclaving. Biological activity of the surfactants produced in the presence of the yeast inducer was more specific towards yeast test cultures, which can be explained by the nature of the studied inducer.

Conclusion. The addition of the yeast inducer into the medium for cultivation of *R. erythropolis* IMV Ac-5017 contributed to significant increase of biological activity of surfactants compared to those synthesized in the medium without an inducer.

Introduction

Microbial biofilms pose a significant challenge to humanity as they can cause diseases associated with chronic and acute infections, as well as food spoilage and equipment damage (Shineh et al., 2023). Most often, several species of microorganisms are involved in the formation of biofilms, and such biofilms exhibit significantly higher resistance to disinfectants and antimicrobials than single-species ones (Yuan et al., 2020).

Natural surfactants are promising biofilm destructors due to their antimicrobial activity. Microbial surfactants can be considered as a potential component of antimicrobial drugs containing antibiotics (Pirog and Kliuchka, 2024) or as an alternative to antibiotics, which is currently relevant due to the emergence of multidrug-resistant strains.

The surfactants produced by *Rhodococcus erythropolis* IMV Ac-5017 are characterized by lower antimicrobial activity compared to known surface-active amino-, rhamno-, and sophorolipids. However, it was established that the biological activity of *R. erythropolis* IMV Ac-5017 surfactants could be significantly increased by introducing into the medium for cultivation live *Escherichia coli* IEM-1 and *Bacillus subtilis* BT-2 cells (Pirog et al., 2020). Limited literature data indicate that the biological activity of microbial surfactants can be increased not only by introduction in medium for cultivation of bacterial inducers, but also by eukaryotic ones.

Cultivation with biological inducers is one of the approaches to regulate the activity of secondary metabolites during their synthesis (Sharma et al., 2017).

The challenge in regulating the biological properties of secondary metabolites by the method of cultivation with other microorganisms is that every combination of microorganisms requires separate investigation. It is currently impossible to predict the result in advance, as there are no clear rules for the metabolites production, and the induction mechanism during co-cultivation remains poorly studied and unclear (Liang, et al., 2020; Song et al., 2020).

Therefore, the aim of this study was to determine the antimicrobial and antibiofilm activity of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of cells of *Saccharomyces cerevisiae* BTM-1 being at different physiological states as a biological inducer.

Materials and methods

Objects of research

The main object of research was a strain of oil-oxidizing bacteria *Rhodococcus erythropolis* EK-1, isolated from a soil sample contaminated with oil. The EK-1 strain is registered in the Microorganisms Depository of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, under the number IMV Ac-5017. Chemically, the extracellular *R. erythropolis* IMV Ac-5017 surfactants are a complex of glycolipids (trehalose mono- and dimycolates), neutral lipids (cetyl alcohol, palmitic acid, methylpentadecanoic acid, triglycerides, mycolic acids), and aminolipids (Pirog et al., 2023). The biological inducer used in this research was *Saccharomyces cerevisiae* BTM-1 yeast.

Bacteria (*Staphylococcus aureus* BMS-1, *Pseudomonas* sp. MI-2, *Bacillus subtilis* BT-2, *Escherichia* coli IEM-1) and yeast (*Candida albicans* D-6, *Candida utilis* BVS-65, *S. cerevisiae* BTM-1) from the collection of live cultures of microorganisms of the Department

of Biotechnology and Microbiology, National University of Food Technologies, were used as test cultures to determine the antimicrobial activity of surfactants, as well as their ability to destroy single- and dual-species biofilms.

Cultivation of R. erythropolis IMV Ac-5017

R. erythropolis IMV Ac-5017 was cultivated in a liquid mineral medium (Pirog et al., 2020) with 2% (*v/v*) ethanol in 750 ml Erlenmeyer flasks containing 100 ml of the medium. Cultivation was performed for 5 days in a shaking incubator at 30°C and 320 rpm.

The inoculum was prepared in the same medium with 0.5% (v/v) ethanol in 750 ml Erlenmeyer flasks containing 100 ml of the medium. It was grown for 2 days in a shaking incubator at 30°C and 320 rpm and then added to the cultivation medium at 10% (v/v).

Preparation of the yeast inducer

The strain *S. cerevisiae* BTM-1 was cultivated in a liquid mineral medium with 0.5% (v/v) glucose in 750 ml Erlenmeyer flasks containing 100 ml of the medium. It was grown for 1 day in a shaking incubator at 30°C and 320 rpm. The resulting culture liquid was poured into 1.5 ml sterile Eppendorf tubes and centrifuged in an ultracentrifuge (10 000g, 15 min). After centrifugation, the supernatant was poured into sterile test tubes and added at the rate of 2.5 ml per 100 ml of the culture medium of the surfactants producer. The biomass (sediment) remaining in the Eppendorf tubes was resuspended in sterile tap water to a final volume corresponding to the volume of culture liquid taken for centrifugation. The resuspended biomass was poured into sterile test tubes (live inductor cells were added at the rate of 2.5 ml of the suspension per 100 ml of the culture medium of the surfactants producer). The rest of the resuspended biomass was sterilized in an autoclave at 131°C for 1 hour to obtain inactivated inductor cells (which were added at the rate of 10 ml of the suspension per 100 ml of the culture medium of the surfactants producer).

Isolation and preparation of the surfactants

To obtain the supernatant, the culture liquid was centrifuged (5000 g, 20 min). Surfactants were isolated from the supernatant by the Bligh and Dyer method, with a modified Folch mixture (chloroform – methanol – hydrochloric acid = 4:3:2), as described in the article (Pirog et al., 2020). The extracts were evaporated on a IP-1M2 rotary evaporator at 50ºC and an absolute pressure of 0.4 atm to constant weight. The concentration of surfactants was determined gravimetrically.

The dry surfactant residue was dissolved in preheated distilled water (25 ml). Surfactant solutions were sterilized in an autoclave at 112 ºC for 30 min.

Determination of antimicrobial activity of surfactants

The antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants was determined using the minimum inhibitory concentration (MIC) (Chebbi et al., 2017).

MIC was determined by the method of two-fold serial dilutions in meat-peptone broth (MPB) for bacterial and liquid wort for yeast test cultures (Pirog et al., 2020). Under sterile conditions, 90 µl of MPB or liquid wort were placed in the wells of a microplate. Then, 100 µl of surfactant solution at the initial concentration was added to the first well, mixed, and 100 µl of this liquid was taken and added to the second well, mixed, 100 µl of this liquid was

taken and added to the third well, etc. Thus, in each subsequent well, the concentration of surfactants decreases by 2 times compared to the previous one. After that, 10 µl of an aqueous suspension of a day-old test culture grown on meat-peptone agar (MPA) or wort agar was added to the wells, and 10 µl of sterile tap water was added for control. The microplates were incubated for 24 hours at 37°C for bacteria and 30 °C for yeast. The results were assessed visually by the turbidity of the medium: (+) – the well in which turbidity was observed (presence of growth), (-) – turbidity was not observed (absence of growth). The MIC of surfactants was determined as the surfactants concentration in the first well in which no growth of test cultures was observed. The permissible error of the experiment was ±5%.

Determination of destruction of single-species biofilms under the action of surfactants

Biofilms of test cultures were treated with the surfactant solutions of different concentrations (1.25-640 µg/ml) in the wells of microplates as described in the work (Pirog et al., 2020). The degree of destruction of biofilms (%) was determined spectrophotometrically as the difference between cell adhesion in untreated and surfactant-treated wells. The permissible error of the experiment was ±5%.

Determination of destruction of dual-species biofilms under the action of surfactants

To form biofilms 100 µl of MPB (for two species of bacteria) or 50 µl of MPB and 50 µl of liquid wort (for bacteria and yeast), as well as 10 µl of aqueous suspensions of test cultures (proportions selected experimentally), were placed into polystyrene microplates and incubated for 24 hours at the optimal temperature for the test culture. After that, the culture liquid was drained, the medium and suspensions of microorganisms were reintroduced, and the microplates were incubated for another 24 hours. The culture liquid was drained again, the wells of microplates (with dual-species biofilms preformed in them) were washed twice with sterile tap water, and then 100 µl of the surfactant solutions of different concentrations (1.25–640 µg/ml) were added. In the control wells, 100 µl of sterile tap water was added instead of surfactants. The microplates were incubated for another 24 hours.

The degree of destruction of biofilms (%) was determined spectrophotometrically as the difference between cell adhesion in untreated and surfactant-treated wells. The permissible error of the experiment was ±5%.

Results and discussion

Antimicrobial activity of the surfactants

At the first stage of our research, the antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the medium with the yeast inducer in different physiological states was determined (Table 1).

Table 1

Influence of the physiological state of yeast inducer on antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants

Test cultures	Minimum inhibitory concentrations (μg/ml) of surfactants synthesized in the presence of *S. cerevisiae* BTM-1:			
	live cells	inactivated cells	supernatant	without an inducer (control)
Escherichia coli IEM-1	10	85	41.3	300
Pseudomonas sp. MI-2	10	85	41.3	300
Staphylococcus aureus BMS-1	40	170	82.5	300
Bacillus subtilis BT-2	40	170	82.5	300
Candida albicans D-6	5	42.5	20.7	300
Candida utilis BVS-65	5	42.5	20.7	300
Saccharomyces cerevisiae BTM-1	1.25	21.3	10.3	300

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

The results showed that cultivation of *R. erythropolis* IMV Ac-5017 in the medium with all studied biological inducers was accompanied by synthesis of the surfactants with increased antimicrobial activity.

Thus, the introduction of live *S. cerevisiae* BTM-1 cells into the culture medium of *R. erythropolis* IMV Ac-5017 was accompanied by the synthesis of surfactants, the MICs of which in relation to test cultures of gram-negative (*E. coli* IEM-1, *Pseudomonas* sp. MI-2) and gram-positive (*B. subtilis* BT-2, *S. aureus* BMS-1) bacteria were 7.5-30 times lower than those established for surfactants produced without an inducer. Similar patterns were observed for yeast test cultures (*C. albicans* D-6, *C. utilis* BVS-65, and *S. cerevisiae* BTM-1): the minimum inhibitory concentration of surfactants synthesized in the medium with the live inducer was two orders of magnitude lower compared to the control (1.25-5 and 300 μg/ml).

In the presence of *S. cerevisiae* BTM-1 supernatant in the medium, surfactants were synthesized, the MIC values of which in relation to bacterial and yeast test cultures were, respectively, 3.6-7.3 and 14.5-29.1 times lower compared to surfactants obtained using a monoculture. The addition of inactivated inductor cells into the culture medium also affected the antimicrobial activity of the synthesized surfactants – the MICs for bacteria and yeast were 1.8-3.5 and 7.1-14.1 times lower compared to the control.

The obtained results on the higher efficiency of live inducer cells and supernatant compared to inactivated cells may indicate that induction requires both chemical and biological interaction between the surfactant producer and the inducer. It is likely that during autoclaving of the yeast cell suspension, proteins and other macromolecules of the inducer cells are denatured, which partially inhibits potential biochemical interactions.

Thus, the data presented in Table 1 indicate the possibility of increasing the antimicrobial activity of *R. erythropolis* IMV Ac-5017 surfactants against various bacterial and yeast test cultures due to the introduction of *S. cerevisiae* BTM-1 in different physiological states into the culture medium. Surfactants of *R. erythropolis* IMV Ac-5017 synthesized without inducers acted nonspecifically on test cultures. At the same time, the surfactants obtained in the presence of the yeast inducer were more effective against yeast test cultures, especially against the inducer itself, which can be explained by specific

competitive interactions between *R. erythropolis* IMV Ac-5017 and *S. cerevisiae* BTM-1 in the culture medium.

In previous studies by scientists of the Department of Biotechnology and Microbiology (Pirog et al., 2020), live *E. coli* IEM-1 and *B. subtilis* BT-2 cells were used as inducers to increase the biological activity of *R. erythropolis* IMV Ac-5017 surfactants. Under such conditions, the synthesized surfactants were characterized by the MIC of 3-12 μg/ml against bacterial test cultures (*E. coli* IEM-1, *B. subtilis* BT-2, *S. aureus* BMS-1). Comparing with our data (10-40 μg/ml), it can be stated that depending on the inducer in the presence of which surfactants are synthesized, the specificity of their action on the test cultures changes.

Similar study (Pirog et al., 2023) was devoted to the effect of prokaryotic inducers *E. coli* IEM-1 and *B. subtilis* BT-2 in different physiological states on the biological activity of *Acinetobacter calcoaceticus* IMB B-7241 and *Nocardia vaccinii* IMB B-7405 surfactants. Thus, surfactants of the IMB B-7241 and IMB B-7405 strains synthesized in the presence of live inducer cells were characterized by the MIC values that were 3-23 and 2.9-16 times lower, respectively, in relation to bacterial test cultures compared to surfactants synthesized without inductors. We observed a more significant decrease in the minimum inhibitory concentrations of microbial surfactants produced in the medium with live *S. cerevisiae* BTM-1cells in relation to bacterial test cultures – by 7.5-30 times compared to the control.

The minimal inhibitory concentrations of *A. calcoaceticus* IMB B-7241 surfactants synthesized in the presence of live *B. subtilis* BT-2 cells against yeast test cultures were 2.8-5.6 times lower compared to surfactants obtained without any inducers. Because of our research, the MICs of *R. erythropolis* IMV Ac-5017 surfactants produced in the medium with live *S. cerevisiae* BTM-1cells against *Candida* yeast were as much as 60 times lower compared to the control. Again, the same trend is observed: in the presence of prokaryotic inducers, surfactants with more effective action against bacterial test cultures are synthesized, while in the presence of eukaryotic inducers, surfactants are more effective against yeast.

The effect of the supernatant was less effective: in its presence, the *A. calcoaceticus* IMB B-7241 surfactants were synthesized with minimum inhibitory concentrations for most test cultures of bacteria and yeast only 2 times lower than those for surfactants produced without any inducers. In contrast, the presence of *S. cerevisiae* BTM-1 supernatant in the culture medium of *R. erythropolis* IMV Ac-5017 was accompanied by the synthesis of surfactants with minimum inhibitory concentrations reduced by as much as 3.6-29 times.

In the presence of inactivated prokaryotic inducer cells in the culture media of *A. calcoaceticus* IMB B-7241 and *N. vaccinii* IMB B-7405, the surfactants were synthesized with MICs against bacterial test cultures that were 2-8 and 2-13.3 times lower compared to the controls. However, it should be noted that the authors obtained inoculum of *E. coli* IEM-1 and *B. subtilis* BT-2 on a meat-peptone agar medium, while we cultivated *S. cerevisiae* BTM-1 in a liquid medium, which is a significant advantage when scaling the technology to an industrial level.

Our data indicate that the regulation of the biological activity of *R. erythropolis* IMV Ac-5017 surfactants can be achieved by introducing biological inducers and activators of key enzymes involved in the biosynthesis of surfactant components responsible for antimicrobial activity into the medium. In the available foreign scientific literature, there is also information on secondary metabolites (including surfactants of microbial origin) synthesized in the presence of inducers in the culture medium, but the data are limited. Live inducer cells are used most often in experiments and inactivated or supernatant cells are used less frequently.

Alves et al. (2019) studies the impact of live cells of *Pseudomonas aeruginosa* ATCC 27853 and *Listeria innocua* NCTC 11288 on the concentration of rhamnolipids produced by *Pseudomonas sp.* 74. The work (DeFilippi et al., 2018) focuses on the effect of live *Rhizopus*

stolonifer 198 cells on the synthesis of *B. subtilis* B9-5 lipopeptides, while in the research (Bagheri et al., 2022) the impact of live *Azospirillum oryzae* NBT506 cells and the corresponding supernatant on the biosynthesis process of *Bacillus velezensis* UTB96 surfactin was studied. However, the authors did not investigate the biological activity of the obtained surfactants. Additionally, there is also information on the influence of *S. cerevisiae* in different physiological states on the concentration of the antibiotic natamycin produced by *Streptomyces natalus* N5 (Shi et al., 2017) and *Streptomyces natalensis* HW-2 (Wang et al., 2013), valinomycin produced by *Streptomyces lavendulae* ACR-DA1 (Sharma et al., 2017), and rimocidin by *Streptomyces rimosus* M527 (Song et al., 2020), but the authors also did not investigate the biological activity of the obtained substances.

To date, there are several articles describing studies on the antimicrobial activity of microbial surfactants. Dusane et al. (2011) studied the antimicrobial activity of surface-active lipopeptides produced by *Bacillus sp.* S3, *Bacillus pumilus* S8, *Bacillus licheniformis* D1, and *Serratia marcescens* V1, obtained in the presence of live *P. aeruginosa* (strain number not specified), *B. pumilus* FJ938166, *C. albicans* (strain number not specified), and *Yarrowia lipolytica* (strain number not specified) cells. Fifani et al. (2022) studied *B. velezensis* GA1 lipopeptides synthesized in the presence of live, heat-inactivated *Trichoderma harzianum* IHEM5437 cells, as well as the corresponding supernatant, against the test cultures of the inducers themselves. However, to assess antimicrobial activity, the authors used the standard disk diffusion method and determination of the dry weight of the biomass of test cultures, which makes it impossible to directly compare their results with ours. The authors note that when cultivating producer bacteria in a medium with biological inducers, surfactants with significantly higher antimicrobial activity were synthesized: the inhibition zones of the test cultures increased by 2-4 mm, and the amount of dry biomass decreased by 50-100% compared to the effect of surfactants obtained using monocultures.

There is also information in the literature regarding co-cultivation or cultivation with inducers, which results in an increase in the antimicrobial activity of other secondary metabolites. Li with co-authors (2022) showed an increase in the inhibition coefficient of the ethyl acetate extract of the metabolite complex (phytohormones, aminoglycosides, amines, macrolides, peptides, terpenoids, alkaloids, steroids, coumarins, phenolic acids) from the co-culture of *Streptomyces albireticuli* MDJK11 and *Streptomyces albofavus* MDJK44 against the fungal test cultures *Fusarium moniliforme*, *Fusarium solani*, and *Fusarium graminearum* (strain numbers not specified) by 2-43.6% compared to that of the extracts produced by the corresponding monocultures. Serna-Cock with co-authors (2019) reported an increase in the growth inhibition zone of *Listeria monocytogenes* ATCC 13932 by 1-3.5 mm due to the supernatant of the metabolite complex from the co-culture of *Lactobacillus plantarum* and *Weissella cibaria* (strain numbers not specified).

As a result of cultivating *Trichoderma asperellum* GDFS1009 with *Bacillus amyloliquefaciens* 1841 (Karuppiah et al., 2019) and *B. amyloliquefaciens* ACCC11060 (Wu et al., 2018), metabolite complexes were obtained, the antimicrobial activity of which against *F. graminearum* (strain number not specified) was an order of magnitude higher (4.5×10^2 spores/ml) compared to the action of the metabolites produced by the monoculture of *T. asperellum* GDFS1009 (7×10^3 spores/ml), and against *Botrytis cinerea* (strain number not specified), the inhibition coefficient increased by 36% (67% and 31%, respectively, for the metabolites obtained in co-culture and monoculture). The article Liu et al. (2022) is dedicated to the cultivation of *Trichoderma atroviride* SG3403 with *B. subtilis* 22, resulting in a metabolite complex that also exhibited an increased inhibition coefficient against *F. graminearum* MN396567 by 19-57% compared to the influence of the metabolites from monocultures (62% and 5-43%, respectively).

In the study (Luti and Yonis, 2013) the author investigated the effect of live and thermally inactivated cells of *E. coli*, *B. subtilis*, and *S. cerevisiae* on the synthesis of phenazine by *P. aeruginosa* (strain numbers not specified). Although the influence of inducers in different physiological states on the antimicrobial activity of the antibiotic was not studied, we were intrigued by the suggestion that the metabolite 'might recognize' the test culture of the inducer and be specific to it. A similar thought arose during our analysis of the minimum inhibitory concentration of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of *S. cerevisiae* BTM-1 against the inducer itself.

Consequently, there is little literature on the antimicrobial activity of surfactants synthesized in the presence of inducers in different physiological states; however, our results for the minimum inhibitory concentration of *R. erythropolis* IMV Ac-5017 surfactants obtained in the presence of *S. cerevisiae* BTM-1 are comparable to similar studies. Furthermore, using the method of co-cultivation with inducers or competing microorganisms can significantly enhance the antimicrobial activity of secondary metabolites.

Determination of the degree of destruction of single-species biofilms

Besides antimicrobial activity, surfactants of microbial origin also possess the ability to disrupt biofilms. Therefore, in the next stage, we studied the degree of destruction of mono-species bacterial and yeast biofilms under the action of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of a yeast inducer in different physiological states during cultivation.

The results showed that surfactants synthesized in media with inducers were more effective at disrupting bacterial and yeast biofilms than surfactants obtained without any inducers (Tables 2–5).

Table 2

Destruction of Gram-positive bacteria biofilms under the action of the surfactants synthesized in the presence of *Saccharomyces cerevisiae* BTM-1

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Bacillus subtilis BT-2	Live cells	75.2	64.8	62.4	60.5	50.2	42.3	31.7	28.2	27.4	24.5
	Inactivated cells	71.0	63.1	56.8	38.7	29.0	26.9	28.8	21.8	20	18
	Supernatant	78.2	75.0	69.5	56.6	50.2	43.8	33.2	36.7	31.8	29.6
	Control	61.9	54.1	42.4	34.0	25.2	20.2	16.0	17.4	14.5	11.4
Staphylococcus aureus BMS-1	Live cells	79.6	66.8	58.3	57.7	46.4	48.3	35.8	23.5	22.7	26.4
	Inactivated cells	76.1	65.4	55.2	34.8	25.6	24.1	24.7	24.8	23.9	19.6
	Supernatant	80.4	73.9	62.3	59.1	52.3	42.3	31.7	34.2	34.1	23.9
	Control	63.7	53.6	37.7	33.6	20.6	17.1	16.7	17.6	15.6	10.6

Note. Tables 2–5: during the determination of the degree of biofilm destruction, the error did not exceed 5%.

Table 3

Destruction of Gram-negative bacteria biofilms under the action of the surfactants synthesized in the presence of the yeast inducer

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Escherichia coli IEM-1	Live cells	85.0	86.8	72.6	67.9	67.4	62.6	61.8	58.9	50.8	50.8
	Inactivated cells	78.4	64.1	60.8	58.9	47.5	39.8	38.9	28.4	20.8	13.1
	Supernatant	88.9	74.1	71.8	57.5	54.6	52.5	51.3	54.6	48.4	32.6
	Control	63.1	57.9	40.5	38.9	26.0	20.0	16.4	16.2	14.1	11.0
Pseudomonas sp. MI-2	Live cells	82.6	79.6	70.3	66.3	59.2	58.6	55.1	57.5	50.0	42.7
	Inactivated cells	74.9	67.6	65.6	55.7	42.2	36.1	35.7	25.8	24.3	19.8
	Supernatant	81.8	76.4	66.2	53.9	51.1	50.6	53.5	46.1	49.3	35.7
	Control	60.2	56.7	41.8	37.9	25.0	17.3	15.8	15.3	13.0	12.1

Table 4

Degree of destruction of *Candida* yeast biofilms under the action of surfactants synthesized in the presence of the biological inducer

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Candida albicans D-6	Live cells	96.4	96.4	83.0	78.4	78.1	63.2	61.3	58.7	55.5	53.3
	Inactivated cells	83.9	80.0	70.0	60.8	56.1	45.8	39.5	28.3	27.7	26.7
	Supernatant	93.9	83.3	80.9	78.1	75.3	63.7	66.7	59.3	50.9	48.0
	Control	64.5	53.9	43.9	39.7	28.3	18.9	15.6	16.2	15.3	14.3
Candida utilis BVS-65	Live cells	91.7	92.8	86.7	71.7	73.5	64.2	62.4	50.9	52.4	44.7
	Inactivated cells	83.5	77.2	71.2	67.0	56.0	42.1	33.4	33.9	27.7	25.8
	Supernatant	93.0	87.5	83.6	73.1	74.8	62.7	52.4	55.3	51.5	41.0
	Control	64.6	55.8	41.7	38.7	23.9	19.5	18.5	16.8	14.4	13.7

It was established that the introduction into the culture medium of live cells of *S. cerevisiae* BTM-1, as well as the corresponding supernatant, was accompanied by the synthesis of surfactants, which in the entire studied concentration range (1.25-640 μg/ml) by 9.1-31.2 and 13.3-31.7%, respectively, more effectively destroyed single-species biofilms of gram-positive bacteria *B. subtilis* BT-2 and *S. aureus* BMS-1 than preparations obtained without an inducer.

Table 5

Degree of destruction of yeast inducer biofilms under the influence of surfactants synthesized in its presence

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Saccharomyces cerevisiae BTM-1	Live cells	99.6	98.1	91.3	80.5	80.5	74	76.3	63.1	63.2	51.1
	Inactivated cells	87.4	82.1	75.1	74.2	67.8	51.1	40.2	30.9	28.9	27.8
	Supernatant	93.6	95.9	87.4	83.3	71.0	61.6	60.0	57.7	41.3	44.0
	Control	65.5	57.5	43.7	32.3	22.4	18.5	17.4	12.8	14.6	11.0

The most pronounced increasing destruction of these biofilms was observed with surfactants (20–160 μg/ml) obtained in the presence of live yeast cells, and those synthesized in the presence of the supernatant at concentrations of 20-320 μg/ml. The maximum degree of destruction (75.2–80.4%) was observed under the influence of surfactants at the highest concentration studied (640 μg/ml).

At the same time, in the presence of inactivated inducer cells, surfactants were synthesized, under the action of which the destruction Gram-positive bacteria biofilms increased by only 3.8–14.5% (Table 2). Similar patterns were observed with surfactants (1.25-640 μg/ml) obtained in a medium with live yeast cells and supernatant on mono-species biofilms of Gram-negative bacteria.

Under these conditions, the destruction of *E. coli* IEM-1 and *Pseudomonas sp.* MI-2 biofilms increased by 16–45.4%, compared to the control. The degree of biofilm disruption increased the most under the action of surfactants in low concentrations (1.25–40 μg/ml). The destruction of these biofilms reached a maximum degree of 81.8-88.9% under the influence of surfactants at high concentrations (320–640 μg/ml).

The addition of inactivated cells to the medium was accompanied by the synthesis of surfactants, which at concentrations of 10–160 μg/ml also effectively disrupted these mono-species biofilms, with an increase in the degree of destruction ranging from 17.2 to 23.8%. At other concentrations, the increase was only 2.1–15% (Table 3).

Surfactants synthesized in the presence of all studied inducers exhibited a more specific action towards yeast test cultures across the entire concentration range studied, which can be explained by their antimicrobial activity. The degree of destruction of biofilms formed by yeast of the genus *Candida* after treatment with solutions of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of live inducer cells increased by 27.1–49.8, inactivated cells – by 12.1–32.1, and supernatant – by 27.3–50.9% (Table 4).

The most specific action of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of yeast was observed against the biofilms of the inducer itself. The destruction of *S. cerevisiae* BTM-1 biofilms under the action of surfactants obtained in the presence of live cells increased by as much as 34.1–58.9, the supernatant – by 26.7–51, and inactivated cells – by 14–45.4% (Table 5).

To summarize all of the above, the obtained experimental data (Tables 2-5) demonstrate the possibility of significantly enhancing the ability of *R. erythropolis* IMV Ac-5017 surfactants to disrupt both bacterial and yeast biofilms by the addition of live *S. cerevisiae*

BTM-1 cells, as well as the corresponding supernatant, into the cultivation medium of the producer.

The possibility of enhancing the anti-biofilm activity of *R. erythropolis* IMV Ac-5017 (Pirog et al., 2020), *N. vaccinii* IMB B-7405 (Pirog et al., 2023), and *A. calcoaceticus* IMV B-7241 (Pirog and Ivanov, 2022) surfactants was shown by the addition of live and inactivated *B. subtilis* BT-2 and *E. coli* IEM-1 cells, as well as the supernatant, to the cultivation medium. Comparing these results with ours, it can be stated that the action of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of live yeast cells was generally no less effective against single-species bacterial biofilms.

In the available foreign scientific literature, information regarding the disruption of single-species biofilms using microbial surfactants is limited; however, we managed to find several articles. Gómez et al. (2016) showed complete inhibition of single-species biofilms of *E. coli* O157*:H7* ATCC 35150, *L. monocytogenes* ATCC 7644, and *Salmonella typhimurium* ATCC 14028 under the action of surfactants synthesized in a co-culture of lactic acid bacteria *Lactococcus lactis* 368 with *Lactobacillus curvatus* MBSa3 or *Lactobacillus sakei* MBSa1. Additionally, Kimelman et al. (2019) showed strong inhibition of the formation of single-species *S. aureus* ATCC 25923 biofilms, as evidenced by a reduction in the optical density of the samples by approximately 1.6 times after treatment with the *B. subtilis* YC189 lipopeptides synthesized during co-cultivation with *L. plantarum*.

In the study (Hamza et al., 2018), the authors investigated the effect of the supernatant of *Staphylococcus lentus* SZ2 glycolipids synthesized in the presence of *Vibrio harveyi* MTCC 7771 on the mono-species biofilms of the competitive bacteria themselves. The increase in the degree of biofilm destruction ranged from 1.26% to 39.36%, depending on the duration of exposure. In contrast, *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of yeast inducer destroyed the biofilms of the inducer by several tens more efficiently.

Consequently, our results on the anti-biofilm activity of *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of *S. cerevisiae* BTM-1 are on par with studies conducted by both domestic and foreign colleagues.

Determination of the degree of destruction of dual-species biofilms

At the next stage, we decided to use surfactants produced by *R. erythropolis* IMV Ac-5017 for the destruction of dual-species biofilms.

The results showed that surfactants (1.25–640 μg/mL) synthesized in the presence of an inducer were more effective in destroying bacterial and bacterial-yeast combined biofilms than surfactants synthesized without the inducer (Tables 6–7).

It was found that in the presence of living cells of *S. cerevisiae* BTM-1 in the culture medium of *R. erythropolis* IMV Ac-5017, surfactants were synthesized, which in a wide range of concentrations (1.25-640 μg/ml) by 3-29% more effectively destroyed two species of bacteria (*B. subtilis* BT-2 + *Pseudomonas* sp. MI-2 and *S. aureus* BMS-1 + *E. coli* IEM-1) and bacterial and yeast (*Pseudomonas* sp. MI-2 + *C. albicans* D-6 and *S. aureus* BMS-1 + *C. utilis* BVS-65) biofilms compared to the effect of surfactants synthesized without an inducer.

The addition of the supernatant was accompanied by the synthesis of surfactants, which were 1-22% more effective in destroying combined bacterial biofilms and 7–26% more effective in destroying bacterial-yeast biofilms compared to the effect of surfactants obtained using a monoculture.

Table 6

Effect of surfactants on bacterial combined biofilms

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Bacillus subtilis BT-2 + *Pseudomonas* sp. MI-2	Live cells	69	61	53	52	49	43	39	40	31	35
	Inactivated cells	58	50	48	48	48	41	31	31	31	28
	Supernatant	72	63	67	60	59	39	39	36	38	33
	Control	52	53	45	49	40	38	27	20	23	18
Escherichia coli IEM-1 + *Staphylococcus aureus* BMS-1	Live cells	68	68	56	49	46	41	40	32	35	38
	Inactivated cells	52	53	47	44	32	36	33	29	28	26
	Supernatant	67	63	54	46	47	48	37	33	33	27
	Control	51	50	44	33	32	29	23	18	19	12

Note. Tables 6-7: during the determination of the degree of biofilm destruction, the error did not exceed 5%.

Table 7

Action of surfactants on bacterial-yeast dual-species biofilms

Test cultures	Physiological state of the inducer	Destruction (%) under the action of the surfactants (μg/ml)									
		640	**320**	**160**	**80**	**40**	**20**	**10**	**5**	**2.5**	**1.25**
Pseudomonas sp. MI-2 + *Candida albicans* D-6	Live cells	79	76	61	62	54	50	46	31	27	23
	Inactivated cells	66	57	56	49	42	40	36	30	26	19
	Supernatant	80	71	67	60	61	51	49	35	39	24
	Control	56	47	41	46	40	33	26	20	20	17
Staphylococcus aureus BMS-1 + *Candida utilis* BVS-65	Live cells	68	52	48	48	47	40	38	33	30	27
	Inactivated cells	60	45	48	42	40	33	35	23	25	20
	Supernatant	65	53	42	45	43	43	42	38	35	22
	Control	55	42	42	32	30	25	23	17	18	13

The degree of destruction of dual-species biofilms under the action of surfactants obtained in the presence of inactivated yeast cells increased by only 1–15% compared to the control.

The maximum degree of destruction, 53–72% and 60–80% for bacterial and bacterial-yeast biofilms, respectively, was observed under the influence of high concentrations (320–640 μg/mL) of surfactants synthesized in the presence of the inducer. Overall, the action of surfactants synthesized in the presence of yeast was more specific to dual-species biofilms of yeast with Gram-negative bacteria, as explained by the results of studies on the destruction of single-species biofilms.

Thus, the experimental data obtained (Tables 6–7) show the possibility of significantly enhancing the ability of *R. erythropolis* IMV Ac-5017 surfactants to destroy combined biofilms by adding live *S. cerevisiae* BTM-1 cells, as well as the corresponding supernatant, to the cultivation medium of the producer.

In the available literature, there is little information regarding the use of microbial surfactants to combat combined biofilms. For example, in the study Ceresa et al. (2021), it was shown that the action of rhamnolipid AC7BS produced by *P. aeruginosa* 89 on a dual-species biofilm of *C. albicans* ATCC 10231 with *S. aureus* ATCC 25923 resulted in 94% growth inhibition. In the study Keyhanian et al. (2023), after treating biofilms of *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 with a mixture of *Bacillus cereus* and *Serratia nematodiphila* (strain numbers not specified) surfactants, their degree of destruction was 60% and 80%, respectively, while the individual components of the agent were less effective.

Consequently, after analyzing the available literature data, it can be concluded that the *R. erythropolis* IMV Ac-5017 surfactants synthesized in the presence of the yeast is comparable in biofilm-inhibitory action to other antimicrobial agents.

Conclusions

The obtained results indicate the possibility of a significant increase in the biological activity of *R. erythropolis* IMV Ac-5017 surfactant, provided that it is synthesized in a medium with *S. cerevisiae* BTM-1. Furthermore, the activity of the surfactants produced in the presence of the inducer was more specific towards yeast test cultures, which can be explained by the nature of the studied inducer.

The introduction into the culture medium of *R. erythropolis* IMV Ac-5017 *S. cerevisiae* BTM-1 in different physiological states (living cells, as well as the corresponding supernatant) was accompanied by the synthesis of surfactants, the MICs of which in relation to bacterial and yeast test cultures were one to two orders of magnitude lower, the destruction of single-species bacterial and yeast biofilms by 9–59%, and of two-species bacterial-bacterial and bacterial-yeast biofilms by 1–29% higher compared to the indicators established for surfactants obtained without an inductor.

References

Alves A.R., Sequeira A.M., Cunha Â. (2019), Increase in bacterial biosurfactant production by co-cultivation with biofilm-forming bacteria, *Letters in Applied Microbiology*, 69(1), pp. 79–86, https://doi.org/10.1111/lam.13169

Bagheri N., Ahmadzadeh M., Mariotte P., Jouzani G.S. (2022), Behavior and interactions of the plant growth-promoting bacteria *Azospirillum oryzae* NBT506 and *Bacillus*

velezensis UTB96 in a co-culture system, *World Journal of Microbiology & Biotechnology*, 38(6), 101, https://doi.org/10.1007/s11274-022-03283-8

Ceresa C., Rinaldi M., Tessarolo F., Maniglio D., Fedeli E., Tambone E., Caciagli P., Banat I.M., Diaz De Rienzo M.A., Fracchia L. (2021), Inhibitory effects of lipopeptides and glycolipids on *C. albicans* - *Staphylococcus* spp. dual-species biofilms, *Frontiers in Microbiology*, 11, 545654, https://doi.org/10.3389/fmicb.2020.545654

Chebbi A., Elshikh M., Haque F., Ahmed S., Dobbin S., Marchant R., Sayadi S., Chamkha M., Banat I. M. (2017), Rhamnolipids from *Pseudomonas aeruginosa* strain W10 as antibiofilm/antibiofouling products for metal protection, *Journal of Basic Microbiology*, 57(5), pp. 364–375, https://doi.org/10.1002/jobm.201600658

DeFilippi S., Groulx E., Megalla M., Mohamed R., Avis T.J. (2018), Fungal competitors affect production of antimicrobial lipopeptides in *Bacillus subtilis* strain B9-5, *Journal of Chemical Ecology*, 44(4), pp. 374–383, https://doi.org/10.1007/s10886-018-0938-0

Dusane D.H., Matkar P., Venugopalan V.P., Kumar A.R., Zinjarde S.S. (2011), Cross-species induction of antimicrobial compounds, biosurfactants and quorum-sensing inhibitors in tropical marine epibiotic bacteria by pathogens and biofouling microorganisms, *Current Microbiology*, 62(3), pp. 974–980, https://doi.org/10.1007/s00284-010-9812-1

Fifani B., Steels S., Helmus C., Delacuvellerie A., Deracinois B., Phalip V., Delvigne F., Jacques P. (2022), Coculture of *Trichoderma harzianum* and *Bacillus velezensis* based on metabolic cross-feeding modulates lipopeptide production, *Microorganisms*, 10(5), 1059, https://doi.org/10.3390/microorganisms10051059

Gómez N.C., Ramiro J.M., Quecan B.X., de Melo Franco B.D. (2016), Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of *Listeria monocytogenes*, *Salmonella typhimurium*, and *Escherichia coli* O157:H7 biofilms formation, *Frontiers in Microbiology*, 7, 863, https://doi.org/10.3389/fmicb.2016.00863

Hamza F., Kumar A.R., Zinjarde S. (2018), Coculture induced improved production of biosurfactant by *Staphylococcus lentus* SZ2: role in protecting *Artemia salina* against *Vibrio harveyi*, *Enzyme and Microbial Technology*, 114, pp. 33–39, https://doi.org/10.1016/j.enzmictec.2018.03.008

Karuppiah V., Sun J., Li T., Vallikkannu M., Chen J. (2019), Co-cultivation of *Trichoderma asperellum* GDFS1009 and *Bacillus amyloliquefaciens* 1841 causes differential gene expression and improvement in the wheat growth and biocontrol activity, *Frontiers in Microbiology*, 10, 1068, https://doi.org/10.3389/fmicb.2019.01068

Keyhanian A., Mohammadimehr M., Nojoomi F., Naghoosi H., Khomartash M. S., Chamanara M. (2023), Inhibition of bacterial adhesion and anti-biofilm effects of *Bacillus cereus* and *Serratia nematodiphila* biosurfactants against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, *Iranian Journal of Microbiology*, 15(3), pp. 425–432, https://doi.org/10.18502/ijm.v15i3.12903

Kimelman H., Shemesh M. (2019), Probiotic bifunctionality of *Bacillus subtilis*-rescuing lactic acid bacteria from desiccation and antagonizing pathogenic *Staphylococcus aureus*, *Microorganisms*, 7(10), 407, https://doi.org/10.3390/microorganisms7100407

Li J., Zhang L., Yao G., Zhu L., Lin J., Wang C., Du B., Ding Y., Mei X. (2022), Synergistic effect of co-culture rhizosphere *Streptomyces*: A promising strategy to enhance antimicrobial activity and plant growth-promoting function, *Frontiers in Microbiology*, 13, 976484, https://doi.org/10.3389/fmicb.2022.976484

Liang L., Wang G., Haltli B., Marchbank D.H., Stryhn H., Correa H., Kerr R.G. (2020), Metabolomic comparison and assessment of co-cultivation and a heat-killed inducer strategy in activation of cryptic biosynthetic pathways, *Journal of Natural Products*, 83(9), pp. 2696–2705, https://doi.org/10.1021/acs.jnatprod.0c00621

Liu H., Li T., Li Y., Wang X., Chen J. (2022), Effects of *Trichoderma atroviride* SG3403 and *Bacillus subtilis* 22 on the biocontrol of wheat head blight, *Journal of Fungi (Basel, Switzerland)*, 8(12), 1250, https://doi.org/10.3390/jof8121250

Luti K., Yonis R. (2013). Elicitation of *Pseudomonas aeruginosa* with live and dead microbial cells enhances phenazine production, *Romanian Biotechnological Letters*, 18(6), 7169.

Pirog T.P., Kliuchka I.V. (2024), Synergism of antimicrobial activity of antibiotics with biocides of natural origin, *Biotechnologia Acta*, 17(1), pp. 5-19, https;//doi.org/10.15407/biotech17.01.005

Pirog T., Kluchka L., Skrotska O., Stabnikov V. (2020), The effect of co-cultivation of *Rhodococcus erythropolis* with other bacterial strains on biological activity of synthesized surface-active substances, *Enzyme and Microbial Technology*, 142, 109677, https://doi.org/10.1016/j.enzmictec.2020.109677

Pirog T., Stabnikov V., Stabnikova O. (2023), Microbial surfactants production from industrial waste, In: O. Stabnikova, O. Shevchenko, V. Stabnikov, O. Paredes-López (Eds.), *Bioconversion of Waste to Value-Added Products*, pp. 272-304, CRC Press, Boca Raton, https://doi.org/10.1201/9781003329671-10

Pirog T., Ivanov M. (2022), Destruction of biofilms under the influence of *Acinetobacter calcoaceticus* IMV B-7241 surfactants, synthesized in the presence of competitive microorganisms, *Ukrainian Food Journal*, 11(2), pp. 291-301, https://doi.org/10.24263/2304-974X-2022-11-2-9

Serna-Cock L., Rojas-Dorado M., Ordoñez-Artunduaga D., García-Salazar A., García-González E., Aguilar C.N. (2019), Crude extracts of metabolites from co-cultures of lactic acid bacteria are highly antagonists of *Listeria monocytogenes*, *Heliyon*, 5(9), e02448, https://doi.org/10.1016/j.heliyon.2019.e02448

Sharma R., Jamwal V., Singh V.P., Wazir P., Awasthi P., Singh D., Vishwakarma R.A., Gandhi S.G., Chaubey A. (2017), Revelation and cloning of valinomycin synthetase genes in *Streptomyces lavendulae* ACR-DA1 and their expression analysis under different fermentation and elicitation conditions, *Journal of Biotechnology*, 253, pp. 40-47, https://doi.org/10.1016/j.jbiotec.2017.05.008

Shi S., Tao Y., Liu W. (2017), Effects of fungi fermentation broth on natamycin production of *Streptomyces*, *Progress in Applied Microbiology,* 1(1), pp. 15-22.

Shineh G., Mobaraki M., Perves Bappy M.J., Mills D.K. (2023), Biofilm formation, and related impacts on healthcare, food processing and packaging, industrial manufacturing, marine industries, and sanitation – A review*, Applied Microbiology,* 3(3), pp. 629-665, https://doi.org/10.3390/applmicrobiol3030044

Song Z., Ma Z., Bechthold A., Yu X. (2020), Effects of addition of elicitors on rimocidin biosynthesis in *Streptomyces rimosus* M527, *Applied Microbiology and Biotechnology*, 104(10), pp. 4445–4455, https://doi.org/10.1007/s00253-020-10565-4

Wang D., Yuan J., Gu S., Shi Q. (2013), Influence of fungal elicitors on biosynthesis of natamycin by *Streptomyces natalensis* HW-2, *Applied Microbiology and Biotechnology*, 97(12), pp. 5527–5534, https://doi.org/10.1007/s00253-013-4786-0

Wu Q., Ni M., Dou K., Tang J., Ren J., Yu C., Chen J. (2018), Co-culture of *Bacillus amyloliquefaciens* ACCC11060 and *Trichoderma asperellum* GDFS1009 enhanced pathogen-inhibition and amino acid yield, *Microbial Cell Factories*, 17(1), 155, https://doi.org/10.1186/s12934-018-1004-x

Yuan L., Hansen M.F., Røder H.L., Wang N., Burmølle M., He G. (2020), Mixed-species biofilms in the food industry: Current knowledge and novel control strategies, *Critical Reviews in Food Science and Nutrition*, 60(13), pp. 2277-2293, https://doi.org/10.1080/10408398.2019.1632790

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Effect of competitive eukaryotic microorganisms on anti-adhesive activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants

Tetyana Pirog[1,2], Mykyta Ivanov[1]

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 aim of the study was to investigate the effect of eukaryotic inductors on the antiadhesive activity and biofilm destruction ability of the microbial surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241.

Materials and methods. Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in liquid mineral media containing purified glycerol or waste of biodiesel production (crude glycerol) as substrates. The biological inductors were live and inactivated cells of the yeast *Saccharomyces cerevisiae* BTM-1, as well as the supernatant after cultivation of the BTM-1 strain. Microbial surfactants were extracted from the culture supernatant using a modified Folch mixture. The anti-adhesive activity and the degree of biofilm destruction were determined by spectrophotometric method.

Results and discussion. Surfactants synthesized in the presence of all the studied inducers in a medium with glycerol of different quality were more effective anti-adhesive agents compared to surfactants obtained without an inductor. Thus, the adhesion of *Proteus vulgaris* PA-12, *Bacillus subtilis* BT-2 and *Candida albicans* D-6 on steel, tiles and linoleum treated with surfactant solutions synthesized by introducing into the medium with both substrates of all types of inductors was on average 10-70% lower than under the action of surfactants formed without an inductor. The introduction of live *S. cerevisiae* BTM-1 cells and the corresponding supernatant into a medium with glycerol of different degrees of purification was accompanied by the formation of surfactants, under the influence of which the destruction of biofilms of bacterial test cultures was on average 5-30% higher compared to the use of surfactants synthesized without an inductor. An increase of 12-35% in the destruction of *Candida* yeast biofilms was observed only under the action of surfactants synthesized in the presence of an inductor in a medium with waste of biodiesel production.

Conclusion. The results demonstrate the potential to regulate the anti-adhesive activity of *A. calcoaceticus* IMV B-7241 surfactants and their ability to destroy biofilms by adding live or inactivated *S. cerevisiae* BTM-1 yeast cells and the corresponding supernatant to the producer culture medium.

Introduction

In the modern world, increasing attention is being paid to the study of interactions between different microorganisms, particularly the impact of co-cultivation on the biosynthesis of various metabolites. Examples of such metabolites include antibiotics, immunosuppressive agents, anticancer medicines, and other bioactive compounds synthesized by microbial cultures. In the context of current biotechnological research, much focus is placed on the potential of microbial surfactants due to their unique antimicrobial, anti-adhesive properties, and ability to disrupt biofilms (Hamza et al., 2018). To date, most studies on the regulation of biological activity of microbial surfactants have utilized prokaryotic inductors, and there are only a few reports on the impact of competitive eukaryotic microorganisms on the synthesis and biological activity of surfactants (Song et al., 2020).

It was found that co-cultivation of surfactant-producing microorganisms with competitive microorganisms can significantly increase the antimicrobial, anti-adhesive and biofilm-destroying activity of the synthesized metabolites (Pirog et al., 2023a). Our earlier results demonstrated that the presence of live and inactivated cells of *Bacillus subtilis* BT-2 in the culture medium of *Acinetobacter calcoaceticus* IMV B-7241 provided a significant increase in the antimicrobial and antiadhesive activity of surfactants (Pirog & Ivanov, 2022a; Pirog et al., 2023a). It is important to mention that the effect of biological inductors on synthesis and biological activity depends on the type of microorganism and its physiological state.

Recently, there has been a growing number of publications highlighting the positive impact of eukaryotic microorganisms, particularly yeast, on the ability of prokaryotic microorganisms to synthesize metabolites with antimicrobial activity (Bai et al., 2023; Shen et al., 2024). However, there is no information in the literature on the use of eukaryotic cells as inductors to increase the antiadhesive properties of secondary metabolites or the destruction of biofilms.

Given that the mechanisms of biofilm destruction and antiadhesive activity are based on their antimicrobial activity, it was assumed that the antiadhesive activity of microbial surfactants could be increased by introducing yeasts as a biological inductor.

In view of the above, the aim of this study was to investigate the antiadhesive activity and biofilm destruction ability of *A. calcoaceticus* surfactants IMV B-7241 synthesized in the presence of *Saccharomyces cerevisiae* BTM-1.

Materials and methods

Acinetobacter calcoaceticus and its cultivation

The main object of the study was a strain of petroleum-oxidising bacteria, *Acinetobacter calcoaceticus*, isolated from an oil-contaminated soil sample and registered in the Depository of microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the IMB B-7241 number.

A. calcoaceticus IMV B-7241 was grown in liquid mineral medium as described (Pirog et al., 2023b). The carbon sources used were (%, volume fraction): purified glycerol - 3, waste of biodiesel production (crude glycerol) - 5. Glycerol concentrations of different quality are equimolar in carbon.

Cultivation of *A. calcoaceticus* IMV B-7241 was carried out in 750 ml flasks with 100 ml of medium on a shaker (320 rpm) at 30°C for 7 days.

***Saccharomyces cerevisiae* and its cultivation**

For the cultivation of the yeast strain *Saccharomyces cerevisiae* BTM-1, a liquid mineral medium similar to that used for the cultivation of surfactant producers was used, but with the replacement of the carbon source with glucose (0.5%). Cultivation was carried out for 24 hours on a shaker at 320 rpm. At the end of this period, the culture liquid was centrifuged in sterile Eppendorf microtubes (10,000 g, 10 minutes) to obtain a supernatant, which was used as an inductor by adding 2.5 ml per 100 ml of surfactant production medium. After centrifugation, the biomass (live inducer cells) was resuspended in sterile tap water to the original volume, and then 2.5 ml of this suspension was added per 100 ml of culture medium.

A portion of the resuspended biomass was sterilised at 131°C for one hour to obtain inactivated cells and 10 ml of the suspension was added to 100 ml of culture medium.

Determination of extracellular surfactant concentration

Extracellular surfactants were isolated from the culture supernatant by extraction with a mixture of chloroform and methanol (2:1) as described in (Pirog et al., 2023a). The culture liquid was centrifuged at 5000 g for 20 minutes to obtain the supernatant. Due to the fact that *A. calcoaceticus* IMV B-7241 produces a complex of various lipids, the classical Folch method (chloroform and methanol, 2:1 ratio) was modified by adding 1 N HCl (chloroform-methanol-water = 4:3:2). The described system allows the maximum separation of both polar and non-polar lipids.

Obtaining surfactant preparations

In the studies, solutions of surfactants of various concentrations were used as preparations. For this purpose, the dry surfactant residue was dissolved in sterile phosphate buffer (0.1 M, pH 7.0) to the original volume (25 ml) and further diluted with this buffer to the required concentration. The surfactant solutions were sterilized in an autoclave at 112 °C for 30 min.

Study of the degree of biofilm destruction under the action of surfactants

To determine the anti-adhesion activity of surfactants, as well as their ability to destroy microbial biofilms, bacteria (*Staphylococcus aureus* BMC-1, *Enterobacter cloacae* C-8, *Proteus vulgaris* PA-12, *Bacillus subtilis* BT-2) and yeasts (*Candida albicans* D-6 and *Candida tropicalis* PE-2) from the collection of live cultures of microorganisms of the Department of Biotechnology and Microbiology of the National University of Food Technologies were used.

The effect of surfactants on biofilm destruction was evaluated by the method described in (Pirog and Ivanov, 2022a). The spectrophotometric method was used to estimate the number of adherent cells. The degree of biofilm destruction was determined as a percentage by comparing cell adhesion in untreated and surfactant-treated wells on a polystyrene plate.

Study of the anti-adhesive activity of surfactants

The determination of the surfactant anti-adhesion properties was carried out as described in (2014). Identical plates (1 cm^2) of the tested materials were pre-cleaned with detergent, rinsed with distilled water, air-dried, and sterilised (steel plates, tiles - at 121 °C, linoleum - at 112 °C for 30 min). After sterilisation, the abiotic surfaces were treated with a surfactant solution (sterile phosphate buffer in the control variant) and kept at 30 °C for 18-24 hours. Next, the control and surfactant-treated materials were rinsed with sterile phosphate buffer or distilled water to remove the remaining surfactant.

The test cultures of microorganisms were suspended in 100 ml of sterile tap water, then pre-treated and untreated (control) materials were placed in the suspension for 2 hours at 30 °C. The control and pre-treated materials were rinsed with phosphate buffer to remove non-adherent cells. The materials with adherent cells were air-dried, after which the adherent cells were fixed by immersing the materials in methanol (99%) for 15 minutes and stained with a 1% solution of crystal violet for 5 minutes. The material plates were rinsed with tap water and left to dry at room temperature. Subsequently, the adherent cells with dye were washed from the surface of the materials using 1 ml of glacial acetic acid, to which 9 ml of distilled water was added, and the optical density of the resulting suspension was measured using a photoelectric colorimeter at a wavelength of 540 nm.

The number of adherent cells (degree of adhesion) was determined spectrophotometrically as the ratio of the optical density of the suspension obtained from the surfaces treated with surfactants (tile, steel, linoleum) to the optical density of control samples (untreated with surfactants) and expressed as a percentage.

Results and discussion

The anti-adhesive activity of microbial surfactants may be associated with the reduction of material surface hydrophobicity and changes in surface tension. Additionally, the action of most microbial surfactants promotes an increase in the hydrophobicity of test culture cell surfaces. This leads to changes in the permeability of cell membranes and can affect the reduction of the microbial cell charge, resulting in the disruption of their biological functions (Patel et al., 2021).

Effect of the physiological state of eukaryotic inductor on the anti-adhesive activity of *A. calcoaceticus* IMV B-7241 surfactants

Table 1 shows the number of cells of microbial test cultures adhered to steel plates treated with solutions of surfactants synthesized *by A. calcoaceticus* IMV B-7241 on glycerol of various degrees of purification in the presence of a yeast inductor.

Studies have shown that, regardless of the physiological state of the inductor (live, inactivated cells, supernatant), surfactants (88 μg/ml) synthesized in its presence in the media with purified or crude glycerol proved to be more effective antiadhesive agents for all test cultures studied compared to preparations obtained without the inductor. The adhesion of *P. vulgaris* PA-12, *B. subtilis* BT-2, and *C. albicans* D-6 on steel treated with surfactant solutions synthesized by adding all types of inductors to the medium with both substrates was 10-43, 8-54, and 4-18 % lower, respectively, than under the action of surfactants obtained in the medium without inductor.

Table 1

The effect of surfactants synthesized in the presence of *S. cerevisiae* BTM-1 on the degree of adhesion of bacterial and yeast test cultures to steel

Substrate	Inductor	Adhesion, %		
		***Proteus vulgaris* PA-12**	***Bacillus subtilis* BT-2**	***Candida albicans* D-6**
Purified glycerol	Control (without inductor)	70	71	48
	Live cells	60	40	30
	Inactivated cells	34	40	31
	Supernatant	53	17	44
Crude glycerol	Control (without inductor)	73	85	43
	Live cells	30	70	33
	Inactivated cells	34	77	31
	Supernatant	40	72	31

Notes. Tables 1-3: the surfactant concentration was 88 μg/ml; the error in the adhesion determination did not exceed 5 %.

Similar results to those presented in Table 1 were observed in the case of adhesion of bacterial and yeast test cultures on tiles (Table 2) and linoleum (Table 3).

Thus, after treating tiles with surfactant solutions synthesized in the presence of *S. cerevisiae* BTM-1 (live, inactivated cells, supernatant) in a medium with glycerol of various degrees of purification, the adhesion of *P. vulgaris* PA-12, *B. subtilis* BT-2 and *C. albicans* D-6 decreased by 27-71, 13-63 and 11-21 %, respectively, compared to the effect of surfactants obtained without inductors.

Table 2

Adhesion of bacteria and yeast on tiles after treatment with *A. calcoaceticus* IMV V-7241 surfactants synthesized in the presence of *S. cerevisiae* BTM-1

Substrate	Inductor	Adhesion, %		
		***Proteus vulgaris* PA-12**	***Bacillus subtilis* BT-2**	***Candida albicans* D-6**
Purified glycerol	Control (without inductor)	96	88	50
	Live cells	25	25	30
	Inactivated cells	36	37	29
	Supernatant	35	28	36
Crude glycerol	Control (without inductor)	77	78	50
	Live cells	31	N.d.	30
	Inactivated cells	50	55	39
	Supernatant	29	65	38

Note. Tables 2 and 3: n.d. - not determined

The introduction of live, inactivated yeast cells or supernatant into the media with purified or crude glycerol was accompanied by the formation of surfactants that reduced the number of bacterial cells of *P. vulgaris* PA-12 and *B. subtilis* BT-2 by 22-50 and 20-64 %, respectively, compared to the effect of surfactants synthesized without yeast inductors (Table 3).

Table 3

Adhesion of bacterial and yeast cells to linoleum treated with surfactant preparations synthesized in the presence of *S. cerevisiae* BTM-1

Substrate	Inductor	Adhesion, %		
		***Proteus vulgaris* PA-12**	***Bacillus subtilis* BT-2**	***Candida albicans* D-6**
Purified glycerol	Control (without inductor)	74	74	50
	Live cells	24	10	30
	Inactivated cells	41	54	49
	Supernatant	52	12	48
Crude glycerol	Control (without inductor)	71	75	50
	Live cells	N.d.	33	30
	Inactivated cells	N.d.	42	49
	Supernatant	31	38	48

It should be noted that the adhesion of *C. albicans* D-6 yeast on linoleum was minimal (30 %) after treatment of this surface with solutions of surfactant synthesized in the presence of live inductor cells in the medium with both substrates. The presence of inactivated *S. cerevisiae* BTM-1 cells or supernatant had practically no effect on the antiadhesive activity of surfactants against *C. albicans* D-6. The adhesion of strain D-6 cells was 48-50 % on linoleum treated with surfactant preparations synthesized both in the medium without yeast inductor and in the presence of its live cells or supernatant.

Based on the data presented in Tables 1-3, it can be concluded that the addition of yeast inductors in media with purified or crude glycerol enhances the synthesis of surfactants with high antiadhesive activity.

Destruction of biofilms under the action of *A. calcoaceticus* IMV B-7241 surfactants synthesized in the presence of eukaryotic inductor

One of the mechanisms of antiadhesive activity of microbial surfactants and the ability to destroy biofilms is their antimicrobial activity. It is known from the literature that the destruction of biofilms by lipopeptides can occur due to disruption of cytoplasmic membranes, which leads to cell lysis and metabolic leakage, and by changing the conformation of proteins, which critically affects important membrane functions (Ohadi et al., 2019).

Tables 4-6 show the summary results of the effect of surfactants synthesized with the addition of *S. cerevisiae* BTM-1 cells as an inductor during the cultivation of *A. calcoaceticus* IMV B-7241 in media with purified or crude glycerol on the destruction of bacterial and yeast biofilms.

Studies have shown that the introduction of live *S. cerevisiae* BTM-1 cells and the corresponding supernatant into the medium with glycerol of various degrees of purification led to the synthesis of surfactants that were significantly more effective in destroying biofilms of Gram-negative bacteria compared to surfactants synthesized without an inductor (Table 4). The destruction of biofilms of *E. cloacae* C-8 and *P. vulgaris* PA-12 by surfactants synthesized in the presence of live yeast cells and supernatant was 9-24 and 6-14 % higher, respectively, than by surfactants formed without inductors. The use of inactivated *S. cerevisiae* BTM-1 cells as an inductor was less effective than live cells and supernatant: the destruction of Gram-negative bacterial biofilms after treatment with such surfactants was almost the same as under the action of surfactants obtained without an inductor.

Table 4

Destruction of biofilms of Gram-negative bacterial test-cultures under the action of surfactants synthesized *by A. calcoaceticus* IMV B-7241 in the presence of *S. cerevisiae* BTM-1

Test culture	Substrate	Inductor	Destruction of biofilm, %
Enterobacter cloacae C-8	Purified glycerol	Control (without inductor)	52
		Supernatant	61
		Live cells	76
		Inactivated cells	51
	Crude glycerol	Control (without inductor)	62
		Supernatant	84
		Live cells	81
		Inactivated cells	59
Proteus vulgaris PA-12	Purified glycerol	Control (without inductor)	55
		Supernatant	61
		Live cells	69
		Inactivated cells	54
	Crude glycerol	Control (without inductor)	61
		Supernatant	67
		Live cells	71
		Inactivated cells	59

Notes. Tables 4-6: surfactant concentration 88 μg/ml; the error in determining the degree of biofilm destruction did not exceed 5%.

Under the action of surfactants obtained with the addition of live cells of the yeast *S. cerevisiae* BTM-1 or the corresponding supernatant to the media with purified or crude glycerol, the degree of destruction of biofilms of Gram-positive bacteria *B. subtilis* BT-2 and *S. aureus* BMS-1 was higher by 5-27 and 13-30 %, respectively, compared to surfactants synthesized without the addition of inductors (Table 5). The use of inactivated cells had almost no effect on the activity of surfactant preparations in the destruction of biofilms of Gram-positive test cultures.

Table 5

Effect of surfactants synthesized by *A. calcoaceticus* IMV B-7241 in presence of *S. cerevisiae* BTM-1 on the destruction of biofilms of Gram-positive test cultures

Test culture	Substrate	Inductor	Destruction of biofilm, %
Bacillus subtilis BT-2	Purified glycerol	Control (without inductor)	70
		Supernatant	N.d.
		Live cells	95
		Inactivated cells	75
	Crude glycerol	Control (without inductor)	61
		Supernatant	76
		Live cells	89
		Inactivated cells	60
Staphylococcus aureus BMC-1	Purified glycerol	Control (without inductor)	86
		Supernatant	98
		Live cells	98
		Inactivated cells	85
	Crude glycerol	Control (without inductor)	65
		Supernatant	95
		Live cells	95
		Inactivated cells	60

Note. Tables 5 and 6: N.d. - not determined

Increased destruction of biofilms of the yeast test cultures *C. tropicalis* PE-2 and *C. albicans* D-6 was found only under the action of surfactants synthesized in the presence of yeast inductors in the medium with crude glycerol (Table 6). The destruction of yeast biofilms did not depend on type of inductor (live, inactivated cells or the corresponding supernatant).

The introduction of live, inactivated *S. cerevisiae* BTM-1 cells or the corresponding supernatant into the medium with purified glycerol was accompanied by the formation of surfactants, after action of which the destruction of *C. tropicalis* PE-2 and *C. albicans* D-6 biofilms did not differ from that established for the preparations synthesized without the inductor. Our further studies will be devoted to the analysis of such an effect of the "quality" of the substrate and the inductor on the biological activity of the synthesized surfactants.

It is known from the literature that co-cultivation of bacteria with eukaryotic inductors (micromycetes, yeast) can significantly affect the synthesis and activity of secondary metabolites. There are three different categories of results that can be identified when various strains of bioactive metabolite producers are co-cultured with eukaryotic inductors: 1) increased synthesis of biologically active compounds (Bai et al., 2023; DeFilippi et al., 2018; Fifani et al., 2022; Li et al., 2020; Liu et al., 2022 a, b; Pan et al., 2021; Ramchandran et al., 2020; Sharma et al., 2022; Song et al., 2020; Wu et al., 2018); 2) synthesis of metabolites with cytotoxic activity (Cowled et al., 2023; Meng et al., 2024; Sun et al., 2021); 3) synthesis of new secondary metabolites, or synthesis of metabolites that are not synthesized in monocultures (Cowled et al., 2023; Meng et al., 2024; Shen et al., 2024; Sun et al., 2021).

Table 6

Degree of destruction of yeast biofilms under the action of surfactants synthesized in the presence of yeast inductors

Test culture	Substrate	Inductor	Destruction of biofilm, %
Candida tropicalis PE-2	Purified glycerol	Control (without inductor)	57
		Supernatant	52
		Live cells	60
		Inactivated cells	53
	Crude glycerol	Control (without inductor)	60
		Supernatant	95
		Live cells	95
		Inactivated cells	92
Candida albicans D-6	Purified glycerol	Control (without inductor)	64
		Supernatant	59
		Live cells	64
		Inactivated cells	57
	Crude glycerol	Control (without inductor)	54
		Supernatant	N.d.
		Live cells	75
		Inactivated cells	66

At the same time, there are few reports in the literature on the effect of eukaryotic inductors on the synthesis and especially the biological activity of microbial surfactants. In recent years, several studies have been published on the effect of yeast inductors on the synthesis of surfactants and their antimicrobial activity (Bai et al., 2023; DeFilippi et al., 2018; Fifani et al., 2022; Liu et al., 2022 a, b; Pan et al., 2021; Ramchandran et al., 2020; Wang et al., 2022).

The effect of fungal inductors *Fusarium sambucinum* 2351, *Rhizopus stolonifer* 198 and *Verticillium dahliae* 175 on the synthesis of lipopeptides by *Bacillus subtilis* strain B9-5 showed an increase in the concentration of fengicin and surfactin by 15-30 % when co-cultured with *R. stolonifer* 198 (DeFilippi et al., 2018).

In the study (Pan et al., 2021), it was found that when *Bacillus amyloliquefaciens* HM618 was co-cultivated with micromycetes (*Aspergillus oryzae* BNCC338380, *Trichoderma reesei* BNCC337997, and *Aspergillus nidulans* BNCC190203), an increase in the level of surfactin synthesis by more than 2 times and an increase in its antifungal activity against the pathogenic fungi *Rhizoctonia solani* and *Botrytis cinerea* were observed. The researchers suggested that this effect may be due to the formation of hydrolytic enzymes by micromycetes.

It was found (Fifani et al., 2022) that the addition of supernatant or autoclaved mycelium of *Trichoderma harzianum* IHEM5437 to the culture medium of the lipopeptide producer *Bacillus velezensis* GA1 slightly increased the concentration of iturin, fengicin and surfactin. The researchers explained it by the ability of fungi to produce amino acids and proteins that bacteria use as a source of nitrogen.

In the works (Liu et al., 2022 a, b), it was found that the addition of live *Magnaporthe oryzae* Guy11 cells or their supernatant to the culture medium of *Streptomyces bikiniensis* HD087 led to an increase in the concentration of lipopeptides in crude extract by 107.4 % compared to cultivation without inductors. The authors noted that the inductor activates the tricarboxylic acid cycle, which in turn leads to an increase in the level of reducing equivalents (NADH and FADH), as well as ATP and pyruvate, key metabolites for fatty acid biosynthesis.

In a study (Ramchandran et al., 2020), it was shown that in the presence of autoclaved *C. albicans* SC 5314 cells in the culture medium of the surfactant producer *B. subtilis* RLID 12.1, the concentration of AF3 and AF5 lipopeptides increased by 1.4 and 2 times, respectively, compared to cultivation without an inductor. In addition, these lipopeptides demonstrated a high level of antifungal activity against *Candida auris* yeast strains (minimum inhibitory concentrations were 4-16 μg/ml).

In the study (Bai et al., 2023), it was found that co-cultivation of the recombinant *Yarrowia lipolytica* YL21 strain with *B. amyloliquefaciens* HM618 was accompanied by an increase in the synthesis of fengicin, surfactin and iturin A by 7, 12 and 3 times, respectively, compared to the monoculture of *B. amyloliquefaciens* HM618.

Wang with co-authors (2022) found that co-cultivation of the fungicin producer *B. amyloliquefaciens* HM618 with the genetically modified yeast *Pichia pastoris* GS115 led to a 2-7-fold increase in the concentration of synthesized fengicin compared to the concentration obtained in bacterial monoculture.

In recent years, the literature has reported an increase in the synthesis of secondary metabolites other than surfactants (in particular, antibiotics) using eukaryotic inductors (Li et al., 2020; Song et al., 2020; Sharma et al., 2017; Wu et al., 2018).

A study (Song et al., 2020) showed that the addition of live *Fusarium oxysporum* f. sp. *cucumerinum* cells or supernatant after cultivation of this strain to *Streptomyces rimosus* M527 culture medium led to a 1.8- and 1.5-fold increase in the concentration of the antibiotic rimocidin compared to cultivation without inductor.

Sharma et al. (2017) found that in the presence of live *S. cerevisiae* cells, the synthesis of valinomycin by *Streptomyces lavendulae* strain ACR-DA1 increased by 34% compared to the one without inductor.

There are also studies that report the synthesis of new secondary metabolites, including those with cytotoxic activity, with the use of eukaryotic inductors (Cowled et al., 2023; Meng et al., 2024; Shen et al., 2024; Sun et al., 2021).

In the study (Cowled et al., 2023), two new compounds with cytotoxic activity (myctospiromide A and kitrinomycin A) were identified in the co-culture of the micromycetes *Penicillium brasilianum* MST-FP1927 and *Aspergillus nomius* MST-FP2004. The compound kitrinomycin A showed significant biological activity against NS-1 murine melanoma cells (LD_{99} 7.8 μM) and against the cattle parasite *Tritrichomonas foetus* (LD_{99} was 4.8 μM).

Shen et al. (2024) showed that the co-cultivation of *A. oryzae* (strain was not shown) and *Epicoccum dendrobii* (strain was not shown) was accompanied by the synthesis of new secondary metabolites, such as epiclactone A, epiclactone B, epioxochromane and aoergostane. It was found (Meng et al., 2024) that the addition of *A. oryzae* cells to the culture

of *Monascus purpureus* resulted in the formation of two new cyclohexylfurans, monaspins A and B. Monaspin B showed potent antiproliferative activity against the leukemic cell line HL-60, inducing apoptosis, with an IC_{50} of 160 nM.

At the same time, it should be noted that to date there is a limited number of publications on the effect of surfactants synthesized in the presence of inducers on the destruction of microbial biofilms (Hamza et al., 2018; Kimelman and Shemesh, 2019; Mohamed et al., 2020; Pirog and Ivanov, 2022a) or the antiadhesive activity of surfactants (Pirog and Ivanov, 2022b).

Our previous results (Pirog and Ivanov, 2022a) showed that the introduction of *B. subtilis* BT-2 (live or inactivated cells or supernatant) into the *A. calcoaceticus* IMB B-7241 cultivation medium enhanced the antibiofilm activity of the synthesized surfactants. The degree of destruction of bacterial and yeast biofilms by the *A. calcoaceticus* IMB B-7241 surfactants obtained with the introduction of a prokaryotic inducer was 1.5-3 times higher compared to the use of surfactants synthesized in a medium without inductors.

The results reported in this article showed that even in the presence of a eukaryotic inductor, surfactants were obtained with a higher ability to destroy biofilms compared to surfactants synthesized by monoculture (see Tables 4-6). However, it should be noted that the destruction of biofilms under the action of surfactants synthesized with the addition of a yeast inductor was 1.1-1.7 times higher compared to the use of preparations obtained without the addition of inductors. Therefore, the eukaryotic inductor was slightly less effective than the prokaryotic inductor, in the presence of which surfactants were synthesized that increased biofilm destruction by 1.5-3 times (Pirog and Ivanov, 2022a).

Meanwhile, the level of increase in the antiadhesive activity of *A. calcoaceticus* IMV B-7241 surfactants synthesized in the presence of both prokaryotic and eukaryotic inductors was almost the same (Pirog and Ivanov, 2022b) (Tables 1-3 of this article). In particular, when abiotic materials (steel, linoleum, tiles) were treated with surfactant preparations obtained by adding biological inductors, the adhesion of bacterial and yeast test cultures decreased by 1.1-5.9 times (Pirog and Ivanov, 2022b) and 1.1-6 times (Tables 1-3 of this article).

Conclusions

The results obtained demonstrate that it is possible to regulate not only the antimicrobial but also the antiadhesive activity of surfactants synthesized by *A. calcoaceticus* IMV B-7241 and their ability to destroy biofilms by introducing live or inactivated cells of the yeast *S. cerevisiae* BTM-1 and the corresponding supernatant into the media containing crude or purified glycerol. These studies are important for understanding the complex interactions between competing microorganisms and the effect of inductors on the biological activity of microbial secondary metabolites, which is essential for the development of new strategies in the microbiological and biotechnological industries.

References

Allegrone G., Ceresa C., Rinaldi M., Fracchia L. (2021), Diverse effects of natural and synthetic surfactants on the inhibition of *Staphylococcus aureus* biofilm, *Pharmaceutics*, 13(8), 1172, https://doi.org/10.3390/pharmaceutics13081172

Bai S., Qiao B., Hou Z. J., Gao G. R., Cao C. Y., Cheng J. S., Yuan Y. J. (2023), Mutualistic microbial community of *Bacillus amyloliquefaciens* and recombinant *Yarrowia lipolytica* co-produced lipopeptides and fatty acids from food waste, *Chemosphere*, 310, 136864, https://doi.org/10.1016/j.chemosphere.2022.136864

Chen W., Wang M., Gong Y., Deng Q., Zheng M., Chen S., Wan X., Yang C., Huang F. (2021), The unconventional adverse effects of fungal pretreatment on iturin A fermentation by *Bacillus amyloliquefaciens* CX-20, *Microbial Biotechnology*, 14(2), pp. 587–599, https://doi.org/10.1111/1751-7915.13658

Cowled M. S., Kalaitzis J. A., Crombie A., Chen R., Sbaraini N., Lacey E., Piggott A. M. (2023), Fungal duel between *Penicillium brasilianum* and *Aspergillus nomius* results in dual induction of miktospiromide A and kitrinomycin A, *Journal of Natural Products*, 86(10), pp. 2398–2406, https://doi.org/10.1021/acs.jnatprod.3c00593

DeFilippi S., Groulx E., Megalla M., Mohamed R., Avis T.J. (2018), Fungal competitors affect production of antimicrobial lipopeptides in *Bacillus subtilis* strain B9–5, *Journal of Chemical Ecology*, 44, pp. 374—383, https://doi.org/10.1007/s10886-018-0938-0

Fifani B., Steels S., Helmus C., Delacuvellerie A., Deracinois B., Phalip V., Delvigne F., Jacques P. (2022), Coculture of *Trichoderma harzianum* and *Bacillus velezensis* based on metabolic cross-feeding modulates lipopeptide production, *Microorganisms*, 10(5), 1059, https://doi.org/10.3390/microorganisms10051059

Hamza F., Kumar A. R., Zinjarde S. (2018), Coculture induced improved production of biosurfactant by *Staphylococcus lentus* SZ2: Role in protecting *Artemia salina* against *Vibrio harveyi*, *Enzyme and Microbial Technology*, 114, pp. 33-39, https://doi.org/10.1016/j.enzmictec.2018.03.008

Kimelman H., Shemesh M. (2019), Probiotic bifunctionality of *Bacillus subtilis*-rescuing lactic acid bacteria from desiccation and antagonizing pathogenic *Staphylococcus aureus*, *Microorganisms*, 7, 407, https://doi.org/10.3390/microorganisms7100407

Li T., Tang J., Karuppiah V., Li Y., Xu N., Chen J. (2020), Co-culture of *Trichoderma atroviride* SG3403 and *Bacillus subtilis* 22 improves the production of antifungal secondary metabolites, *Biological Control*, 140, 104122, https://doi.org/10.1016/j.biocontrol.2019.104122

Liu W., Wang J., Li S., Zhang H., Meng L., Liu L., Ping W., Du C. (2022a), Genomic and biocontrol potential of the crude lipopeptide by *Streptomyces bikiniensis* HD-087 against *Magnaporthe oryzae*, *Frontiers in Microbiology*, 13, 888645, https://doi.org/10.3389/fmicb.2022.888645

Liu W., Wang J., Zhang H., Qi X., Du C. (2022b), Transcriptome analysis of the production enhancement mechanism of antimicrobial lipopeptides of *Streptomyces bikiniensis* HD-087 by coculture with *Magnaporthe oryzae* Guy11, *Microbial Cell Factories*, 21(1), pp. 1—11, https://doi.org/10.1186/s12934-022-01913-2

Meng Z., Tan Y., Duan Y., Li M. (2024), Monaspin B, a novel cyclohexyl-furan from cocultivation of *Monascus purpureus* and *Aspergillus oryzae*, exhibits potent antileukemic activity, *Journal of Agricultural and Food Chemistry*, 72(2), pp. 1114-1123, https://doi.org/10.1021/acs.jafc.3c08187

Mohamed S.H., Hossam M., Mohammed R., Mohamed M.F., Ahmed S.M., Ahmed A.H., Sameh F.A., Mostafa E.R., Hani A.A., Ramadan A.U. (2020), Induction of antibacterial metabolites by co-cultivation of two red-sea-sponge-associated *Actinomycetes micromonospora* sp. UR56 and *Actinokinespora* sp. EG49, *Marine Drugs*, 18(5), 243, https://doi.org/10.3390/md18050243

Ohadi M., Forootanfar H., Dehghan-Noudeh G., Eslaminejad T., Ameri A., Shakibaie M., Adeli-Sardou M. (2019), Antimicrobial, anti-biofilm, and anti-proliferative activities of lipopeptide biosurfactant produced by *Acinetobacter junii* B6, *Microbial Pathogenesis*, 138, 103806, https://doi.org/10.1016/j.micpath.2019.103806

Pan F.D., Liu S., Xu Q.M., Chen X.Y., Cheng J.S. (2021), Bioconversion of kitchen waste to surfactin via simultaneous enzymolysis and fermentation using mixed-culture of enzyme-producing fungi and *Bacillus amyloliquefaciens* HM618, *Biochemical Engineering Journal*, 172, 108036, https://doi.org/10.1016/j.bej.2021.108036

Patel M., Siddiqui A.J., Hamadou W.S., Surti M., Awadelkareem A. M., Ashraf S.A., Alreshidi M., Snoussi M., Rizvi S.M.D., Bardakci F., et al. (2021), Inhibition of bacterial adhesion and antibiofilm activities of a glycolipid biosurfactant from *Lactobacillus rhamnosus* with its physicochemical and functional properties, *Antibiotics*, 10(12), 1546, https://doi.org/10.3390/antibiotics10121546

Pirog T., Ivanov M. (2022a), Destruction of biofilms by surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 in the presence of competitive microorganisms, *Ukrainian Food Journal*, 11(2), pp. 302-314, https://doi.org/10.24263/2304-974X-2022-11-2-9

Pirog T., Ivanov M. (2022b), Influence of biological inductors on the anti-adhesive activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants, *Scientific Works of NUFT*, 28(4), pp. 36–46, https://doi.org/10.24263/2225-2924-2022-28-4-5

Pirog T.P., Ivanov M.S., Shevchuk T.A. (2023a), Biological activity of *Acinetobacter calcoaceticus* IMV B-7241 surfactants synthesized in the presence of competitive bacteria *Bacillus subtilis* BT-2, *Microbiological Journal*, 4, pp. 21−33, https://doi.org/10.15407/microbiolj85.04.021

Pirog T., Stabnikov V., Stabnikova O. (2023b), Microbial surfactants production from industrial waste, In O. Stabnikova, O. Shevchenko, V. Stabnikov, O. Paredes-López (Eds.), *Bioconversion of waste to value-added products* (pp. 271-304), CRC Press, Boca Raton, https://doi.org/ 10.1201/9781003329671-10

Ramchandran R., Ramesh S., A A., Thakur R., Chakrabarti A., Roy U. (2020), Improved production of two anti-*candida* lipopeptide homologues co-produced by the wild-type *Bacillus subtilis* RLID 12.1 under optimized conditions, *Current Pharmaceutical Biotechnology*, 21(5), pp. 438–450, https://doi.org/10.2174/1389201020666191205115008

Shen X., Lei C., Zhang A., Wang L., Chen D., Qi L., Hu Y., Chen G., Ran H., Yin W.B. (2024), Stimulating novel and bioactive metabolite production by cocultivation of two fungi─*Aspergillus oryzae* and *Epicoccum dendrobii*, *Journal of Agricultural and Food Chemistry*, 72(30), pp. 16825-16834, https://doi.org/10.1021/acs.jafc.4c04764

Song Z., Ma Z., Bechthold A., Yu X. (2020), Effects of addition of elicitors on rimocidin biosynthesis in *Streptomyces rimosus* M527, *Applied Microbiology and Biotechnology*, 104, pp. 4445-4455, https://doi.org/10.1007/s00253-020-10565-4

Sun Y., Liu W.C., Shi X., Zheng H.Z., Zheng Z.H., Lu X.H., Xing Y., Ji K., Liu M., Dong Y.S. (2021), Inducing secondary metabolite production of *Aspergillus sydowii* through microbial co-culture with *Bacillus subtilis*, *Microbial Cell Factories*, 20(42), 42, https://doi.org/10.1186/s12934-021-01527-0

Wang D., Yuan J., Gu S., Shi Q. (2013), Influence of fungal elicitors on biosynthesis of natamycin by *Streptomyces natalensis* HW-2, *Applied Microbiology and Biotechnology*, 97, pp. 5527–5534, https://doi.org/10.1007/s00253-013-4786-0

Wang X. F., Miao C. H., Qiao B., Xu S. J., Cheng J. S. (2022), Co-culture of *Bacillus amyloliquefaciens* and recombinant *Pichia pastoris* for utilizing kitchen waste to

produce fengycins, *Journal of Bioscience and Bioengineering*, 133(6), pp. 560—566, https://doi.org/10.1016/j.jbiosc.2022.02.009

Wu Q., Ni M., Dou K., Tang J., Ren J., Yu C., Chen J. (2018), Co-culture of *Bacillus amyloliquefaciens* ACCC11060 and *Trichoderma asperellum* GDFS1009 enhanced pathogen-inhibition and amino acid yield, *Microbial Cell Factories*, 17, 155, https://doi.org/10.1186/s12934-018-1004-x

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Marketing in field of vegetarian food products

Svitlana Tkachuk, Svitlana Stakhurska, Larysa Kapinus, Andrii Nykonenko

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Svitlana Tkachuk
E-mail: lollalag@i.ua

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Abstract

Introduction. The aim of the study was to identify problems and prospects for the development of marketing policies for vegetarian food brands.

Materials and methods. Data were collected using desk research from open sources of vegetarian food brands and retailers. The study was conducted using the methods of generalization, analysis and synthesis of information. The analysis based on J. McCarthy's 4P model was applied.

Results and discussion. India has the highest level of vegetarianism in the world (about 38% of the population). A high level of vegetarianism is also observed in Mexico (19% of the population). Since the beginning of the 21st century, vegetarianism has been spreading in Ukraine. Vegetarianism is present in almost all countries of the world. Even in countries with a high percentage of animal products consumption (the United States (88%), Australia (86%), Argentina (85%)), the movement to protect the rights of farm animals is gaining popularity (46% of global funding for this movement comes from the United States), which is one of the motivations for the transition to vegetarianism. According to Fortune Business Insights, the global vegan food market is expected to grow by 163%, or $38.05 billion. Out of the total number of vegetarians, 6% are vegans, 8% are typical vegetarians, 9% refuse to eat meat, 33% limit their meat consumption, 15% limit their consumption of milk and dairy products, and 29% limit their consumption of both meat and dairy products. Among the motives for switching to vegetarianism, it is worth highlighting the environmental motive (64%), the health motive (24%), and the ethical motive (12%). Marketing strategies of vegetarian food producers include niche (according to F. Kotler) and concentrated differentiation strategies (according to M. Porter). The marketing product policy of vegetarian brands is represented by such categories as vegetable alternatives to meat and meat products (25%), vegetable alternatives to milk and dairy products (15%), vegetarian sweets (35%) and other categories, such as fast food, ready meals, and baked goods (25%). Considering the marketing mix of vegetarian brands according to J. McCarthy's "4P" model, a problem in the marketing pricing policy was identified. The average percentage of excess prices for vegan alternatives compared to conventional products is 236%, in particular by 87% for plant-based meat, by 170% for dairy products and by 450% for sweets. Such a price difference is one of the barriers to a faster transition to vegetarianism and the development of vegan products.

Conclusions. The problem points in the marketing mix of food brands can be eliminated through an improved marketing policy mechanism that will provide economic, environmental and marketing effects.

Introduction

There are many studies devoted to marketing issues. Scientific approaches to forming marketing strategies, developing marketing mixes and mechanisms for improving marketing policy are adapted to most areas of activity. Scientists have developed specialized marketing models for various industries. Factors that may prevent people from adopting a vegetarian diet or negatively impact the quality of life of those who follow it have been identified and investigated (Hargreaves et al., 2021). Vegetarianism has been found to have positive effects on humans: better physical health, positive feelings associated with adopting a morally correct attitude towards animals, an increased sense of belonging to the vegetarian community, and a reduced impact on the environment. Factors beyond human control have also been identified, including economic aspects, as well as socio-cultural (membership of social (cultural) groups) and natural aspects (restricted access to products) (Baroni et al., 2024). Vegetarianism is also defined from the perspective of a person's social identity, values, beliefs, and views (Nezlek et al., 2020).

However, marketing models and mechanisms that could be adapted and used in the field of vegetarian food products remain unexplored in many aspects. The solution to this issue is relevant, because, although it is niche marketing, a relatively significant segment of the world's population is to some extent a vegetarian diet. The theory of vegetarianism originated in India and the eastern Mediterranean in the middle of the first millennium BC (Petruzzello et al., 2024). In the 17th - 19th centuries AD, certain aspects of vegetarianism were practiced and popularized in the UK (Petruzzello et al., 2024). In 1847, the first vegetarian society was established in England, and in 1908, the International Vegetarian Union was founded in Germany with its headquarters in the UK (Petruzzello et al., 2024). In the West, until the beginning of the 20th century, vegetarianism was mostly seen as a dietary supplement to the usual diet, for example, for medicinal purposes (Petruzzello et al., 2024). The impetus for the further development of vegetarianism in the second half of the 20th century was the work of Peter Singer, an Australian ecophilosopher. Supporters of this theory were guided by an ethical motive. Opponents of P. Singer's views appealed to the lack of adequate protein in the human diet with a vegetarian diet, but further research largely refuted this statement (American et al., 2003; Petruzzello et al., 2024). At the beginning of the twentieth century, the vegetarian movement was developing in Ukraine, which could have become one of the centres of vegetarianism in Europe (Pyvovarenko, 2019). The basis was primarily ideological, although there were both medical and ideological (ethical) trends of vegetarianism. In total, there were 11 vegetarian societies in Ukraine. The most popular was lacto-ovo vegetarianism. In the early twentieth century, there were about 12 vegetarian canteens in Kyiv, about 10 in Odesa, 2 in Kharkiv, and 2 in Poltava. There was a price differentiation, and local culinary peculiarities of each city were taken into account. The promoters of vegetarian cuisine were people with high incomes, but the consumers were quite different, with students making up a significant segment of the vegetarian canteen customers. Vegetarian canteens were located near large educational institutions (Pyvovarenko, 2019). During the totalitarian era, the Ukrainian vegetarian movement was virtually stopped, but at the beginning of the twenty-first century, it is being restored in line with global trends, so it is important to study, among other countries, the experience of Ukraine in marketing vegetarian brands, taking into account the specifics of the history, decline and revival of vegetarianism in this country. The study of marketing in the field of vegetarian food products will identify existing trends, as well as problems to be solved and prospects for solving these problems, which will enable domestic and foreign vegetarian brands to improve their operations and lead to economic, environmental and marketing effects.

The purpose of the research was to identify the problems and prospects for the development of marketing policy of vegetarian food brands.

Materials and methods

Materials

Publicly available electronic resources of more than 50 vegetarian food brands and retailers were used to assess the marketing policy in the field of vegetarian food products.

To assess the importance of such a study, the works of scholars and practitioners in the field of marketing and vegetarianism were used. To determine the methodological tools that formed the basis of the study, the scientific works of leading marketers were used.

Methods

In the process of studying the marketing of vegetarian food brands, the methods of desk research, observation, analysis and synthesis, and generalisation were used.

The desk research method (Ratchford, 2020) was used to study the marketing policy of vegetarian brands. In the course of desk research, information on product, pricing and communication marketing policies was collected, systematised and analysed from more than 50 electronic resources. The data obtained were further generalised to identify key trends, problems and prospects for the development of marketing tools for vegetarian food brands.

In the course of the study, the methods of analysis and synthesis (Silva, 2022) were used, which allowed to identify the main problems of the marketing policy of vegetarian brands and formulate recommendations for their elimination.

Methodof observation (Silva, 2022) contributed to identifying current practices of vegetarian food brands in the field of marketing communications.

Method of modelling (Silva, 2022) was used to develop a mechanism for improving the marketing policy of vegetarian food brands.

As a basis for the study of the marketing complex of vegetarian food brands, the J. McCarthy's "4Ps" model (Mada, 2024) was used (Figure 1).

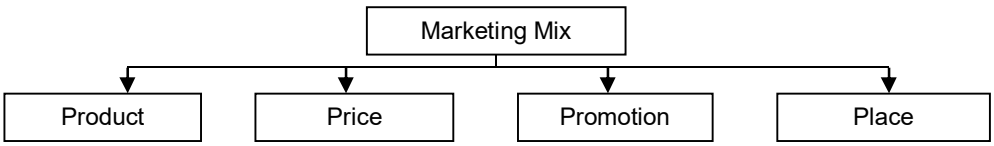

Figure 1. J. McCarthy's "4P" model
Source: (Mada, 2024).

J. McCarthy's "Marketing Mix" or "4P" model is one of the fundamental concepts of marketing management, which defines the key elements of an effective marketing strategy. With its help, the following elements were analyzed:

– Product: tangible or intangible goods and services that a company offers to consumers. Important aspects include quality, design, packaging, assortment, product life cycle and unique characteristics that shape its competitiveness;

- Price: The cost of a product to consumers and includes pricing strategies, price levels, discounts, payment and credit terms. Price affects demand, profitability and brand positioning in the market;
- Place: The ways in which a product is delivered to the end user. This includes distribution channels, logistics, inventory management, and the choice of outlets (retail, online, etc.);
- Promotion: communication strategies aimed at attracting and retaining customers. The main tools are advertising, PR, personal selling, sales promotion and digital marketing. In terms of current trends, digital marketing methods in the field of vegetarian brands were separately investigated.

In order to make the proposals for the marketing policy of vegetarian brands more customer-oriented, the study was based on an improved transformation of J. McCarthy's model, the SIVA model (Mada, 2024) (Figure 2).

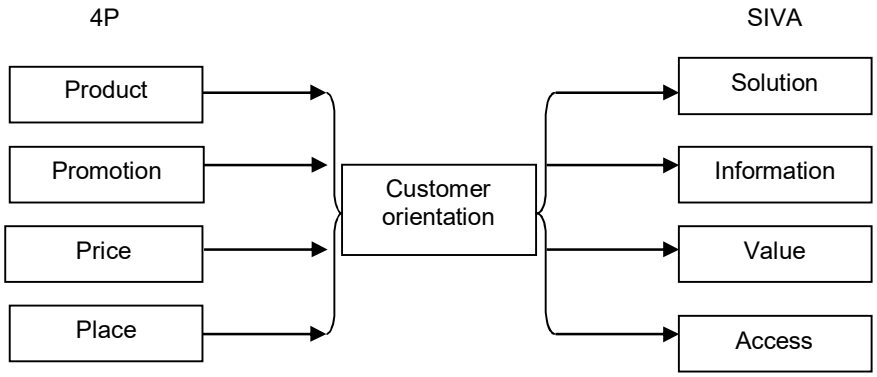

Figure 2. Transformation of 4P model into SIVA model
Source: formed by the authors based on the source (Mada, 2024).

The Solution element was considered from the standpoint of the value of the marketing offer solution for the consumer, the ability to solve the client's problems, and ensure the elimination of key undesirable phenomena.

Promotion was considered in terms of maximum nativeness and targeting of marketing messages for customers: the correspondence of communication channels, the concept of advertising messages and other types of promotion to the target audience.

The Price element was studied taking into account not only the price, but also the value for the customer, as a set of complex interrelated elements, which involves taking into account the following factors:

- the ratio of the marketing offer and the price of the product;
- alternatives for spending money and time on purchasing goods;
- the availability of alternatives in the form of substitute goods.

The Place element was considered from the standpoint of the availability of purchasing goods by end consumers.

To assess the effectiveness of SMM (social media marketing) in the aspect of digital promotion, the following indicators were calculated:

1. Average Number of Reels Views: the indicator is calculated by dividing the total number of views by the total number of Reels published during a certain period (Trunfio et al., 2021):

$$\text{Average Reels Views} = \frac{\text{Total Views on Reels}}{\text{Total Number of Reels Posted}} \quad (1)$$

2. Average Number of Comments on Social Media: is determined by dividing the total number of comments received by the total number of posts published over a certain period of time (Trunfio et al., 2021).

$$\text{Average Number of Comments} = \frac{\text{Total Comments}}{\text{Total number of posts}} \quad (2)$$

3. Engagement Rate (Activity Level on Social Media): Engagement rate measures how actively users interact with content. It is calculated using various engagement metrics, such as likes, comments, shares, and saves, relative to the number of followers or impressions. The overall level of engagement is calculated using all these indicators (Trunfio et al., 2021).

$$\text{Engagement Rate} = \frac{\text{Likes+Comments+Shares+Saves}}{\text{Total Followers}} \quad (3)$$

The higher the value of each of the indicators, the more effective the SMM strategy, and the higher the level of subscriber engagement.

Results and discussion

Competitive strategies of vegetarian food market players

Competitive strategies of vegetarian brands can be attributed to strategies that involve the formation of high-order competitive advantages, namely differentiation, uniqueness of the offer in a niche segment (Figure 3).

Thus, it can be argued that, according to the method of M. Porter (Dieffenbacher, 2023), most market participants use a competitive focus strategy based on differentiation or an optimisation strategy. According to the method of F. Kotler (Kotler, 2021), most producers are niche players in the overall food market, mostly striving for niche leadership. The share of vegetarians ranges from 1% to 38% around the world (Geeksforgeeks, 2024; Palyvoda, 2021), meaning that the target audience of market players is quite narrow, so the competitive strategy of market players is niche. Within the niche, the strategies of niche leaders are also used: market expansion through variations in positioning (search for new needs), reaching new consumers and intensifying promotion (Kotler, 2021). According to the method of I. Ansoff (Heubel, 2024), strategies of deep market penetration (within a niche), market expansion (entering a market related to an existing one), product line expansion, or product development are usually used. In terms of diversification, there is limited (modified product, related market) or partial (new product, related market) diversification. Full diversification is rarely used. The STP (segmentation, targeting, positioning) marketing strategy (Kotler, 2021) of vegetarian food brands is characterised by targeted concentrated marketing and positioning of the marketing offer based on three main motives: environmental friendliness, health, ethics, and an emphasis on modernity and progressiveness.

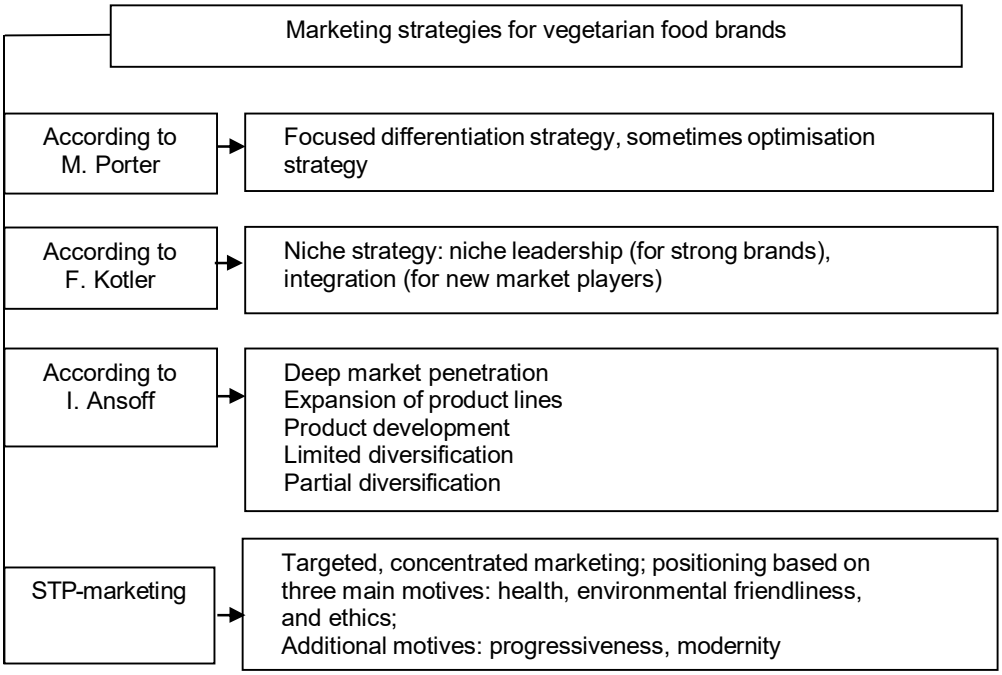

Figure 3. Characteristics of marketing strategies of vegetarian restaurants food brands
Source: Own study based on the approaches to marketing strategies (Dieffenbacher, 2023; Heubel, 2024; Kotler, 2021) and open source materials of vegetarian brands.

Characteristics of vegetarian food consumers and motives for switching to vegetarianism

The global plant-based meat market was worth $8.6 billion as of 2020. According to forecasts, by 2026, the market is expected to grow to $10.1 billion (Mishler, 2023). According to research by Sana Ijaz, as of 2018, about 5% of the world's population identifies as vegetarians and 3% as vegans (Ijaz, 2023). The percentage of vegetarians is on the rise. According to a Statista Consumer Insights survey conducted in 21 countries, as of 2023, 86% of the population adheres to a meat diet. That is, about 14% limit or exclude meat and other livestock products from their diets to some extent. The main consumer segment that prefers a vegetarian diet by age is young people, and women predominate by gender. According to Fortune Business Insights (as of 2021), the global vegan food market is expected to grow by 163%, or $38.05 billion (Ijaz, 2023). According to research by the Food and Agriculture Organisation of the United Nations, the highest level of vegetarianism in the world is observed in India, followed by Mexico, then Brazil, Taiwan, Israel, Australia, Argentina, Finland, Sweden, and Austria in descending order (Figure 4) (Geeksforgeeks, 2024).

According to research by Glanbia Nutritionals and UA Plant-Based, the following structure of vegetarianism was observed in Western Europe (Figure 5).

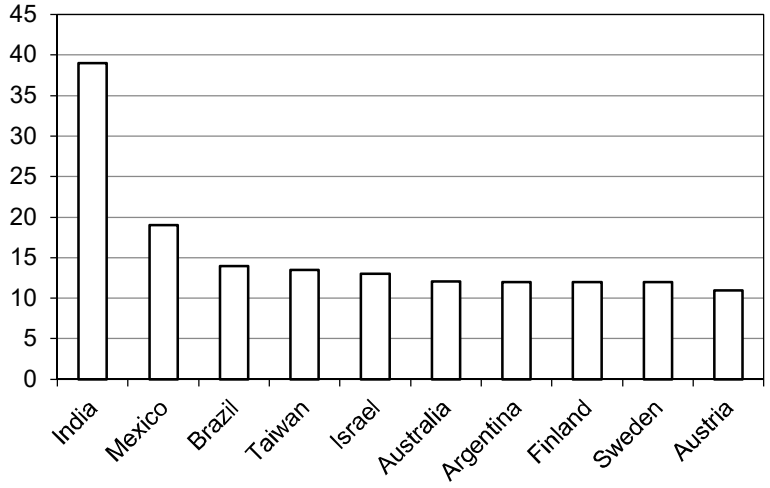

Figure 4. Level of vegetarianism in the world (% of the total population)
Source: (Geeksforgeeks, 2024).

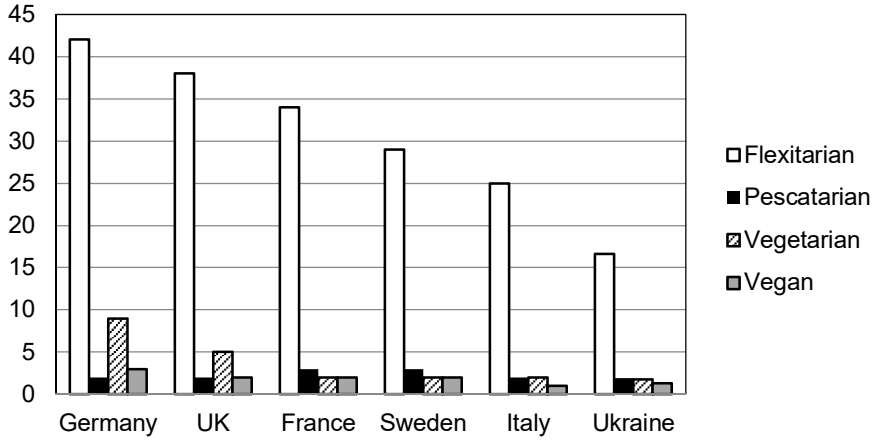

Figure 5. Level of vegetarianism in European countries (% of the total population)
Sources: (Glanbia, 2023; Kozachok, 2022).

The diagram shows that vegetarianism is present in all the countries analysed, and the level of flexitarianism (conditional vegetarianism, which involves limiting meat without completely giving it up) is generally. This indicates that there is a demand in European countries for vegetarian products, including plant-based meat and milk substitutes, and thus the importance of developing this area. Countries with low levels of vegetarianism include the United States, Australia, Argentina, and China (Geeksforgeeks, 2024). However, general global trends in vegetarianism are gradually introducing the popularity of this food concept in these countries as well (Ijaz, 2023).

For example, one of the world's most famous vegetarian brands, which has made a significant contribution to the development of vegetarianism in the world, is the Beyond Meat brand (California, USA). Even McDonald's Corporation has expanded its targeting of vegetarians in some countries by offering dishes with Beyond Meat plant-based burgers. Also, according to Animal Charity Evaluators (2024), the United States is the world leader in funding for the protection of animal rights on farms (45.75% of total global funding), The UK is in second place (8.29%), followed by Germany (6.14%), France (5.87%), Sweden (4.46%), the Netherlands (4.40%), Israel (3.02%), Brazil (2.29%), China (2.17%), and the share of other countries is 17.6% (Figure 6).

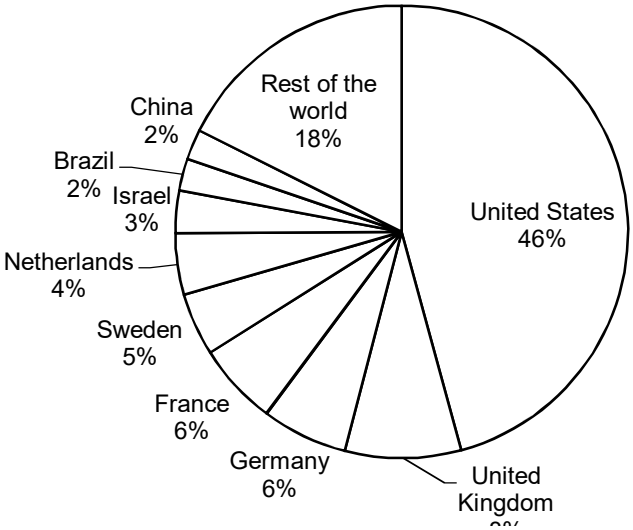

Figure 6. Geographical structure of funding for the protection farm animals
Source: (Animal, 2024).

In other words, even in countries where vegetarianism is not popular, ethical issues of protecting the rights of farm animals are being actively raised, which is an impetus for the development of vegetarianism. Despite the relatively low level of vegetarianism in the United States, many of the world's best-known vegetarian brands belong to this country (Mishler, 2023). The markets of Brazil and China are also promising for the development of vegetarian food brands, in particular due to rapid urbanisation and the need for access to various products, including vegetarian ones (Mishler, 2023).

Figure 5 shows that along with Western European countries that have long been developing on the basis of a market economy, Ukraine, which began to implement a market economy only in early 1990s, has significant potential for the development of vegetarian food brands. Another noteworthy fact is that vegetarianism was actively developing in Ukraine in the early twentieth century, and having been destroyed by the Soviet authorities, it has been gaining popularity again since the beginning of the twenty-first century, which has led to the establishment of a large number of companies producing vegetarian products, some of which are entering the international market. Although the overall level of vegetarianism in Ukraine is slightly lower than in many European countries, the percentage of people who adhere to

this type of diet is still quite high, which has led to the emergence and development of many national vegetarian brands. According to the results of UA Plant-Based research published at the end of 2022, 21.6% of the total number of Ukrainians refuses to consume animal products to some extent (Kozachok, 2022). The largest percentage is made up of people who simply limit their consumption of meat products (7%), followed by people who limit their consumption of meat and dairy products (6.3%) and people who limit their consumption of dairy products (3.3%). Thus, the total share of conditional vegetarians in Ukraine is about 16.6%. The share of people who completely refuse to eat meat but consume other animal products is 1.9%, and vegetarians - 1.8%. Hard vegetarianism, i.e. veganism, is only 1.3% (Kozachok, 2022). At the same time, the overall ratio of supporters of traditional food and supporters of vegetarianism is about 80/20%.

Below is a diagram of the structure of vegans and vegetarians for Ukraine (Figure 7).

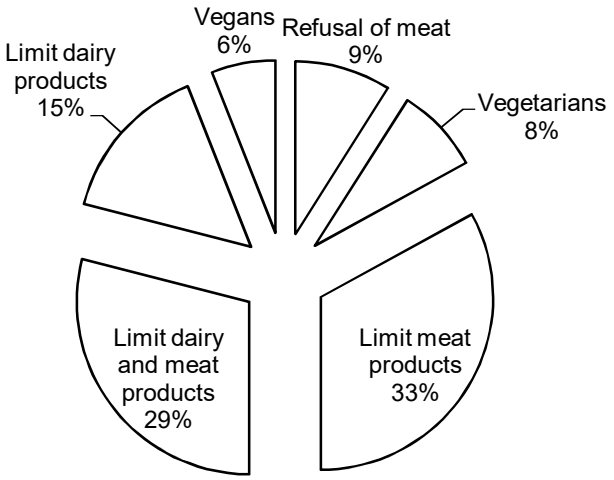

Figure 7. Approximate structure of vegetarians and vegans in Ukraine
Source: (Kozachok, 2022).

What motivates people to choose a vegetarian diet? According to the pyramid of Maslow (Dar, 2022), these will be higher-order needs, as physiological needs are mostly easier to satisfy with classical food: vegetarian brands focus on differentiation and concentration, which leads to a high-price marketing policy. This judgement is also supported by research by scientists from Poland and the United States. For example, John B. Nezlek, Catherine A. Forestell consider social identification, including values, life philosophy, motivation, media influence, friends, relatives, and religious beliefs, among the factors influencing the decision to become a vegetarian (Nezlek et al., 2020).

Let's describe the portrait of the target consumer of vegetarian food producers. Segmentation should be based on the factor of motives for switching to vegetarianism. Three motives are considered to be the most important (Figure 8): concern for the environment (64%), concern for health (24%), and the philosophy of "Cruelty Free" (12%).

Eco-lovers (64%): the main motive is love of nature, environmental friendliness, and the desire to preserve the environment. Healthy eaters (24%): the main motive is health care. Cruelty Free (12%): the main motive is humane, not harming living beings. Thus, concern

for the environment is the main motivation for consumers to go vegetarian. The negative impact of livestock on the Earth's ecosystem is confirmed by numerous studies: UN News, World Wildlife Fund, Florida International University (Machovina et al., 2015) and other studies (Figures 9–10).

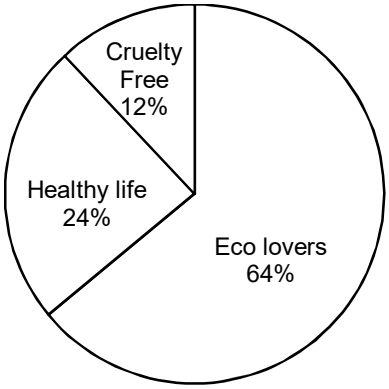

Figure 8. Segmentation of vegetarian food consumers by motivational component Source: (Bielkina, 2020).

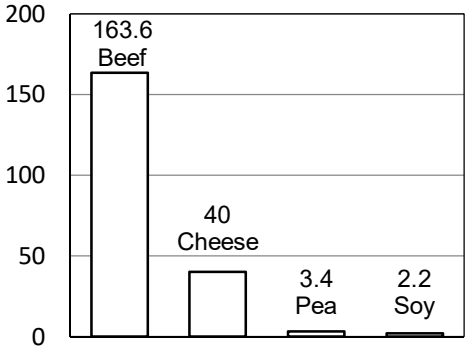

Figure 9. Area of land required to produce 100 grams of protein from different sources, m^2
Source: (Ritchie, 2017).

It takes 48 times as land to produce 100 grams of protein from beef as it does from peas, and it takes 12 times as much land to produce 100 grams of protein from cheese (dairy animal protein) as it does from 100 grams of plant protein (Ritchie, 2017).

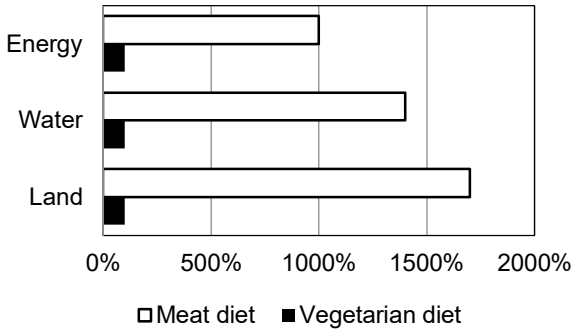

Figure 10. The prevalence of necessary natural resources meat diets compared to vegetarian diets, %.
Source: (Lukianova, 2021).

To provide the population with a meat diet compared to a vegetarian diet, 17 times more land resources, 14 times more water resources, and 10 times more energy resources are required (Lukianova, 2021). Harmful air emissions from mass livestock production account for 8% of CO2, 29% of methane, and 39% of nitrous oxide in the total amount of harmful air emissions (Figure 11) (Baran, 2020).

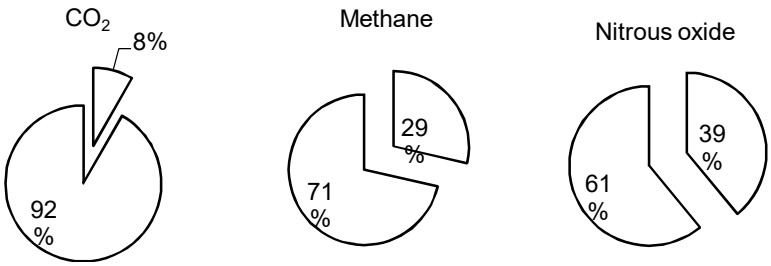

Figure 11. Percentage of air emissions from livestock farming (out of the total air emissions of the respective substance)
Source: (Baran, 2020).

According to Food & Nutrition Research, food should be considered as an issue that affects the environment (Jackson et al., 2024). Research shows that vegetarian diets are one of the key drivers of lifestyle changes that can reduce greenhouse gas emissions (Krpan et al., 2020).

The ethical motive for switching to vegetarianism has also been confirmed (Roser, 2023). Thus, annual number of animals killed for human consumption is about 772 billion, including about 4.5 billion farmed mammals, 86 billion farmed poultry, and more than 100 billion farmed fish. The daily consumption of meat worldwide is about 360 million tonnes, which means that about 900,000 cows, 1.5 million goats, 1.7 million sheep, 3.8 million pigs, 12 million ducks and 202 million chickens (Figure 12) (Roser, 2023; Millstein, 2024).

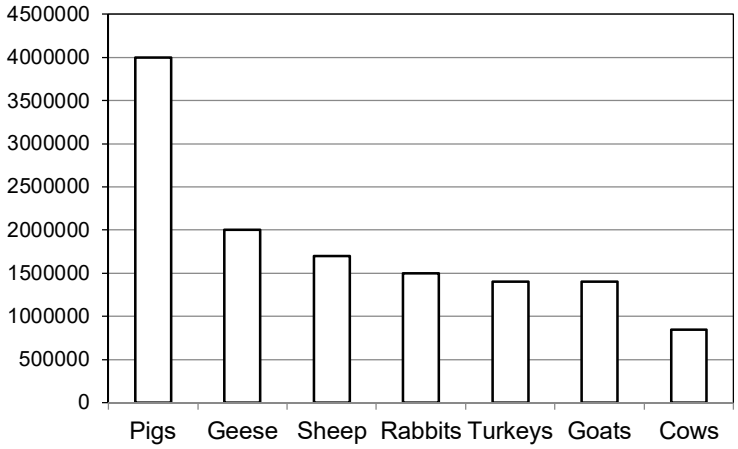

Figure 12. Number of animals killed per day for consumption in the world meat, individuals
Source: (Millstein, 2024).

It is sad from both ethical and economic perspectives that about 24% of livestock (about 18 billion animals per year) that die are not used for food (Millstein, 2024). The global average is 2.4 animals per person per year (Millstein, 2024). In addition to slaughter, the ethical motive for going vegetarian is also related to the suffering of animals during their confinement, restriction of movement, and medical manipulations, including castration, without anaesthesia.

Characteristics of Ukrainian market of vegetarian food brands as new market in the international arena of vegetarian brands

Let's move on to analysing the demand for vegan alternatives to meat products in Ukraine. 73% of the supply of vegan food alternatives (by number of brands) on the market is made up of Ukrainian brands, and only 27% is imported, which is positive. Imported brands include those from Poland, Italy, Germany, the UK, Romania, the Czech Republic, Greece, Spain, Denmark, India, Thailand, Belgium, France, Sweden, and Mexico. Poland has the largest share of imported brands (about 29%), Italy, Germany and the UK account for 8% each, and other countries account for 4% each (Figure 13).

Thus, Ukraine is actively engaged with the global market of vegetarian food brands.

The largest numbers of producers of vegetarian food brands are located in Kyiv and the region (up to 60%). The percentage of producers in other regions of Ukraine is approximately the same. The largest share belongs to Kharkiv and its region (8%), Lviv and its region (6%), Chernihiv and Volyn regions (5% each), Vinnytsia and Dnipro regions (3% each). The share of other regions varies from 1 to 2%.

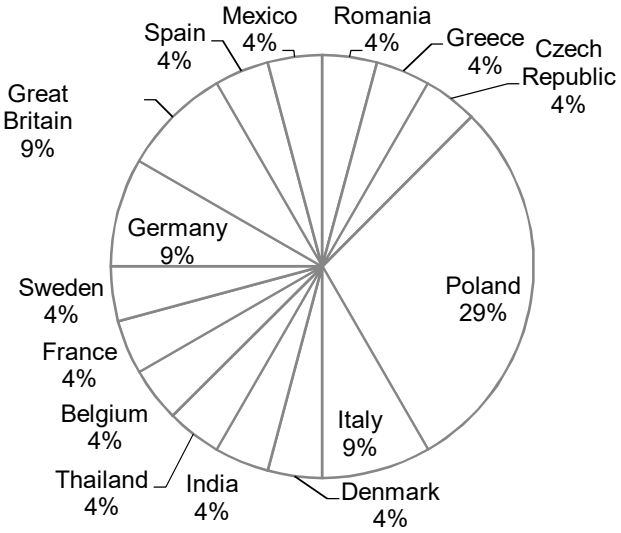

Figure 13. Structure of imports of vegetarian food alternatives to Ukraine (by number of brands)
Source: Own study based on the desk (Internet resources) and field (retail outlets) research

Characteristics of the marketing mix of vegetarian food brands

Let us characterise the marketing complex of vegetarian food brands according to J. McCarthy's 4P model (Mada, 2024).

Marketing product policy of vegetarian brands. Let's take a look at the vegetarian alternatives available on the market (Figure 14).

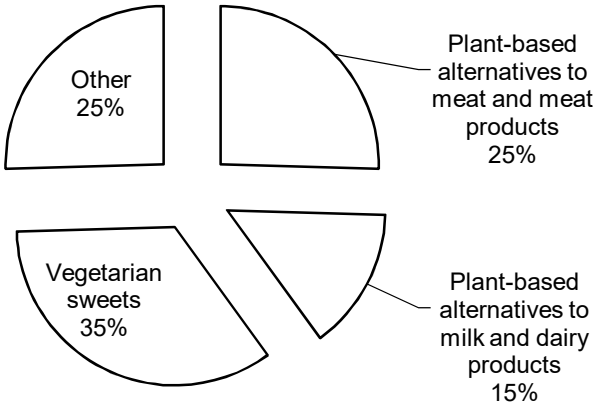

Figure 14. Structure of vegetarian food brands by product type (by number of brands)
Source: *Own study based on the desk (Internet resources) and field (retail outlets) research*

The most relevant category for meeting the demand of vegetarians is the category of alternatives to meat and meat products. In terms of, the share of brands offering such alternatives is 25%. The assortment is characterised by sufficient width and depth, which practically meets vegetarian demand for plant-based meat substitutes. In addition, some manufacturers offer plant-based alternatives to fish and seafood. In addition to meat, fish and dairy vegetarian alternatives sweets, other vegetarian products are available on the market: super foods, convenience foods, ready-to-eat meals, natural food for children, sauces, hummus, bread and pastries, balanced diets, polenta, vegan food pastes, etc.

The emphasis is on uniqueness, differentiation of the offer, as well as on the naturalness of the products and their health benefits. In general, the marketing product policy of vegetarian brands is well developed. There is room for improvement in this area, especially since 64% of vegetarians are motivated by environmental concerns (Bielkina, 2020).

Marketing price policy of vegetarian brands. There is a problem of significant price overcharging compared to non-vegetarian products. Compared to conventional meat, fish and dairy products, as well as sweets, vegetarian alternatives follow a high-price policy for the following reasons: focused strategy, niche segment; low economies of scale; unique offer, competitive advantage based on differentiation; natural ingredients, sometimes unique recipes and cooking methods; little choice for consumers, and virtually no similar offers outside the niche segment. Figure 15 shows the percentage of prices for vegan and conventional products in the categories of sausages, milk, and sweets.

Thus, the pricing policy of vegetarian food brands is one of high and even premium prices. The average percentage of price excess is 87% for vegetable sausages, 170% for vegetable milk, and 450% for vegan sweets. This price difference is one of the barriers to transition to vegetarianism, as well as the inability to meet the nutritional needs of vegetarians.

Figure 15. Percentage of for vegan products compared to conventional products
Source: Own study based on the desk (Internet resources) and field (retail outlets) research

Marketing policy of promotion. Vegetarian brands are mainly promoted in the SMM format and are targeted. The marketing communication channels used are Instagram, Facebook, TikTok, and sometimes YouTube. In addition, many vegetarian brands have their own website, where they also use Google analytics tools, SEO optimisation, Google Ads, branding, etc. (official websites and social media pages of vegetarian food brands, 2024). SMM is the most popular promotion tool for vegetarian brands. Let's analyse the activity of vegetarian brands on Instagram. The brands chosen for the study are Beyond Meat (USA), Veganz (Germany), Alpro (UK), RËBA (Poland), and Wanted Vegan (Ukraine). For clarity, let's build a polygon of the studied brands' activity on Instagram in terms of absolute and relative indicators (Table 1).

Table 1

Polygon of absolute indicators of vegan brands' activity in social media

Indicator, units	Vegan brand				
	«Wanted Vegan», Ukraine	«Beyond Meat», USA	«RËBA», Poland	«Veganz», Germany	«Alpro», UK
Number of publications	275	2420	76	3054	1216
Number of subscribers	8547	995000	535	154000	332000
Average number of «likes»	111	3923	36	368	5462
Average number of comments	2	295	3	103	16
Reels average views	5855	104056	1395	25244	237887

Source: Own study based on the open Internet resources of RËBA, Beyond Meat, Veganz, Alpro, Wanted Vegan brands

The highest level of social media activity is demonstrated by Beyond Meat (USA), which is confirmed by the largest number of publications, subscribers, and the average number of likes, comments, and views of video content. This indicates the brand's strategic

focus on digital communication and promotion in the global space. The lowest indicators of social activity were recorded for the RĒBA brand (Poland), which may indicate the limited use of digital marketing tools or the early stage of brand development in social media. The Ukrainian brand Wanted Vegan demonstrates a high level of audience engagement relative to the number of subscribers, as evidenced by the ratio between video views and the number of subscribers. This indicates the effective use of the available resource and the potential for growth. Alpro (UK) and Veganz (Germany) have a stable presence in social media, which is reflected in the average level of engagement and audience size. In particular, Veganz stands out for its active interaction with subscribers in the form of comments. However, relative indicators are also important, namely engagement rates. Let's show the engagement of the brands under study on Instagram in the form of a triangle (Table 2).

Table 2

Polygon of relative activity indicators of vegetarian brands in social media

Indicator, %	Vegan brand				
	«Wanted Vegan», Ukraine	**«Beyond Meat», USA**	**«RËBA», Poland**	**«Veganz», Germany**	**«Alpro», UK**
Level of engagement by «likes»	1.299	0.394	0.294	6.687	0.239
Level of engagement by comments	0.021	0.030	0.014	0.498	0.067
Level of engagement by views of reels	68.50	10.46	154.44	260.73	16.39

Source: Own study based on the open Internet resources of RËBA, Beyond Meat, Veganz, Alpro, Wanted Vegan brands

The German brand «Veganz» is clearly the leader in all three engagement indicators. Despite the relatively small number of subscribers, activity of Polish brand «RËBA» is quite high, meaning that the brand's SMM works well in a narrow niche. The highest level of engagement in terms of the number of likes was demonstrated by the Veganz brand (Germany) - 6.687%, which indicates a high interest and emotional response from the audience, regardless of the total number of subscribers. This is a sign of an effective content marketing strategy and the content's compliance with target expectations. The Ukrainian brand Wanted Vegan was ranked second in terms of engagement by likes - 1.299%, which is significantly higher than similar brands from the US, UK and Poland. This indicates a high level of emotional attachment among subscribers and the potential of the local brand to expand its influence. The Veganz brand is the leader in terms of engagement by comments - 0.498%, which confirms active communication and two-way interaction with the audience. This may indicate the high quality of the content that encourages subscribers to discuss. Veganz (260.73%) and RĒBA (154.44%) recorded the highest level of video views in relation to the number of subscribers, which indicates the effective use of video formats to reach both existing and new audiences. RĒBA's high score is especially noteworthy given the low total number of subscribers, which indicates the potential for viral content. The lowest relative scores across all parameters are observed for the Alpro brand (UK), which may indicate the need to revise the social media strategy to increase audience engagement and activity.

Marketing policy of the point of sale (access). Availability in specialised vegan online stores is quite good. However, it is difficult to buy a wide range of vegetarian products from retailers focused on broad market coverage. The widest range of products for vegetarians is available in Silpo supermarkets. Among the Ukrainian vegetarian brands represented there are “Eat me at”, “Wanted Vegan”, “Meet Not Meat”, “Green Chef”, “Kyivkraut”, “Vega's” (Kyiv), “Khors” (Kremenchuk), “Prema” (Rivne region). European brands include “Plenty reasons”, “Wege Siostry”, “Roleski” (Poland), “Liveg Ber” (Italy), “Wasa” (Sweden), “Exquise”, “Bett`r” (Germany), “Remia” (Netherlands), “Ppura”, “Polli” (Italy), “Jealous Sweets” (UK) and “Little Pleasures” (Belgium). In addition, products for vegans and vegetarians are sold under the Lavka Tradytsii brand, which was originally created as a proprietary Silpo brand and later developed into a separate business with outlets in Silpo stores. In general, Silpo stores offer the following categories of vegetarian and vegan products: sausages and deli meats (about 18 items), cheeses (about 7 items), canned food, sauces and condiments (about 8 items), sweets (about 4 items), frozen food (about 28 items), bread and bakery products (about 2 items), pickles, as well as regular fresh vegetables and fruits. Vegetarian and vegan products at Lavka Tradytsii include approximately 13 items.

Marketing mix problems of vegetarian brands and prospects for their solution

Based on the research conducted on the 4P marketing mix model in the field of vegetarian food products, it was found that the most painful point for consumers is price, as prices for vegetarian alternatives are on average 236% higher than prices for similar non-vegetarian products. The second problem is accessibility (point of sale), but in the niche segment (specialised vegetarian shops and specialised vegetarian catering) accessibility is adequate. Accessibility in non-vegetarian catering establishments and in large chain retailers is problematic. Therefore, the main problem is the policy of high prices.

Figure 16 presents a generalised description of the marketing mix of vegetarian food brands.

According to a study by the Kyiv International Institute of Sociology commissioned by UA Plant-Based, 65.3% of consumers are ready to switch to vegan alternatives if they are affordable (Palyvoda, 2021). Currently, on average, about 15% of consumers consume plant-based alternatives to meat, and about 14% consume milk (including not only vegetarians but also those who consume these products as a partial replacement for their meat diet) (Palyvoda, 2021). This means that the consumer segment could potentially grow by 65% only if prices were reduced.

Figure 17 shows the proposed mechanism for improving the marketing policy in the field of vegetarian food products.

The starting point of this mechanism is the marketing policy of promotion: improving and intensifying this policy should lead to the popularisation of vegetarianism and vegetarian brands and, accordingly, to an increase in demand for plant-based alternatives to meat and dairy products among both vegetarians and non- vegetarians. The tools for promotion should mostly be the same as those that are available now: SMM, BTL, event marketing and synthetic elements of promotion (participation in exhibitions and fairs). Improvement requires more active use of these tools, maximum adaptation of strategies and activities to the preferences of the target audience, enhanced targeting, increased native content, improved content planning, further study and detailing of the target audience portrait, and cooperation with influencers.

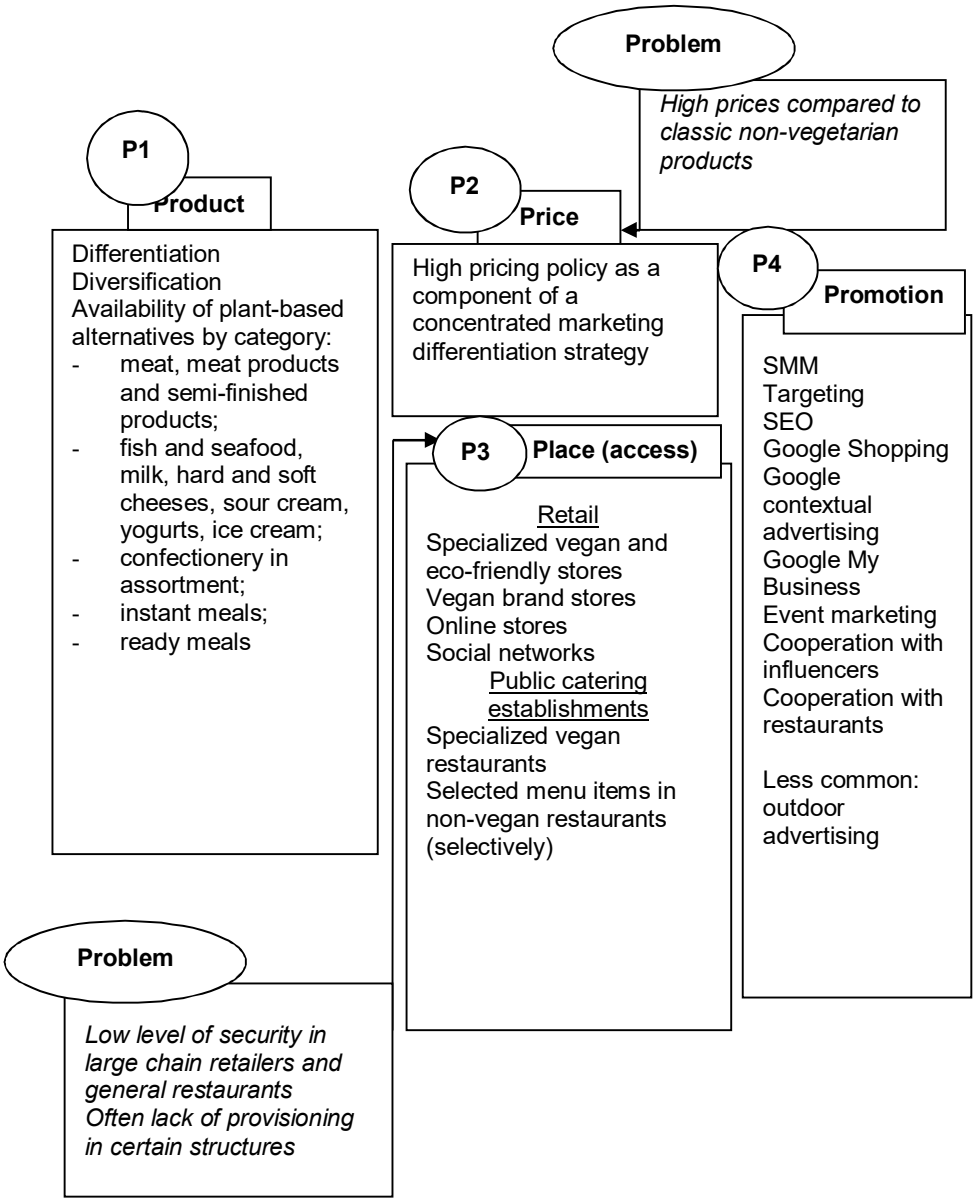

Figure 16. Marketing mix of vegetarian food brands (J. McCarthy's 4P model)
Source: Own study based on the model of J. McCarthy and cabinet research

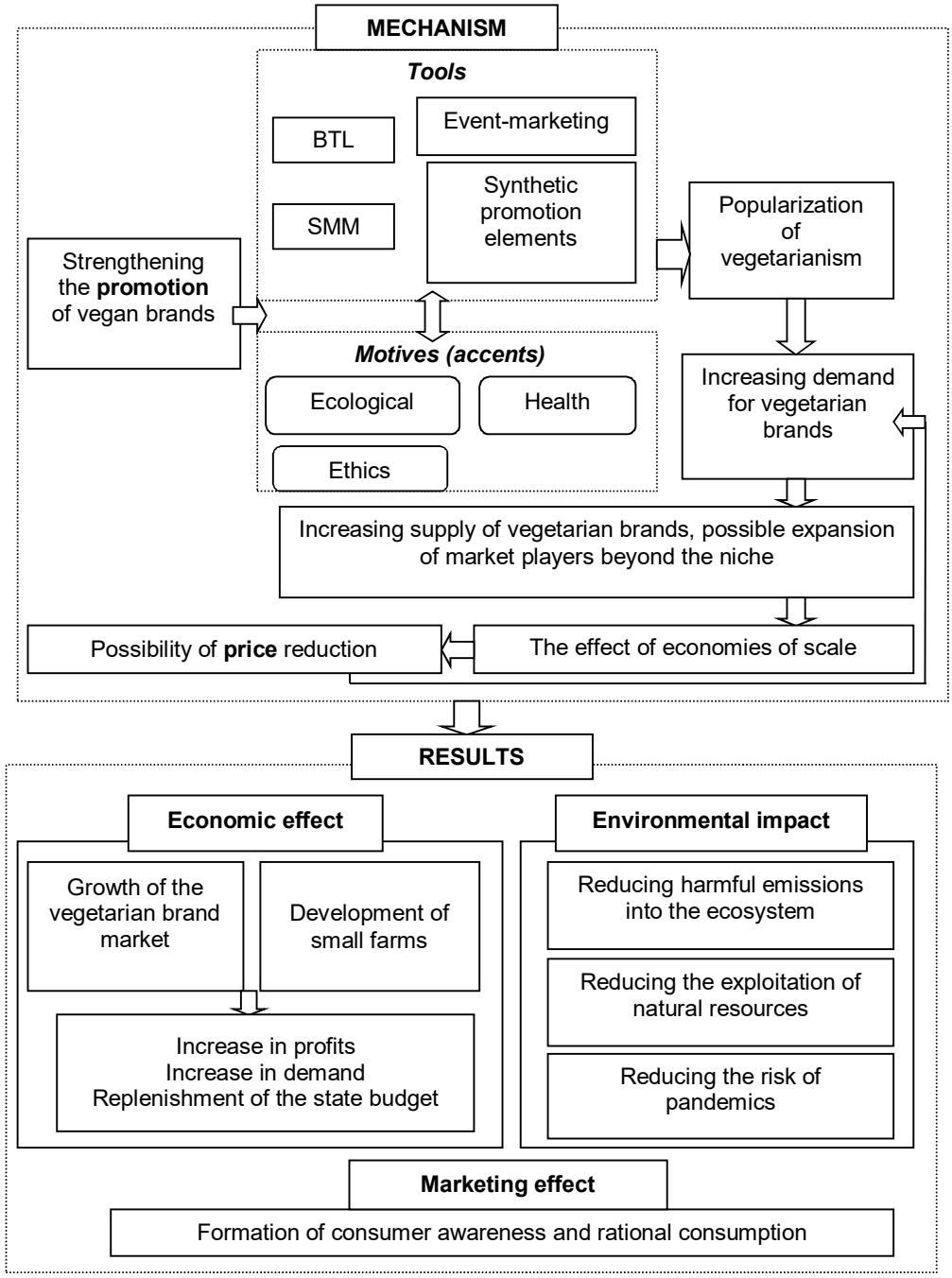

Figure 17. Mechanism for improving marketing policy in the field of vegetarian food products
Source: Own study

It is also necessary to appeal to all three existing motivations that encourage people to consume plant-based alternatives to meat and dairy products: ecology, health, and ethics. The growing demand for vegetarian food will allow vegetarian brands to expand their consumer base and, possibly, in the future, go beyond the niche market to a broader market where the effect of production leverage will be applied. The latter will allow for a slight reduction in prices, which in turn will stimulate an increase in demand. Then the mechanism goes in a circle, leading to even greater production leverage and the possibility of lower prices. It is expected that the market will expand not only due to vegetarians, but also due to consumers with a classical approach to nutrition who will show interest in plant-based meat substitutes, as well as flexitarians. The launched mechanism should lead to the following results:

1) economic effect: increased profits for vegetarian brands, increased contributions to the state budget, and more jobs. In addition, the popularisation of vegetarianism, including the ethical and health and wellness motive, will boost demand for farm products. This is especially true for mild vegetarians, flexitarians and people who do not give up meat at all. The ethical motive appeals not only to slaughter, but also to the conditions of animal welfare and the intense fear and pain of slaughter. The health and wellbeing motive is related, among other things, to the adrenaline that remains in animal meat, to antibiotics, and to the unnatural feeding of animals to increase weight quickly, which also worsens the quality of meat. Farms, providing free range, less antibiotics and natural outdoor nutrition for animals, will produce products, even if they are animal products that will be in demand by the eco- lovers, health-conscious and even partially by the cruelty-free (soft vegetarian, flexitarian) segments. The development of farming will stimulate the state budget, ensure a healthier diet for people and preserve the environment. The main thing is to ensure that farmers comply with the conditions of production and animal husbandry that make them attractive to customers;

2) environmental effect: increasing the consumption of plant-based foods as opposed to animal-based foods will save natural resources, reduce harmful emissions into the atmosphere, reduce the risk of pandemics, and preserve biodiversity;

3) marketing effect: strengthening of the marketing function to form the right needs in terms of conscious and rational consumption, which will have a positive impact on the structure of consumer demand and, accordingly, on the supply (not only of vegetarian brands). As a result, there will be an environmental effect and improved human health.

The proposed mechanism for improving the marketing policy in the field of vegetarian brands takes into account aspects that have not been taken into account before. The works of most authors in this area relate to in-depth studies of a single vegetarian brand (Rybalchenko, 2024) or a wider range of studies from the standpoint of environmental friendliness and ethical marketing policy, but without reference to vegetarianism (Kirnosova, 2023). Authors, who have studied vegetarianism in more detail and extensively, analyze a significant number of factors, including both factors that may prevent people from adopting a vegetarian diet and the positive effects of vegetarianism (Hargreaves et al., 2021; Baroni et al., 2024). But, the issue of an integrated approach to the marketing strategy of vegetarian brands in terms of all elements of the marketing mix has not been considered in recent years, so the identified problems and proposed solutions will allow a broader view of the marketing of vegetarian food brands and the application of the proposed areas of improvement in the marketing policy of enterprises producing vegetarian food products.

Conclusions

1. The study made it possible to determine the specifics of marketing strategies of vegetarian food brands:
   - the basic marketing competitive strategies are focused differentiation and optimisation strategies;
   - niche strategy: niche leadership for strong brands and integration for new market players;
   - product/market ratio: deep market penetration, expansion of product lines, product development, limited and partial diversification;
   - STP marketing strategy: targeted concentrated marketing and positioning based on health, environmental friendliness, and ethics.
2. Features and problem points in the marketing mix of food brands are identified: high price policy due to a focused marketing strategy and to a high level of differentiation.
3. A mechanism for improving the marketing policy in the field of vegetarian food brands is proposed, based on strengthening and optimising the marketing policy of promotion, which should lead to an increase in demand and economies of scale. This will partially solve the problem of high prices, which will also contribute to demand growth. This will result in economic, environmental and marketing benefits.

References

American D.A. (2003), Position of the American Dietetic Association and Dietitians of Canada: vegetarian diets, *Journal of the American Dietetic Association*, 103(6), pp. 748–765, https://doi.org/10.1053/jada.2003.50142

Animal C.E. (2024), The geography of farmed animals and farmed animal advocacy funding, *Animal Charity Evaluators*, Available at: https://animalcharityevaluators.org/research/reports/farmed-animal-advocacy-geography/

Animal C.E. (2018), The effects of diet choices, *Animal Charity Evaluators*, Available at: https://animalcharityevaluators.org/research/reports/dietary-impacts/effects-of-diet-choices/

Baker P., Lacy-Nichols J., Williams O., Labonté R. (2021), The political economy of healthy and sustainable food systems: an introduction to a Special Issue, *International Journal of Health Policy and Management*, 10, pp. 734–744, https://doi.org/10.34172/ijhpm.2021.156

Baran O. (2020), How what we eat affects the Earth: biodiversity vs human eating habits, LLC "IA" Rubric, Available at: https://rubryka.com/article/den-bioriznomanittya/

Baroni L., Rizzo G., Galchenko A.V., Zavoli M., Serventi L., Battino M. (2024), Health benefits of vegetarian diets: an insight into the main topics, *Foods*, 13(15), 2398, https://doi.org/10.3390/foods13152398

Bielkina K. (2020), The number of vegetarians in Ukraine is growing, *Open Cages. Animal Welfare Organization*, Available at: https://cagefree.opencages.com.ua/

Dar S.A., Sakthivel P. (2022), Maslow's hierarchy of needs is still relevant in the 21st century, *Journal of Learning and Educational Policy*, 5(3), pp. 15–22, https://doi.org/10.55529/jlep25.1.9

Dieffenbacher S.F. (2023), Porter's generic strategies: cost leadership, differentiation and focus, *Digital Leadership,* Available at: https://digitalleadership.com/blog/porters-generic-strategies/

Dix C.F., Brennan L., Reid M., McCaffrey T.A., Molenaar A., Barklamb A., Chin S., Truby H. (2021), Nutrition meets social marketing: targeting health promotion campaigns to young adults using the living and eating for health segments, *Nutrients,* 13(9), 3151, https://doi.org/10.3390/nu13093151

Doustmohammadian A., Bazhan M. (2021), Social marketing-based interventions to promote healthy nutrition behaviors a systematic review protocol, *Systematic Reviews*, 10, 75, https://doi.org/10.1186/s13643-021-01625-5

Geeksforgeeks O. (2024), List of top 10 countries with the most vegetarians, *Geeksforgeeks.org*, Available at: https://www.geeksforgeeks.org/countries-with-the-most-vegetarians/

Geeksforgeeks O. (2024), Most non vegetarians countries – list of top 10, *Geeksforgeeks.org*, Available at: https://www.geeksforgeeks.org/most-non-vegetarians-countries/

Glanbia N. (2023), Ways of eating in Europe in 2023, *Glanbia Nutritionals*, Available at: https://www.glanbianutritionals.com/

Hargreaves S.M., Raposo A., Saraiva A., Zandonadi R.P. (2021), Vegetarian diet: an overview through the perspective of quality of life domains, *International Journal of Environmental Research Public Health,* 18(8), 4067, https://doi.org/10.3390/ijerph18084067

Heubel M. (2024), The Ansoff matrix (product-market matrix): definition, model and examples, *Consulterce*, Available at: https://consulterce.com/ansoff-matrix/

Ijaz S. (2023), Fifteen worst countries in the world for vegetarians, *Yahoo.finance*, Available at: http://surl.li/ofnijn

Jackson P., Holm L. (2024), Social and economic dimensions of food sustainability – a background paper for the Nordic Nutrition Recommendations, *Food & Nutrition Research*, 68, https://doi.org/10.29219/fnr.v68.10450

Jenkins E.L., Legrand S., Brennan L., Molenaar A., Reid M., McCaffrey T.A. (2021), Psycho-behavioural segmentation in food and nutrition: a systematic scoping review of the literature, *Nutrients*, 13(6), 1795, https://doi.org/10.3390/nu13061795

Kirnosova M.V. (2023), Green strategies in the marketing product policy of manufacturers: environmental and ethical aspects, *Marketing and Digital Technologies*, 7(4), pp. 53–62, https://doi.org/10.15276/mdt.7.4.2023.3

Kotler P., Kartajaya H., Setiawan I. (2021), *Marketing 5.0: Technology for Humanity*, John Wiley & Sons.

Kozachok A. (2022), Ukrainians try to reduce consumption of animal products: research results, *HoReCa-Ukraine, Analytical reviews*, Available at: https://horeca-ukraine.com/ukrainci-namagajutsja-zmenshiti-spozhivannja-produktiv-tvarinnogo-pohodzhennja-rezultati-doslidzhennja/

Krpan D., Houtsma N. (2020), To veg or not to veg? The impact of framing on vegetarian food choice, *Journal of Environmental Psychology*, 67, 101391, https://doi.org/10.1016/j.jenvp.2020.101391

Lukianova V. (2021), World vegan day: five reasons to switch to a plant-based diet, *Ukrainian truth of life,* Available at: https://life.pravda.com.ua/columns/2021/11/1/246346/

Machovina B., Feeley K.J., Ripple W.J. (2015), Biodiversity conservation: the key is reducing meat consumption, *Science of The Total Environment*, 536, pp. 419–431, https://doi.org/10.1016/j.scitotenv.2015.07.022.

Mada Y.P. (2024), The evolution and revolution of marketing mix from 4P to 4C to 4E to e-marketing mix: a literature overview, *International Journal of Innovations in Engineering Research and Technology*, 11(8), pp. 37-47, https://doi.org/10.26662/ijiert.v11i8.pp37-47.

Maksi S.J., Keller K.L., Dardis F., Vecchi M., Freeman J., Evans R.K., Boyland E., Masterson Tr.D. (2024), The food and beverage cues in digital marketing model: special considerations of social media, gaming, and livestreaming environments for food marketing and eating behavior research, *Frontiers in Nutrition*, 10, 1325265, https://doi.org/10.3389/fnut.2023.1325265

Martinho V.J.P.D. (2020), Food marketing as a special ingredient in consumer choices: the main insights from existing literature, *Foods*, 9(11), 1651, https://doi.org/10.3390/foods9111651

Millstein S. (2024), How many animals are killed for food every day, *Sentient Media,* Available at: https://sentientmedia.org/how-many-animals-are-killed-for-food-every-day/

Mishler J. (2023), Five plant-based meat companies you should know, *Sentientmedia*, Available at: https://sentientmedia.org/plant-based-meat-companies/

News U.N. (2006), Rearing cattle produces more greenhouse gases than driving cars, *UN News,* Available at: https://news.un.org/en/story/2006/11/201222-rearing-cattle-produces-more-greenhouse-gases-driving-cars-un-report-warns

Nezlek J.B., Forestell C.A. (2020), Vegetarianism as a social identity, *Current Opinion in Food Science*, 33, pp. 45–51, https://doi.org/10.1016/j.cofs.2019.12.005

Palyvoda N. (2021), How many vegetarians and vegans there are in Ukraine – research, *Mind.ua,* Available at: https://mind.ua/news/20222628-skilki-v-ukrayini-vegetarianciv-ta-veganiv-doslidzhennya

Peterdy K. (2023), Ansoff matrix, Co*rporate Finance Institute*, Available at: https://corporatefinanceinstitute.com/resources/management/ansoff-matrix/

Petruzzello M. (2024), Modern developments in vegetarianism, *Britannica,* Available at: https://www.britannica.com/science/dieting

Pyvovarenko O.A. (2019), Kyiv public vegetarian canteens in the beginning of the 20th century, *Journal of Ukrainian History*, 39, pp. 20–23, https://doi.org/10.17721/2522–4611.2019.3

Ratchford B.T. (2020), The history of academic research in marketing and its implications for the future, *Spanish Journal of Marketing*, 24(1), pp. 3–36, https://doi.org/10.1108/SJME-11-2019-0096

Ritchie H. (2017), How much of the world's land would we need in order to feed the global population with the average diet of a given country, *Our World in Data,* Available at: https://ourworldindata.org/agricultural-land-by-global-diets

Roser M. (2023), How many animals get slaughtered every day, *Our World in Data*, Available at: https://ourworldindata.org/how-many-animals-get-slaughtered-every-day

Rounsefell K., Gibson S., McLean S., Blair M., Molenaar A., Brennan L., Truby H., McCaffrey T.A. (2020), Social media, body image and food choices in healthy young adults: a mixed methods systematic review, *Nutrition & Dietetics*, 77, pp. 19–40, https://doi.org/10.1111/1747-0080.12581

Rybalchenko A.Y. (2024), Critical discourse analysis of plant-based meat marketing (based on Beyond Meat's instagram marketing), *Study Linguistica*, 15, pp. 112–125, https://doi.org/10.17721/StudLing2024.24.89-100

Silva J.G.C. (2022), Scientific research methods*, International Journal of Science and Research*, 11, pp. 334-350, https://doi.org/10.21275/SR221004074851

Sun Y., Cheah I., Sung B., Lee E.J. (2020), Innovative research methodologies in marketing research, *Asia Pacific Journal of Marketing and Logistics*, 32(5), pp. 1001–1003, https://doi.org/10.1108/APJML-07-2020-738

Trunfio M., Rossi S. (2021), Conceptualising and measuring social media engagement: a systematic literature review, *Italian Journal of Marketing*, pp. 267–292, https://doi.org/10.1007/s43039-021-00035-8

Truman E., Elliott C. (2019), Identifying food marketing to teenagers: A scoping review, *International Journal of Behavioral Nutrition and Physical Activity,* 16, 67, https://doi.org/10.1186/s12966-019-0833-2

Tyler R., Barrocas A., Guenter P., Araujo Torres K., Bechtold M.L., Chan L.N., Collier B., Collins N.A., Evans D.C., Godamunne K., Hamilton C., Hernandez B.J.D., Mirtallo J.M., Nadeau W.J., Partridge J., Perugini M., Valladares A. (2020), Value of nutrition support therapy: impact on clinical and economic outcomes in the United States, *Journal of Parenteral and Enteral Nutrition,* 44, pp. 395–406, https://doi.org/10.1002/jpen.1768

Wilson M.S. (2018), A dual-process motivational model of attitudes towards vegetarians and vegans, *European Journal of Social Psychology*, 49(1), pp. 169–178, https://doi.org/10.1002/ejsp.2386

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Financial dynamics and strategic growth in sugar industry: Comparative analysis of Central and Eastern Europe (2013–2022)

Jaroslav Vlach[1], Luboš Smutka[1], Tomáš Mrkvička[1], Inna Čábelková[2], Dagmar Škodová Parmová[1], Jana Moravcová[1]

1 – University of South Bohemia, Ceske Budejovice, Czech Republic
2 – Czech University of Life Sciences, Prague, Czech Republic

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Corresponding author:

Luboš Smutka
E-mail: smutka@pef.czu.cz

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Abstract

Introduction. It is explored the financial dynamics and strategic growth of the sugar production sector focusing on six countries: the Czech Republic, Austria, Germany, Poland, Hungary, and Slovakia, over the period 2013–2022.

Materials and methods. To analyze the performance of sugar-producing companies in the Czech Republic and neighboring countries, it was employed a two-stage analytical approach, involving the calculation of normalized linear trends and Principal Component Analysis of company characteristics averages and normalized trends.

Results and discussion. The first principal component accounted for 46.43% of the total variance, which clearly indicates that company size plays a dominant role in defining their financial profiles. Larger companies such as Suedzucker AG and Agrana Zucker GMBH score highly on this component, reflecting their substantial financial resources, capital, and operating revenue. The second principal component (PC2), which accounted for 23.10% of the total variance, was crucial in highlighting the growth potential of sugar-producing companies. While large firms like Suedzucker AG demonstrated limited growth (negative PC2), smaller companies demonstrated considerable growth potential. For example, Pfeifer & Langen Polska S.A. The third principal component (PC3), which explained 12.75% of the variance, offered insights into the equity development and financial health of companies. This component is especially important because it highlights how companies manage their liabilities (creditors) and working capital to maintain financial stability. The analysis highlights regional and company-level variations in financial performance, identifying key drivers such as company size, equity management, and growth potential. At the company level, larger firms demonstrate stability but limited growth, while smaller firms show strong development potential, emphasizing strategic adaptability as a critical factor for success. At the country level, the Czech Republic and Poland emerge as dynamic markets with robust financial bases, while Germany and Austria reflect mature industries with lower growth potential. Hungary and Slovakia, despite financial challenges, exhibit opportunities for development. This study underscores the importance of balancing financial health, innovation, and sustainability for the long-term resilience of the sugar sector. Principal Component Analysis (PCA), while powerful for identifying patterns, does not capture all the underlying causes of financial dynamics, such as market competition, consumer preferences, or regulatory changes.

Conclusion. It is highlighted diverse dynamics affecting financial stability, growth, and sustainability. Larger firms dominate the market but show limited growth potential, while smaller firms demonstrate stronger growth trajectories. Countries like the Czech Republic and Poland have strong financial positions, whereas Hungary needs improved financial strategies to enhance competitiveness.

Introduction

The sugar production sector in Central and Eastern Europe (CEE) plays a pivotal role in the region's agricultural and industrial economy (Swinnen et al., 2010). Its importance is underscored by its contribution to rural development, employment, and trade (Kotyza et al., 2019). This study focuses on the dynamics of the sugar market in the Czech Republic and its neighboring countries—Austria, Germany, Poland, Hungary, and Slovakia—during the period from 2013 to 2022. The aim is to examine the factors influencing market fluctuations, the financial performance of major sugar producers, and strategies for sustainable growth amid market volatility.

The market for sugar in the region has been shaped by significant regulatory and economic changes over the past decade (Řezbova et al., 2015). A key turning point was the European Union's sugar market reform and the abolition of production quotas in 2017 (Muir and Anderson, 2022). These changes liberalized the market, exposing producers to heightened global competition and price volatility (Kotyza et al., 2018). Studies indicate that global price dynamics, driven by major sugar-exporting countries like Brazil and India, have had a profound impact on the CEE region, influencing profitability and market stability (Soare et al., 2021). Additionally, regional trade policies and cross-border supply chain dynamics have added complexity to the sector, highlighting the interconnectedness of CEE economies (European Commission, 2017; Nes et al., 2021).

Sustainability has emerged as a critical concern for the sugar industry, with increasing emphasis on reducing its environmental footprint (Garcia-Bustamante et al., 2018). The sector faces scrutiny for its significant carbon emissions, high water usage, and energy demands (Aguilar-Rivera, 2022; Formann et al., 2020; Meghana and Shastri, 2020).

The methodological approach combining time-series analysis and Principal Component Analysis (PCA) is a powerful tool (Chowdhury et al., 2017) for evaluating the financial performance (Liu and Bai, 2021) of sugar-producing companies. Time-series analysis is widely recognized for its ability to identify trends, seasonal patterns, and structural changes in financial data (Diggle and Giorgi, 2024; Hamilton, 2020), making it particularly valuable for industries like sugar production, which are influenced by cyclical market dynamics and external shocks.

PCA complements time-series analysis by reducing the dimensionality of complex datasets while preserving the essential characteristics of the data (Huang et al., 2022). This technique is particularly effective in identifying and prioritizing key financial indicators (Crepey et al., 2022), such as EBITDA, working capital, operating revenue, and creditor management. By focusing on these critical metrics, PCA enables a nuanced comparison of sugar producers, accounting for differences in company size and operational environments. Studies have demonstrated how PCA can reveal patterns in financial performance and group companies based on similar characteristics, facilitating strategic decision-making and benchmarking (Xue et al., 2018).

The evaluation of key financial indicators is central to understanding corporate performance and resilience (Singh et al., 2019). Metrics like EBITDA and operating revenue are essential for assessing profitability and operational efficiency, while working capital and creditor management provide insights into liquidity and financial stability. Normalizing financial trends to account for variations in company size and market conditions ensures a fair and meaningful comparison across firms (Schroeder et al., 2022; Vernimmen, 2022). This normalization process is particularly relevant in the sugar sector, where companies operate under diverse regulatory, environmental, and economic contexts.

At an aggregated level, the analysis of financial trends across sugar-producing companies and countries offers a broader perspective on the industry's health and development trajectories.

The combination of time-series analysis and PCA represents an innovative and robust approach for assessing the financial health and strategic positioning of sugar producers. Time-series analysis captures temporal changes and underlying trends, while PCA identifies the principal factors driving performance and facilitates cross-company and cross-country comparisons. Together, these methods provide a comprehensive understanding of the financial dynamics at both individual and aggregate levels, offering valuable insights into the overall development and sustainability of the sugar industry in the region.

Given the broad historical and economic context, this paper's central objective is to analyze the sugar business within the Central and Eastern European region, focusing on both company-level and country-level dynamics. At the individual company level, the study aims to investigate the causes of changes in financial indicators and their impact on the competitiveness and financial stability of sugar-producing firms operating within six countries: the Czech Republic, Austria, Germany, Poland, Hungary, and Slovakia.

This article, although it primarily focuses on production for the domestic market (i.e., EU countries for the EU market), can be generalized to the context of commodities on global markets. Sugar itself is a commodity, and it is advisable to strengthen vertical production in sugar companies, that is, to produce foods and products with higher added value.

Materials and methods

To analyze the performance of sugar-producing companies in the Czech Republic and neighboring countries over the period 2013–2022, we employed a two-stage analytical approach, involving the calculation of normalized linear trends and Principal Component Analysis (PCA) of company characteristics (Labrin and Urdinez, 2020). This methodology allows for the comparison of trends in various business metrics while ensuring the results are scale-independent and interpretable across countries. Below are the steps involved in our analysis (Deutsch et al., 2019).

Calculation of normalized linear trend

For each company, we calculated the normalized trend (Mahoney, 2005) of a simple linear regression model (De Jong et al., 2012) over the period from 2013 to 2022. The model is represented as:

$$Y_i = a + b \cdot X_i,$$

where:

- Y_i is the enterprise characteristic at time i (such as sales, EBITDA, or working capital),
- X_i represents the years (2013 to 2022),
- a is the estimated value of the characteristic in 2013 (the intercept),
- b is the slope of the trend (the rate of change per year).

The regression analysis is performed separately for each enterprise, where the characteristic Y may represent different company metrics such as sales, operating revenue, number of employees, working capital, creditors, capital, or EBITDA.

Normalization of trends

The calculated trend (b) represents the yearly change in the enterprise characteristic. To standardize the results and ensure comparability across companies of different scales, we normalize this trend by dividing it by the average value of the characteristic over the entire period (2013–2022). This gives us a normalized trend Nb, which represents the percentage increase (or decrease) of the characteristic per year, divided by 100 (Mahoney, 2005).

$$Nb_i = \frac{b}{AverageValueofY_i}$$

This step ensures that trends are comparable on the same scale regardless of the absolute values of the enterprise characteristics, allowing us to focus on the relative performance of companies.

Weighted average of normalized trends

Next, we compute the weighted average of normalized trends (Purushothaman, 2011) for each country and for blocks of countries. The weight of each companies in the calculation is based on their Averaged Working Capital (AWC). The weighted average is calculated for each country and for specific groups of countries:

- Austria and Germany,
- Czechia, Hungary, and Slovakia,
- All countries combined.

$$Nb(Germany) = \frac{AWC(Suedzucker) \cdot Nb(Suedzucker) + AWC(Nordzucker) \cdot Nb(Nordzucker)}{AWC(Suedzucker) + AWC(Nordzucker)}$$

This weighting ensures that larger enterprises, as measured by their working capital, have a greater influence on the overall trend of the country or country block.

Further we have performed principal component analysis of companies from average time series and trends of the following characteristics: Capital, Creditors, EBITDA, Number of employers, Operating revenue, Sales, and Working Capital.

Data collection and variables

This research focuses on 14 sugar-producing companies from six countries within Central and Eastern Europe: Austria (AT), Germany (DE), Czech Republic (CZE), Hungary (HU), Slovakia (SK), and Poland (PL). The financial data used in this analysis are based on average values over a period of five years (2018-2022), ensuring stability in the analysis and reflecting long-term performance trends. The key financial variables used for the Principal Component Analysis (PCA) are:

- Capital (in EUR thousands): Represents the financial resources available to the company.
- Creditors (in EUR thousands): The total amount of liabilities a company owes to external creditors.
- EBITDA (Earnings Before Interest, Taxes, Depreciation, and Amortization, in EUR thousands): A measure of a company's operational profitability.
- Number of Employees (both average and total): Reflects the size of the company's workforce and can give insight into operational scale.
- Operating Revenue (Turnover, in EUR thousands): Represents the total income generated by the company from its regular business activities.

- Sales (in EUR thousands): The total value of products sold, indicative of the company's market share.
- Working Capital (in EUR thousands): A measure of a company's short-term financial health and operational efficiency.

Each company's data was standardized to ensure comparability across companies of varying sizes and financial profiles. ORBIS database was used for relevant above mentioned data collection.

Principal component analysis

Principal Component Analysis (PCA) is a statistical technique used to reduce the dimensionality of large datasets while retaining as much variance as possible. This allows for a more manageable set of variables (principal components) that explain the major patterns within the data. The main steps of the Principal Component Analysis (PCA) applied in this research are as follows (Deutsch et al., 2019):

Standardization of Data: Each financial variable was standardized to have a mean of zero and a standard deviation of one. This step is crucial for ensuring that variables with different units (e.g., revenue in EUR vs. number of employees) contribute equally to the analysis.

Covariance Matrix Calculation: A covariance matrix was computed to explore the relationships between the financial variables. This matrix provides insight into how the variables co-vary with each other.

Eigenvalue and eigenvector calculation

The eigenvalues and eigenvectors of the covariance matrix (Lin et al., 2020) were calculated. The eigenvalues represent the variance explained by each principal component, and the eigenvectors define the direction of the components.

Component Selection: The number of components to retain was determined based on the eigenvalues (e.g., components with eigenvalues greater than one were retained).

Interpretation of results

After, the components were analyzed to determine which financial variables contributed most to each component. The companies' scores on the principal components were calculated, allowing for the assessment of their relative positioning within the financial landscape, using biplot (Gabriel, 1971; Gabriel and Odoroff, 1990).

Data analysis

The results of the Principal Component Analysis (PCA) were analyzed at two levels:

Company-Level Principal Component Analysis (PCA): Principal Component Analysis (PCA) was applied to the individual companies to identify how their financial characteristics contribute to their overall financial performance, size, and growth potential. Each company was represented by a point in a multidimensional space, where the position of each company reflected its financial attributes.

Country-Level Principal Component Analysis (PCA): In addition to the company-level analysis, Principal Component Analysis (PCA) was also applied to the aggregated data of companies in each country to understand broader regional trends. This allowed for

comparisons across countries, revealing how the financial dynamics of the sugar production sector differ in each state.

The analysis identified the main components that explain the financial variance across companies and countries. The results were visualized using scatter plots and component loading charts, allowing for easy interpretation of the relationships between the financial variables and the principal components.

The companies are plotted in a 3D space based on their scores in the first, second, and third principal components. This allows for a visual representation of the companies' performance across the most important business dimensions. The resulting figure helps identify patterns, such as clustering of companies with similar growth or equity profiles and provides a clear overview of how companies from different countries or country blocks compare.

Results and discussion

Principal component analysis of companies

The Principal Component Analysis (PCA) of the companies yielded insightful results, with the first three principal components (PC1, PC2, and PC3) explaining 82.28% of the total variance. The breakdown is as follows:

PC1 explained 46.43% of the total variance, representing the size of the company. Larger companies generally exhibited higher values on PC1, while smaller companies demonstrated lower values. See the rotations in the Table 3 for the given interpretations of the components.

The size of the company may be related to the variables that have a big rotation coefficient in Table 3 for PC1 (average values of capital, creditors, number of employees, operating revenue, sales, working capital).

PC2 accounted for 23.10% of the variance, reflecting the growth or development potential of the company. Companies with a positive PC2 value demonstrated significant growth, while those with negative values exhibited stagnation or shrinkage.

The label growth or development potential of PC2 we obtained from big rotations of PC2 in variables trend of operating revenue, trend of sales, and trend of number of employees.

PC3 explained 12.75% of the variance, capturing the equity development trends of the companies.

The trend in the label development of PC3, which we derived from significant rotations of PC3, includes variables such as the trend of capital, the trend of working capital, and the negative trend of creditors.

Table 1

Importance of components

	PC1	PC2	PC3
Standard deviation	2.5497	1.7982	1.3360
Proportion of variance	0.4643	0.2310	0.1275
Cumulative proportion	0.4643	0.6953	0.8228

Source: own processing

Table 2

PCs with respect to key sugar producing companies

PC1	PC2	PC3	Company
-0.96591	-1.79322	-2.95359	Agrana Zucker GMBH
-1.61952	-1.48201	1.86055	Moravskoslezke Cukrovary
-1.65217	0.28348	1.44341	Cukrovar Vrbatky a.s.
-2.26005	-1.33810	0.96678	Litovelska Cukrovarna
0.06189	1.98233	0.13341	Tereos a.s
6.92069	-2.24695	1.01244	Suedzucker AG
3.07329	-0.46317	-0.39555	Nordzucker AG
-1.59659	0.29054	-1.41489	Magyar Cukorgyarto es Forg
1.93971	1.13487	-1.30579	Krajowa Grupa Spozywcza S.A.
1.27704	4.77504	0.65072	Pfeifer and Langen Polska
-0.55261	0.02023	-1.34400	Sudzucker Polska S.A.
-1.03807	0.25494	0.10056	Nordzucker Polska S.A.
-1.71824	-0.31222	0.42547	Povazsky Cukor a.s.
-1.86943	-1.10576	0.82047	Slovenske Cukrovary s.r.o.

Source: own processing

Table 3

Rotations

	PC1	PC2	PC3
Capital average	0.32328	-0.05179	-0.20187
Capital trend	-0.02968	-0.21616	0.38770
Creditors average	0.37279	-0.11678	0.01899
Creditors trend	-0.01975	0.17736	-0.64081
EBITDA average	0.06270	0.37823	-0.24664
EBITDA trend	-0.30572	0.24338	-0.16893
Number of employees average	0.37624	0.00287	-0.07696
Number of employees trend	0.17618	0.33108	0.29225
Operating revenue (Turnover) aver.	0.37757	-0.12615	-0.05940
Operating revenue trend	0.14876	0.48733	0.05491
Sales average	0.37767	-0.12325	-0.06129
Sales trend	0.15537	0.48363	0.11081
Working capital aver.	0.37302	-0.10467	-0.09038
Working Capital trend	0.12092	0.28304	0.43182

Source: own processing

Company-specific interpretations

The scores for each company on the principal components provide a snapshot of their financial health and growth prospects:

– Suedzucker AG (point 6) is the largest company in the region (high score on PC1) but exhibits minimal development potential (negative PC2 value). This indicates that the

company is in a mature stage, focusing on maintaining market dominance rather than pursuing aggressive expansion.

- Pfeifer & Langen Polska S.A. (point 10), despite being smaller, demonstrates strong development potential with a positive PC2 score, indicating a dynamic market strategy.
- Agrana Zucker GMBH (point 1), while large, demonstrates weak growth potential, reflected in its negative PC2 score, signaling possible challenges in adapting to market changes.
- Litovelská Cukrovarna A.S. (point 4), the smallest company, demonstrates similar development potential to Suedzucker AG, indicating that size alone does not determine a company's growth trajectory.
- Companies such as Tereos A.S. (point 5) and Magyar Cukorgyártó és Forgalmazó Zrt. (point 8) display a balance of size and equity development, with moderate scores across PC1 and PC3.

Rotated component loadings

The rotation of the components revealed further insights into the relationships between financial variables:

Capital and Operating Revenue consistently correlated with PC1, confirming their importance in determining the overall size and financial stability of companies.

EBITDA and Working Capital were linked to PC2 and PC3, reflecting the need for profitability and efficient resource management to support growth.

Principal component analysis of states (Countries)

To understand regional trends, Principal Component Analysis (PCA) was also applied at the state level, based on the aggregated data from companies in each country. The first three components accounted for 90.61% of the variance, broken down as follows:

PC1 explained 61.14% of the variance, reflecting the overall financial strength of each country's sugar production sector (size).

PC2 accounted for 18.39% of the variance, representing the developmental trends and market growth in each state. Negative value of growth or development potential.

PC3 explained 11.08% of the variance, capturing equity development trends. Negative value of development trend.

The interpretation of PCAs for companies and countries differs slightly because they are calculated from different input data. We analyze 14 companies but only 6 states (narrower sample).

Table 4

Importance of components

	PC1	PC2	PC3
Standard deviation	2.9257	1.6047	1.2452
Proportion of variance	0.6114	0.1839	0.1108
Cumulative proportion	0.6114	0.7953	0.9061

Source: own processing

Table 5

PCs with respect to selected countries under the analysis

PC1	PC2	PC3	
-2.97677556	2.0364548	1.1019787	AT
0.06452467	-1.4647739	-0.9606211	DE
4.31375674	1.9115372	-0.6420512	CZE
-2.33420439	-0.6708318	0.3685516	HU
2.63897485	-1.5528708	1.6564579	SK
-1.70627631	-0.2595154	-1.5243158	PL

Source: own processing

Table 6

Rotations

	PC1	PC2	PC3
Capital average	0.31714	0.05563	0.25026
Capital trend	0.08068	0.15132	-0.32843
Creditors average	0.30383	0.27303	-0.06121
Creditors trend	-0.19562	0.18136	0.60144
EBITDA average	0.20498	-0.29848	0.43607
EBITDA trend	-0.26384	-0.36947	0.13133
Number of employees average	0.32664	0.01613	0.22277
Number of employees trend	0.25642	-0.30909	-0.11048
Operating revenue (Turnover) aver.	0.31208	0.24993	0.02845
Operating revenue trend	0.23489	-0.40079	0.11399
Sales average	0.31323	0.24522	0.03043
Sales trend	0.25430	-0.38271	0.04184
Working capital aver.	0.32077	0.20029	0.09148
Working Capital trend	0.24493	-0.27450	-0.41223

Source: own processing

Visualizing the results

The visualizations below illustrate the relationships between the size, development, and equity development of companies and countries.

This plot demonstrates that Suedzucker AG (point 6) dominates in size (high PC1 score) but demonstrates minimal development potential (low PC2 score). Pfeifer & Langen Polska S.A. (point 10), on the other hand, demonstrates strong growth despite its smaller size.

Here, the size of the company (PC1) is compared with its equity development (PC3). While larger companies like Suedzucker AG have minimal equity development, smaller companies like Litovelská Cukrovarna A.S. or Cukrovarna Vrbatky, a Moravskoslezké cukrovary demonstrate comparable equity development potential, indicating that equity management plays a key role in smaller companies' financial strategies.

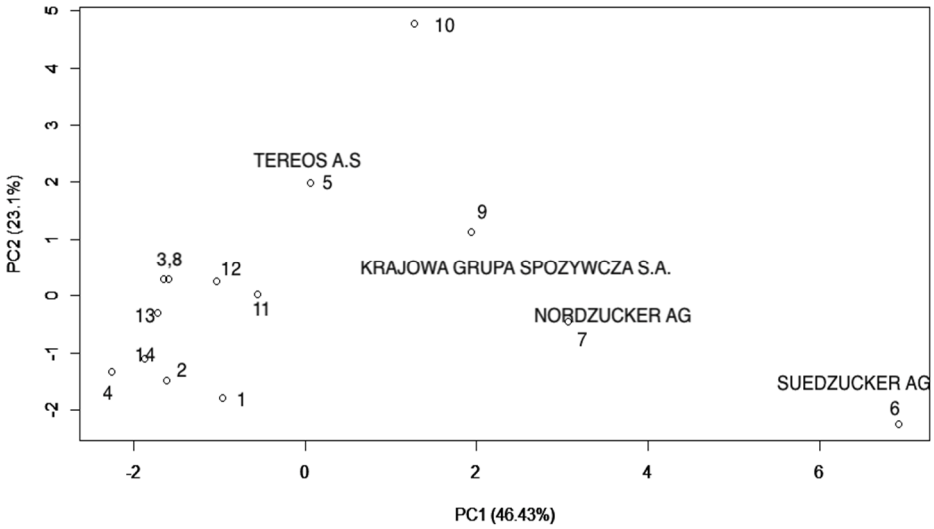

Figure 1. Size of the Company vs. Development (PC1 vs. PC2)
Source: own processing
The legend: 1 – Agrana Zuker GMBH, 2 – Moravskoslezke Cukrovary s.r.o., 3 – Cukrovar Vrbatky a.s., 4 – Litovelska Cukrovarna A.S., 5 – Tereos a.s., 6 – Seuzucker AG., 7 – Nordzucker AG, 8 – Magyar Cukorgyarto es forgalmazo zartk, 9 – Krajowa Grupa Spozywcza a.s., 10 – Pfeifer & Langen Polska s.a., 11 – Sudzucker Polska s.a., 12 – Nordzucker Polska s.a., 13 – Povazsky Cukor a.s., 14 – Slovenske Cukrovary s.r.o.

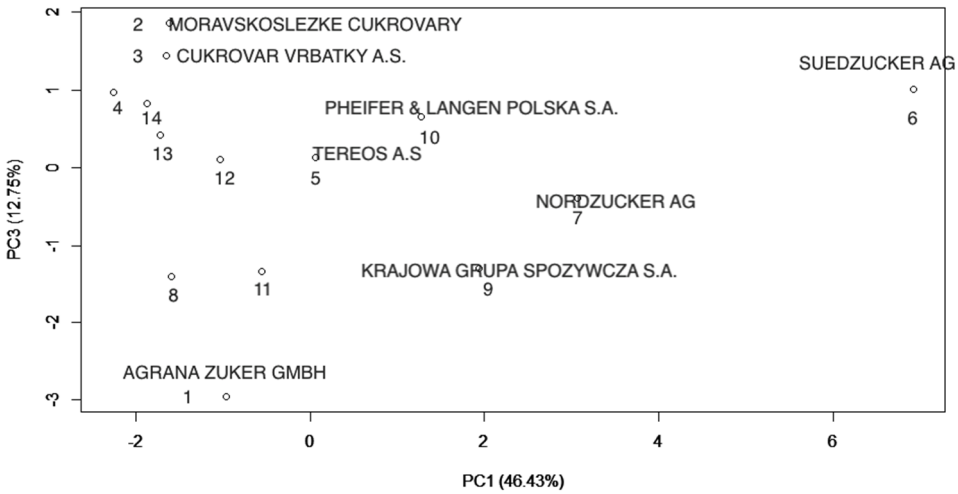

Figure 2. Size of the Company vs. Equity Development (PC1 vs. PC3)
Here, the size of the company (PC1) is compared with its equity development (PC3).
Source: own processing,
The legend: 1 – Agrana Zuker GMBH, 2 – Moravskoslezke Cukrovary s.r.o., 3 – Cukrovar Vrbatky a.s., 4 – Litovelska Cukrovarna a.s., 5 – Tereos a.s., 6 – Seuzucker ag., 7 – Nordzucker ag, 8 – Magyar Cukorgyarto es Forgalmazo Zartk, 9 – Krajowa Grupa Spozywcza a.s., 10 – Pfeifer & Langen Polska s.a., 11 – Sudzucker Polska s.a., 12 – Nordzucker Polska s.a., 13 – Povazsky Cukor a.s., 14 – Slovenske Cukrovary s.r.o.

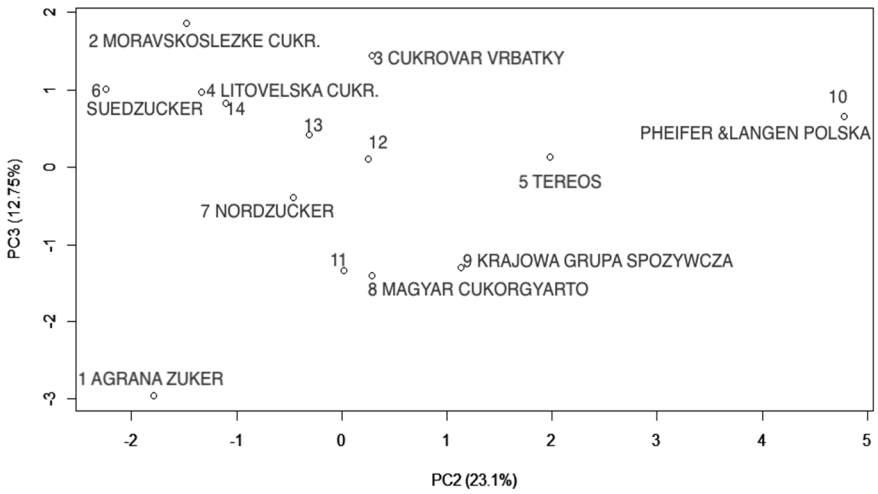

Figure 3. Development potential of the Company vs. Equity Development Trend (PC2 vs. PC3)
Source: own processing
The legend: 1 – Agrana Zuker GmbH, 2 – Moravskoslezke Cukrovary s.r.o., 3 – Cukrovar Vrbatky a.s., 4 – Litovelska Cukrovarna a.s., 5 – Tereos a.s., 6 – Seuzucker ag., 7 – Nordzucker ag, 8 – Magyar Cukorgyarto es Forgalmazo Zartk, 9 – Krajowa Grupa Spozywcza a.s., 10 – Pfeifer & Langen Polska s.a., 11 – Sudzucker Polska s.a., 12 – Nordzucker Polska s.a., 13 – Povazsky Cukor a.s., 14 – Slovenske Cukrovary s.r.o.

Principal Component Analysis components linked to variables like EBITDA, sales, and working capital indicate that there are differences in operational efficiency and financial health among companies. However, these components do not provide direct insights into the technological or operational practices that may underlie these financial results. The financial variables examined in the Principal Component Analysis, do not account for potential shifts in sustainable practices or environmental regulations, which are becoming increasingly important in the sugar production sector.

The Principal Component Analysis results demonstrate that some companies, like Südzucker (Germany) and Agrana Zucker GMBH (Austria), have significantly higher performance in terms of PC1 (size), but their development potential seems limited according to PC2. The findings indicate these companies are large but may not exhibit the same growth trajectory as smaller firms, indicating a potential issue of market concentration or lack of innovation.

Country-specific insights

PCA analysis reveals notable differences: while Austria and Germany demonstrate financial stability characteristic of mature markets, Poland and Hungary show growth potential indicative of dynamic, developing industries.

– Czech Republic (CZE) demonstrated the highest score on PC1, indicating a strong financial position with high capital availability and strong operating revenue. The country's dynamics of the sugar market appears to be dynamic, with a substantial potential for growth.

- Poland (PL) and Slovakia (SK) also scored highly on PC1, signaling robust financial bases in their sugar industries, though Slovakia demonstrated stronger growth potential (positive PC2 score).
- Germany (DE) and Austria (AT), with lower PC1 scores, indicate that these markets are more mature, with less room for expansion but strong financial stability.
- Hungary (HU) exhibited a lower PC1 score, indicating weaker financial fundamentals in its sugar sector, though the country demonstrated some growth potential in PC2.

Rotated component loadings for states

The Principal Component Analysis (PCA) revealed distinct country-specific performance patterns, especially between countries like Austria and Germany versus Czechia, Hungary, and Slovakia. However, the underlying drivers behind these country-level disparities are not fully explained in the Principal Component Analysis (PCA). For example, the variance captured by PC1 (representing company size) and PC2 (representing company development) indicates certain countries' firms exhibit larger size or growth potential.

The country-specific results of Principal Component Analysis (PCA), particularly the differences between Poland and Slovakia, indicate that there may be unique economic or regulatory environments influencing the dynamics of the sugar market outcomes. However, Principal Component Analysis (PCA) alone cannot explain why Poland's sugar-producing companies, despite demonstrating favorable trends in capital and working capital, might perform differently compared to their counterparts in Slovakia or Hungary.

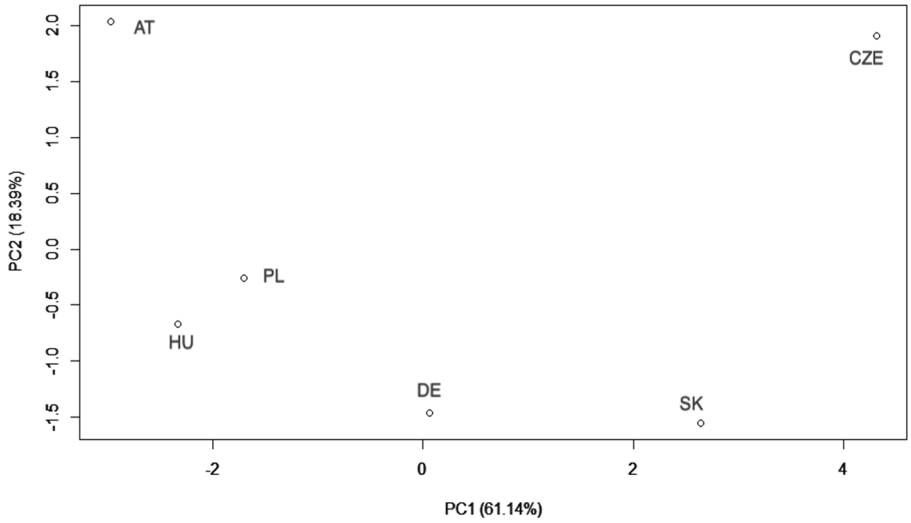

Figure 4. Strength of each State vs. Development trend of the market (PC1 vs. PC2)
Source: own processing
The legend: Czech Republic (CZE), Poland (PL), Slovakia (SK),
Germany (DE) Austria (AT), Hungary (HU)

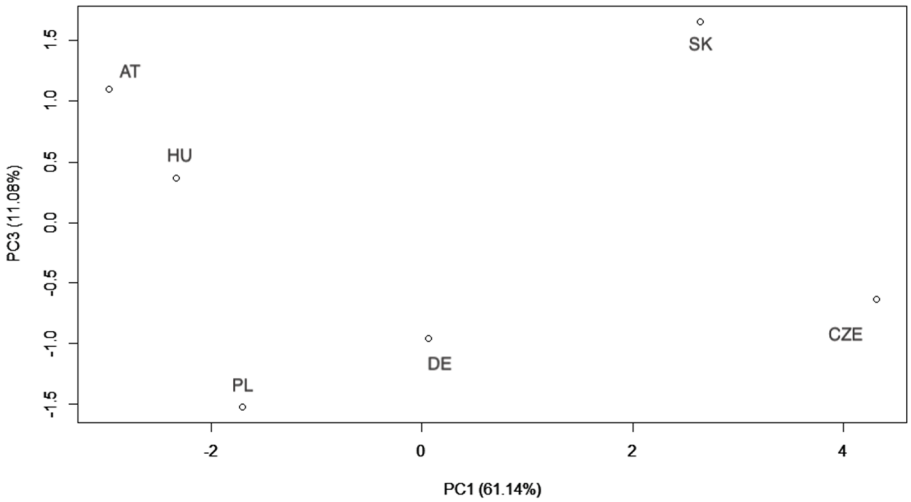

Figure 5. Strength of each State vs Equity Development trend (PC1, PC3)
Source: own processing
The legend: Czech Republic (CZE), Poland (PL), Slovakia (SK),
Germany (DE) Austria (AT), Hungary (HU)

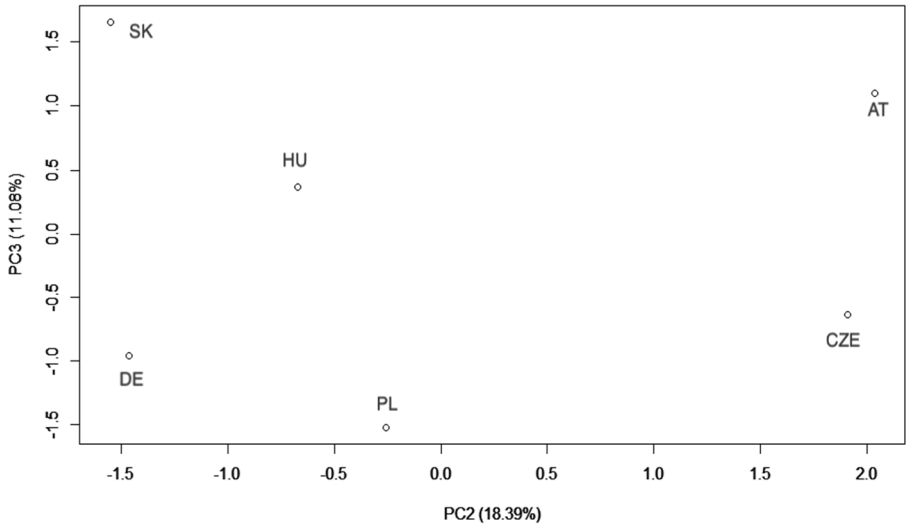

Figure 6. Development Trend of the Market vs Equity Development Trend (PC2, PC3)
Source: own processing
The legend: Czech Republic (CZE), Poland (PL), Slovakia (SK),
Germany (DE) Austria (AT), Hungary (HU)

Implications for the sugar market

The Principal Component Analysis (PCA) results highlight several key trends in the sugar production sector.

Larger companies, such as Suedzucker AG, dominate the market in terms of size but have limited development potential, reflecting a more mature business model that prioritizes market stability.

Smaller companies like Litovelská Cukrovarna A.S. may exhibit similar equity development potential despite their smaller size, indicating that growth is possible in smaller markets with the right management strategies.

The Czech Republic and Poland stand out as dynamic markets with high growth potential, while Germany and Austria demonstrate signs of maturity and lower growth potential.

The findings indicate that smaller, developing markets may offer more opportunities for innovation and growth, while more mature markets focus on maintaining profitability and managing operational efficiency (Motta, 2004).

Financial size and stability

The first principal component (PC1) accounted for 46.43% of the total variance, which clearly indicates that company size plays a dominant role in defining their financial profiles. Larger companies such as Suedzucker AG and Agrana Zucker GmbH score highly on this component, reflecting their substantial financial resources, capital, and operating revenue. These companies benefit from economies of scale, a strong market presence, and the ability to leverage significant capital for long-term stability.

However, the results indicate that simply being large does not guarantee sustained growth or market leadership. Suedzucker AG, despite its size, demonstrates minimal development potential as indicated by its negative score on the second principal component (PC2). This highlights a trend observed in larger companies across various industries: maturity can result in stagnation.

This trend is evident in the company-level Principal Component Analysis, where larger firms generally demonstrate less aggressive growth patterns. Companies like Pfeifer & Langen Polska S.A., despite their smaller size, score higher on PC2, reflecting their more dynamic and growth-oriented strategies. This observation challenges the traditional view that larger companies automatically outperform smaller ones and indicates that strategic adaptability is key to fostering growth.

Growth potential and development

The second principal component (PC2), which accounted for 23.10% of the total variance, was crucial in highlighting the growth potential of sugar-producing companies. While large firms like Suedzucker AG demonstrated limited growth (negative PC2), smaller companies demonstrated considerable growth potential. For example, Pfeifer & Langen Polska S.A.

This finding is consistent with broader trends in the food and beverage sector, where smaller, more agile companies often demonstrate greater flexibility in adapting to market changes and emerging consumer preferences. They are also more likely to experiment with new products, production methods, and marketing strategies, providing them with a competitive edge.

Interestingly, Agrana Zucker GMBH, despite being one of the larger companies, scored low on PC2, signaling that size alone does not guarantee development. It indicates that some companies in the sugar production sector may be facing challenges in adapting to changing

market conditions or may be overly reliant on established practices. In contrast, companies with more aggressive growth strategies, like Tereos a.s.

Equity and financial health

The third principal component (PC3), which explained 12.75% of the variance, offered insights into the equity development and financial health of companies. This component is especially important because it highlights how companies manage their liabilities (creditors) and working capital to maintain financial stability.

Larger companies, which typically have more access to financing and larger capital bases, generally display a stronger capacity to manage their equity development. However, this is not always the case. Companies such as Pfeifer & Langen Polska S.A., despite their smaller size, demonstrate impressive scores on PC3, indicating that effective management of equity and liabilities can also help smaller companies maintain financial health and foster sustainable growth.

The presence of smaller companies like Litovelská Cukrovarna A.S. with a strong performance on PC3 challenges the assumption that larger companies are inherently more stable. In fact, the ability to manage working capital effectively and maintain a healthy balance between equity and liabilities appears to be a key driver of financial success, regardless of company size.

Country-level analysis: regional market dynamics

The analysis at the country level reveals interesting regional variations in the financial dynamics of the sugar production sector. Countries like the Czech Republic (CZE) and Poland (PL) score highly on PC1, reflecting a strong financial base and competitive sugar industries. These countries appear to have a balanced combination of capital availability, operating revenue, and profitability, which positions them well for sustained market leadership.

In contrast, countries like Hungary (HU) and Slovakia (SK) exhibit lower scores on PC1, which may reflect weaker financial positions or less mature dynamics of the sugar markets. However, Slovakia, in particular, stands out with a positive score on PC2, indicating that while its market may be smaller or less financially robust, it is demonstrating potential for growth and development. (Penrose (2009)).

Germany (DE) and Austria (AT) are two countries with lower scores on PC1, indicating that their sugar industries are more mature. This indicates that companies in these countries might face challenges in growing or adapting to rapidly changing market conditions. The low growth potential reflected in PC2 for both countries further supports the idea that these markets may be in a consolidation phase, with companies focusing on maintaining existing market share rather than pursuing significant expansion.

Implications for strategy and policy

The findings of this study have important implications for both sugar-producing companies and policymakers in Central and Eastern Europe.

For Companies: The analysis indicates that while large companies benefit from financial stability and market dominance, they must be vigilant about potential stagnation. To sustain growth, even the largest companies must explore new market opportunities, invest in innovation, and diversify their product offerings. Smaller companies, on the other hand,

may be able to leverage their agility and growth potential to disrupt the market, provided they manage their financial resources effectively and adapt to changing consumer demands.

For Policymakers: The analysis also has important policy implications. Countries with strong financial positions, such as the Czech Republic and Poland, should focus on fostering innovation and supporting the growth of smaller companies that demonstrate potential for expansion. In contrast, policymakers in countries with less robust markets, like Hungary and Slovakia, should focus on improving the financial health of local sugar-producing companies, particularly by offering support for equity development and efficient resource management.

Additionally, there is a clear need for regionally tailored policies that consider the different stages of market maturity across countries. Policymakers should encourage investment in technology, sustainable practices initiatives, and market diversification, particularly in more mature markets where growth has plateaued.

Limitations and future research

While the Principal Component Analysis provided valuable insights, several limitations should be considered. The study focused on financial data over a five-year period (2018-2022), which may not fully capture short-term market fluctuations or sudden shifts in the global dynamics of the sugar market. Additionally, Principal Component Analysis, while powerful for identifying patterns, does not capture all the underlying causes of financial dynamics, such as market competition, consumer preferences, or regulatory changes.

Future research could explore the role of these external factors in shaping the financial performance of sugar-producing companies. Further analysis of the industry's responses to global sugar price trends, sustainable practices concerns, and technological advancements could provide a more comprehensive understanding of the market dynamics.

In addition, the inclusion of non-financial variables, such as consumer sentiment or regulatory factors, could offer a richer, more nuanced view of the sugar production sector in Central and Eastern Europe.

Discussion

The Principal Component Analysis results provide important insights into the financial structure and strategic positioning of sugar-producing companies in Central and Eastern Europe. The first principal component (PC1) explains 46.43% of the variance and is strongly associated with company size, confirming prior research by Kotyza et al. (2019), which found that larger firms benefit from economies of scale, greater capital resources, and stronger market positions. However, despite their financial stability, large companies such as Suedzucker AG and Agrana Zucker GMBH exhibit limited growth potential, as indicated by their negative scores on the second principal component (PC2). This aligns with Muir and Anderson (2022), who demonstrated that post-2017 EU sugar quota abolition led to reduced expansion incentives for established market leaders. In contrast, smaller firms such as Pfeifer & Langen Polska S.A. exhibit higher PC2 scores, suggesting that they are more agile and responsive to market changes, a trend consistent with findings by Severini and Sorrentino (2017), who highlighted the role of flexible business models in adapting to policy shifts.

Growth potential (PC2) accounted for 23.10% of the variance, reinforcing the notion that financial expansion is not solely dictated by company size but by equity management and strategic reinvestment. Similar to observations by Schroeder et al. (2022) in food supply chain financial studies, firms with robust EBITDA trends and efficient working capital management demonstrate higher growth trajectories. This suggests that strategic adaptability

and financial health are key determinants of expansion rather than just scale. Moreover, equity management (PC3), which explains 12.75% of the variance, highlights critical differences in financial stability across firms, reinforcing Labrin and Urdinez (2020) and Nes et al. (2021), who found that effective creditor-liability balancing is fundamental for long-term sustainability in capital-intensive industries. While large companies may rely on financial reserves to sustain operations, mid-sized firms showing strong PC3 scores, such as Tereos a.s., illustrate that dynamic investment in capital and efficient debt structuring are key drivers of sustainable growth.

At the regional level, the PCA analysis confirms significant disparities in financial health and market maturity across CEE countries. The Czech Republic and Poland exhibit high PC1 scores, reflecting their strong financial positioning, which aligns with Pawlak and Smutka (2022), who found that these countries have maintained a competitive edge in agri-food exports post-quota abolition. Conversely, Hungary and Slovakia score lower on PC1, highlighting weaker financial structures, consistent with Soare et al. (2021), who identified external investment constraints and structural inefficiencies as barriers to sugar market growth in these countries. Moreover, Meghana and Shastri (2020) emphasize that sustainability concerns and environmental costs are increasingly shaping financial strategies in the sugar sector, an area that could influence future profitability and competitiveness. These findings suggest that while larger markets emphasize consolidation, emerging markets require targeted policy interventions to enhance financial resilience.

The results reinforce previous literature by demonstrating that sugar market dynamics in CEE are driven by a trade-off between financial stability and growth potential. Larger firms exhibit strong capital bases but limited expansion, whereas smaller firms have higher agility but require strategic financial management to scale successfully. The study also highlights the importance of sustainable business models, as emphasized by Aguilar-Rivera (2022), who argued that resource efficiency and technological innovation will be crucial for future competitiveness in the sugar industry. Policymakers should consider these insights when developing strategies for sectoral sustainability, balancing financial growth with regulatory and environmental constraints.

Conclusions

The analysis of the sugar production sector in Central and Eastern Europe reveals diverse dynamics at both the company and country levels, highlighting critical factors influencing financial stability, growth potential, and sustainability.

At the company level, larger firms such as Südzucker AG and Agrana Zucker GMBH dominate the market in size and financial resources, benefiting from economies of scale and stable capital bases. However, these companies demonstrate limited growth potential, reflecting their mature market position and focus on maintaining stability rather than expansion. In contrast, smaller companies like Pfeifer & Langen Polska S.A. and Litovelská Cukrovarna A.S. exhibit stronger growth trajectories, leveraging their agility to adapt to market changes and seize new opportunities. Companies with effective equity and resource management, such as Tereos A.S., are better positioned for steady growth, regardless of their size.

At the country level, significant regional variations emerge. The Czech Republic and Poland stand out with strong financial positions and high development potential, signaling dynamic and competitive sugar industries. Slovakia, while smaller, demonstrates considerable growth opportunities, suggesting the need for strategic investments and policy

support. Germany and Austria reflect mature markets characterized by financial stability but slower growth rates, indicating a focus on consolidation. Meanwhile, Hungary's weaker financial fundamentals point to the need for improved financial strategies and capital management to enhance its industry's competitiveness.

Key drivers of these dynamics include critical financial indicators such as capital structure, operating revenue, and working capital, which determine company size and stability. Growth potential is closely tied to efficient equity management and operational adaptability. Additionally, the abolition of EU sugar quotas and global market pressures have shaped the sector, emphasizing the need for efficiency, innovation, and sustainability.

Sustainability practices are becoming increasingly important for long-term competitiveness. Larger firms must integrate renewable energy solutions and adopt sustainable resource management, while smaller and medium-sized firms should focus on leveraging innovation and diversifying their offerings to capture new market opportunities. Policymakers play a vital role in tailoring strategies to the specific needs of each country's sugar industry. In mature markets like Germany and Austria, efforts should prioritize fostering innovation and ensuring sustainability. For emerging markets such as Poland and Slovakia, policies should focus on improving financial health, increasing capital investment, and supporting growth-oriented firms.

The study highlights the importance of balancing size with strategic growth and innovation. Larger companies must avoid stagnation by diversifying their portfolios and investing in new technologies, while smaller firms can exploit their flexibility to drive growth. Effective financial management, sustainable practices, and tailored policies will ensure resilience and long-term competitiveness for the sugar production sector across the CEE region.

In conclusion, the sugar industry in Central and Eastern Europe faces distinct opportunities and challenges. By addressing market dynamics and integrating sustainable and innovative practices, companies and policymakers can secure the sector's continued success in an evolving global market.

References

Aguilar-Rivera N. (2022), Bioindicators for the sustainability of sugar agro-industry, *Sugar Tech*, 24(3), pp. 651-661, https://doi.org/10.1007/s12355-021-01105-z

Chowdhury U.N., Rayhan M.A., Chakravarty S.K., Hossain M.T. (2017), Integration of principal component analysis and support vector regression for financial time series forecasting, *International Journal of Computer Science and Information Security*, 15(8), pp. 28-32.

Crepey S., Lehdili N., Madhar N., Thomas, M. (2022), Anomaly detection in financial time series by principal component analysis and neural networks, *Algorithms,* 15(10), 385, https://doi.org/10.3390/a15100385

De Jong R., Verbesselt J., Schaepman M. E., De Bruin S. (2012), Trend changes in global greening and browning: contribution of short-term trends to longer-term change, *Global Change Biology*, 18(2), pp. 642-655, https://doi.org/10.1111/j.1365-2486.2011.02578.x

Deutsch H.P., Beinker M.W. (2019), Principal component analysis, *Derivatives and internal models: Modern risk management*, (pp. 793-804). Palgrave Macmillan Cham.

Diggle P., Giorgi E. (2024), *Time series: A biostatistical introduction*, Oxford University Press.

Formann S., Hahn A., Janke L., Stinner W., Sträuber H., Logroño W., Nikolausz M. (2020), Beyond sugar and ethanol production: value generation opportunities through sugarcane residues, *Frontiers in Energy Research*, 8, 579577, https://doi.org/10.3389/fenrg.2020.579577

Gabriel K.R. (1971), The biplot graphic display of matrices with application to principal component analysis, *Biometrika,* 58(3), pp. 453-467, https://doi.org/10.2307/2334381

Gabriel K.R., Odoroff C.L. (1990), Biplots in biomedical research, *Statistics in Medicine*, 9(5), pp. 469-485, https://doi.org/10.1002/sim.4780090502

García-Bustamante C.A., Aguilar-Rivera N., Zepeda-Pirrón M., Armendáriz-Arnez C. (2018), Development of indicators for the sustainability of the sugar industry, *Environmental Socio-Economic Studies*, 6(4), pp. 22-38, https://doi.org/10.2478/environ-2018-0025

Hamilton J. D. (2020), *Time series analysis*, Princeton University Press.

Huang D., Jiang F., Li K., Tong G., Zhou G. (2022), Scaled PCA: A new approach to dimension reduction, *Management Science*, 68(3), pp. 1678-1695, https://doi.org/10.1287/mnsc.2021.4020

Kotyza P., Hornowski A., Pawlak K., Smutka L. (2018), Czech and Polish sugar industry – concentration of sugar production, *Proceedings of the 2018 International Scientific Conference 'Economic Sciences for Agribusiness and Rural Economy',* No 2, Warsaw, 7–8 June 2018, pp. 136–143, http://dx.doi.org/10.22630/esare.2018.2.17

Kotyza P., Smutka L., Pawlak K. (2019), Changes in sugar beet production in the Czech Republic and Poland after the year 2000, *Journal of Central European Agriculture*, 20(3), pp. 1023–1043, http://dx.doi.org/10.5513/jcea01/20.3.2313

Labrin C., Urdinez F. (2020), Principal component analysis, In: F. Urdinez, A. Cruz (Eds.), *R for Political Data Science,* pp. 375-393, Chapman and Hall/CRC.

Lin R.M., Mottershead J.E., Ng T.Y. (2020), A state-of-the-art review on theory and engineering applications of eigenvalue and eigenvector derivatives, *Mechanical Systems and Signal Processing*, 138, 106536, https://doi.org/10.1016/j.ymssp.2019.106536

Liu Z., Bai Y. (2021), Financial performance evaluation of electric power listed companies based on principal component analysis, *Journal of Applied Science and Engineering Innovation*, 8(1), pp. 18–23, https://doi.org/10.1088/1742-6596/1955/1/012123

Mahoney J. T. (2004), *Economic foundations of strategy*, Sage Publications.

Meghana M., Shastri Y. (2020), Sustainable valorization of sugar industry waste: Status, opportunities, and challenges, *Bioresource Technology*, 303, 122929, https://doi.org/10.1016/j.biortech.2020.122929

Muir B.M., Anderson A.R. (2022), Development and diversification of sugar beet in Europe, *Sugar Tech*, 24(4), pp. 992-1009, http://dx.doi.org/10.1007/s12355-021-01036-9

Nes K., Colen L., Ciaian P. (2021), *Market power in food industry in selected EU Member States*, Publications Office of the European Union, Luxembourg.

Pawlak K., Smutka L. (2022), Does Poland's agri-food industry gain comparative advantage in trade with non-EU countries? Evidence from the transatlantic market *Plos One*, 17(9), e0274692, https://doi.org/10.1371/journal.pone.0274692

Řezbová H., Maitah M., Sergienko O.I. (2015), EU quota sugar market concentration–the main drivers of EU sugar market, *AGRIS on-line Papers in Economics and Informatics*, 7(4), pp. 131-142, http://dx.doi.org/10.7160/aol.2015.070413

Rosario D., Robin C. (2017), *EU sugar quota system comes to an end*, Available at: https://ec.europa.eu/commission/presscorner/detail/en/IP_17_3487

Schroeder R.G., Clark M.W., Cathey J.M. (2022), *Financial accounting theory and analysis: Text and cases*, John Wiley Sons.

Severini S., Sorrentino A. (2017), Efficiency and coordination in the EU agri-food systems, *Agricultural and Food Economics*, 5, 15, https://doi.org/10.1186/s40100-017-0086-9

Singh C.S., Soni G., Badhotiya G.K. (2019), Performance indicators for supply chain resilience: review and conceptual framework, *Journal of Industrial Engineering International*, 15(1), pp. 105-117, https://doi.org/10.1007/s40092-019-00322-2

Smutka L., Pawlak K., Kotyza K., Svatoš M. (2018), Polish sugar industry development, *AGRIS on-line Papers in Economics and Informatics*, 10(1), pp. 71-90, https://doi.org/10.7160/aol.2018.100107

Smutka L., Pawlak K., Kotyza P., Pulkrábek J. (2019), Polish sugar market specifics and development, *Listy Cukrovarnické a Reparské*, 135(4), 154.

Soare E., Dobre I., David L. (2021), Research on sugar beet production and trade-worldwide overview, *Scientific Papers. Series "Management, Economic Engineering in Agriculture and Rural Development",* 21(4), pp. 533-540.

Swinnen J., Van Herck K., Vranken L. (2010), Shifting patterns of agricultural production and productivity in the former Soviet Union and Central and Eastern Europe, In J.M. Alston, B.A. Babcock, P.G. Pardey (Eds.), *The shifting patterns of agricultural production and productivity worldwide* (pp. 279-313), Available at https://www.card.iastate.edu/files/inline-files/chapter10.pdf

Vernimmen P., Quiry P., Le Fur Y. (2022), *Corporate finance: theory and practice*, John Wiley Sons.

Xue J., Zhou S., Liu Q., Liu X., Yin J. (2018), Financial time series prediction using ℓ2, 1RF-ELM, *Neurocomputing*, 277, pp. 176–186, https://doi.org/10.1016/j.neucom.2017.04.076

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Study on consumer behavior towards food waste in Azerbaijan

Tülay Yeniçeri, Tamerlan Akbarov

Bandirma Onyedi Eylul University, Turkey

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Corresponding author:

Tamerlan Akbarov
E-mail: tamerlanakbarov@ogr.bandirma.edu.tr

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Abstract

Introduction. The aim of this study was to investigate consumer behavior towards food waste in Azerbaijan.

Materials and methods. 455 people were determined in accordance with the criteria suggested by Cohen, Manion and Morrison (2018) and can represent the universe with a 95% confidence interval and a 5% margin of error. The relationships between food waste and its determinants were analyzed by Partial Least Squares Structural Equation Modeling (PLS–SEM) method.

Results and discussion. According to the structural equation modeling (PLS–SEM) analysis, only the variable "food characteristics" was found to have a significant effect on the amount of food waste ($\beta = 0.137$, $p = 0.005$). All other variables – food quantity ($\beta = –0.061$, $p = 0.498$), durability ($\beta = –0.014$, $p = 0.791$), frequency of purchase ($\beta = –0.066$, $p = 0.158$), peeling habits ($\beta = –0.090$, $p = 0.101$), purchase method ($\beta = 0.035$, $p = 0.642$) and price ($\beta = 0.016$, $p = 0.749$) – were not statistically significant.

Furthermore, no significant relationship was observed between food waste attitudes and the amount of waste ($\beta = –0.142$, $p = 0.213$). Of the 455 participants in the study, 77% were female and 91% were between the ages of 18–24. 58% of the participants are high school graduates and 32% are undergraduate graduates. 43% of the households consisted of five or more people.

When analyzing the responses to the question about the amount of food waste, 46% of respondents said that they throw away less than 5%, and 27% of respondents said that they throw away 6–15%. These results show that consumers in Azerbaijan have a low level of awareness about food waste and that waste is mostly due to the physical characteristics of food (freshness, appearance, and flavor). Other economic or behavioral factors were not found to be statistically effective. Thus, Azerbaijani consumers often throw away food products depending on their properties, as they do not care about reducing food waste in their country. Changing consumer behavior is one of the key areas to reduce overall food waste.

Conclusion. The results of the study allow raising consumer awareness about food waste and give grounds to believe that it is necessary to improve consumption planning by both consumers and the food sector in Azerbaijan.

Introduction

According to the Waste Management Regulation (2015), "waste" is any substance, product or material that is disposed of or thrown away by its producer or the real or legal person who holds it. Waste product is examined in three definitional frameworks as "food loss", "food waste" and "food waste". Food loss is the decrease in the quantity or quality of food suitable for human consumption (Lipinski et al., 2013), food waste is an inefficiency that occurs mostly at the final stage and is associated with changes in consumer behavior or habits (Parfitt et al., 2010), and food waste is a general term that covers both food loss and food waste (FAO, 2024). 54% of food waste occurs in the post-harvest processing and storage stages of production, while 46% occurs in the processing, distribution and consumption stages. According to the 2024 Food Waste Index Report published by the United Nations Environment Programme (UNEP), the largest share occurred at the household level (631 million tons of food waste) (UNEP, 2024).

According to the 2024 Food Waste Index Report prepared by the United Nations Environment Programme (UNEP) and Waste and Resources Action Programme (WRAP), 1.05 billion tons of food is wasted every year worldwide. This means that one in every five plates consumed is thrown away. Homes play a significant role in increasing food waste (Katsarova, 2011; Stenmarck et al., 2016; Zhang et al., 2018; Veselá et al., 2023). 60% of the waste, or 631 million tons, is caused by households. The remaining waste originates from the production, sales and food and beverage sectors. The report also states that even just half of the wasted food would be enough to solve the hunger problem of 783 million hungry people worldwide (UNEP, 2024).

According to FAO (2023) data, the most wasted products in the world are; bread and cereals (30%), milk and dairy products (20%), fruits and vegetables (40%) and meat products. Household food waste is increasing due to reasons such as faulty planning, excessive shopping, misinterpretation of date labels, inappropriate storage conditions, aesthetic preferences and incorrect portioning, as well as individuals' lack of knowledge about food evaluation (Aschemann et al., 2015; Graham et al., 2014; Hebrok and Boks, 2017; Mnerie et al., 2016; Qi and Roe, 2016; Quested and Johnson, 2009; Szymkowiak et al., 2022). Existing literature reveals that the majority of food waste is generated by consumers (Griffin et al., 2009; Kantor et al., 1997). Food waste is also a result of individuals' consumption behaviors shaped by psychological, economic and social factors (Solomon et al., 2006; Tas and Boyacıoğlu, 2024). While Marshall's Economic Model suggests that excessive purchases of products due to discounts may lead to waste (Marshall, 1890), Freud's Psychoanalytic Model states that excessive food intake due to unconscious motivations is aimed at increasing the sense of security (Freud, 1923). Pavlov's Conditioning Model states that factors such as advertising and social norms can shape individuals' automatic consumption habits and trigger waste (Pavlov, 1927).

The causes and levels of food waste vary across countries. Although West Asian countries are an important area of research on food waste, they have not been sufficiently examined in terms of consumer behavior across countries. Among these countries, Azerbaijan was included in the scope of the study. The main reason for the selection of Azerbaijan is the limited academic research on household food waste in the country and the lack of data in this area. According to the 2024 Food Waste Index Report, Azerbaijan ranks low confidence among West Asian countries in terms of per capita food waste.

Based on the total population of 210,500 out of a total population of 10,460,041 in Azerbaijan (The World Bank, 2024), the country is expected to annually waste 102 kg of food per person in 2024, totalling 1,055,462 tonnes. Compared to other West Asian countries,

Azerbaijan's food waste is higher than Kuwait (420,861 tonnes), Oman (451,415 tonnes), Palestine (534,863 tonnes), United Arab Emirates (930,427 tonnes), Cyprus (88,750 tonnes), Israel (874,433 tonnes). Lower than Yemen (3,490,097 tonnes), Turkey (8,694,318 tonnes), Jordan (1,136,788 tonnes), Saudi Arabia (3,818,681 tonnes), Iraq (6,378,198 tonnes) (UNEP, 2024; p. 171). Azerbaijan ranks in the middle levels among West Asian countries in terms of food waste and is in a remarkable position in terms of regional comparisons. However, empirical research on food waste in the country is limited. There are no comprehensive studies in the literature that examine the awareness levels, purchasing habits and waste management behaviors of consumers in Azerbaijan on food waste.

Azerbaijan is an economically developing country and changes in income levels directly affect consumption patterns. Understanding the impact of changes in food consumption with increasing welfare level on wastage is critical for developing sustainable food management strategies. In this direction, our study aims to determine the food waste behavior of households in Azerbaijan, measure their awareness levels and analyze the factors affecting waste. This research can be a welcome step in uncovering factors that are missing in this field. It is also hoped that this study may be useful in raising public awareness about food waste.

The aim of this study was to identify the behavioral, psychological and structural factors that cause household food waste in Azerbaijan, to assess consumers' awareness of food waste and to contribute to effective waste reduction strategies for policy makers, practitioners and future researchers in line with the findings.

Materials and methods

Research design and sample selection

This research was conducted using quantitative method. The data were collected through a survey method. The survey was conducted in Azerbaijan between December 2023 and February 2024 using Google Forms.

The sample size was determined as 455 people. As of December 2024, the total population of Azerbaijan was calculated as 10.18 million. In order to evaluate the representativeness of the sample from the population in question, the sample size criteria proposed by Cohen, Manion and Morrison (2018; p. 480) were taken into consideration. According to his calculation criteria, 300–500 samples are generally sufficient for large populations. In this context, the sample of 455 people used in the study is representative of household food waste behaviors in Azerbaijan with 95% confidence interval and 5% margin of error. Moreover, it meets the minimum sample size recommended for PLS-SEM analysis (Hair et al., 2022). The sample is considered methodologically reliable to represent the household food waste behavior in Azerbaijan.

Questionnaire design and data collection

A survey form has been prepared to elucidate the reasons for food waste in consumers' homes and to determine their behaviors on the subject. The developed survey form was designed based on scales used in Ananda et al. (2021); Grainger et al. (2018); Ilakovac et al. (2020); Richter and Bokelmann (2017); Russell et al. (2017); Setti et al. (2018); Veselá et al. (2023); Wilson et al. (2017) studies, and 24 variables were identified (Table 1). These include: Amount of food waste (G1_1), amount of food (G2_1, G2_2, G2_3, G2_4), food

durability (G3_1), food characteristics (G4_1, G4_2, G4_3, G4_4, G4_5, G4_6), frequency of food purchasing (G5_1), food preparation (G6_1, G6_2), food shopping (G7_1, G7_2, G7_3), price (G8_1, G8_2, G8_3), and attitude towards food waste (G9_1, G9_2, G9_3). The amount of food waste is measured on a 7-point Likert scale (1 = I don't know the amount thrown away, 2 = 5% or less, 3 = 6–15%, 4 = 16–30%, 5 = 31–50%, 6 = more than 50%, 7 = None of these), while all other variables are measured on a 5-point Likert scale.

Table 1

Variables

G1_1	What percentage of the food you buy do you think goes to waste?
G2_1	I cook a lot of food (more than the number of people).
G2_2	I buy large packages of food.
G2_3	I throw away uneaten food scraps without composting.
G2_4	I ensure that the amount of food to be purchased is planned and prepared.
G3_1	How often did you check ‘use by’ or ‘use by’ dates before buying food?
G4_1	I don't use food that spoils during transportation, I throw it away.
G4_2	If the food is not of sufficient quality, I will not use it and will throw it away.
G4_3	I do not use food that spoils during storage, I throw it away.
G4_4	I don't use food that doesn't appeal to me, I throw it away.
G4_5	If the food is not tasty, I don't continue eating it and throw it away.
G4_6	If the food packaging is damaged, I will not use it and throw it away.
G5_1	Please indicate the frequency of your food purchases.
G6_1	I don't pay attention when peeling fruit.
G6_2	I don't pay attention when peeling vegetables.
G7_1	I use a shopping list when buying food.
G7_2	I check stock before purchasing food.
G7_3	I ensure the consumption of all purchased foods by cooking additional meals.
G8_1	I shop based on the price of the product.
G8_2	I prefer large food packages as I think they are more convenient.
G8_3	I prefer discount grocery shopping.
G9_1	I think the issue of food waste remains relevant.
G9_2	I think food waste poses a risk.
G9_3	I think food waste behavior is bad.

Research model and hypotheses

The research model was developed based on the variables used in previous studies identified through the literature review. The model and hypotheses developed in accordance with the research objectives determined as a result of the literature review are presented below.

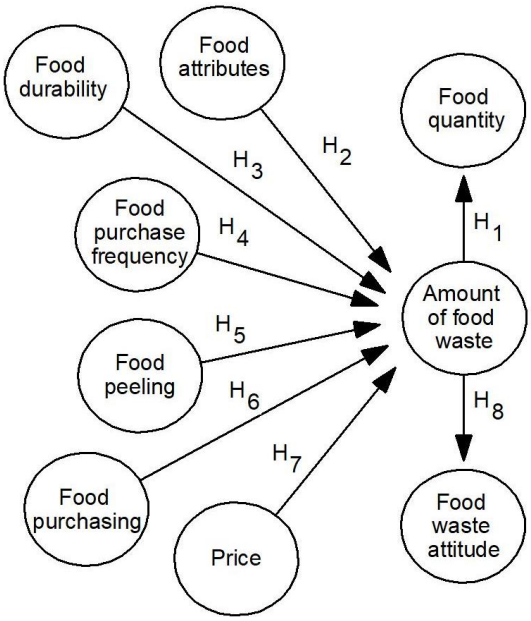

Figure 1. Research model

H1: There is a positive effect of food quantity on food waste amount.
H2: There is a positive effect of food durability on food waste amount.
H3: There is a positive effect of food quality on food waste amount.
H4: There is a positive effect of food purchase frequency on food waste amount.
H5: There is a positive effect of food preparation on food waste amount.
H6: There is a positive effect of food purchasing on food waste amount.
H7: There is a positive effect of price on food waste amount.
H8: There is a positive effect of food waste amount on food waste attitude.

Data analysis method

The research model and the data obtained were tested using Partial Least Squares Structural Equation Modeling (PLS-SEM) analysis. PLS-SEM method is accepted as a suitable method for small sample sizes and multivariate analyses in structural equation modeling (SEM) (Hair et al., 2022). The statistical tests applied in the data analysis phase are as follows: Confirmatory Factor Analysis (CFA) was used to test the validity and reliability of the scales. Internal Consistency Reliability (ICR), the reliability of the scales was evaluated with values of 0.70 and above. Discriminant Validity (Discriminant Validity - HTMT Criterion), HTMT coefficients suggested by Henseler et al. (2015) were examined. Hypothesis Testing and Regression Analysis, t-test and p-values were calculated to evaluate significant relationships between factors affecting food waste.

Results and discussion

Respondent characteristics

The demographic data obtained within the scope of the research are summarized in Table 1.

Table 1

Respondent characteristics

N = 455		Sample	Sample %			Sample	Sample %
Gender	Male	106	23	**Age**	18–24 years old	418	91
	Female	349	77		25–34 years old	27	5
Number of Households (Including you)	Yalnız Yaşıyorum	11	2		35–44 years old	8	2
	2	15	3		45–54 years old	1	1
	3	71	16		55–64 years old	1	1
	4	165	36		65–74 years old	-	-
	5+	193	43		75+ years old	-	-
Household Income (AZN)	1900-less	244	53	**Education**	Primary School		
	901–1500	121	27		Middle School	13	3
	1501–2000	47	10		High School	264	58
	2001–2600	17	4		Undergraduate	148	32
	2601+	26	6		Graduate	30	7

77% of the participants were female and 23% were male. When the age distribution is analyzed, it is seen that 91% of the participants are between the ages of 18-24. The majority of the participants (58%) are high school graduates, 32% are undergraduates and 7% have postgraduate education. When analyzed in terms of household size, 43% of the participants stated that they live with 5 or more people, while 36% stated that they live in households of 4 people.

Validity and reliability analysis of scales

Validity and reliability studies for the constructs in the research were made before the research model has been analyzed. The internal consistency reliability, convergent validity, discriminant validity in the context of the criterion-related validity of the scale were examined. Internal consistency reliability was tested by CR. Factor loadings and average variance extracted (AVE) values were used to assess convergent validity Factor loadings > 0.70, composite reliability coefficients ≥ 0.70 and AVE ≥ 0.50 are expected to occur (Hair et al., 2019, 2022). The measurement model results are presented in Table 2.

Table 2

Validity and reliability analyses of scales

Structure	Expression	Factor Loading	CR	AVE
Amount of food waste	1.1.AFW	1 .000	---	---
Food quantity	2.1.FQ	0 .782	0 .758	0 .611
	2.2.FQ	0 .782		
Food durability	3.1.FD	1 .000	---	---
Food attributes	4.1.FA	0 .726	0 .839	0 .635
	4.2.FA	0 .833		
	4.3.FA	0 .828		
Food purchase frequency	5.1.FPF	1 .000	---	---
Food peeling	6.1.FPE	0 .927	0 .924	0 .859
	6.2.FPE	0 .927		
Food purchasing	7.1.FPU	0 .662	0 .764	0 .520
	7.2.FPU	0 .778		
	7.3.FPU	0 .718		
Price	8.1.PRI	0 .800	0 .780	0 .639
	8.3.PRI	0 .800		
Food waste attitude	9.1.FWA	0 .724	0 .759	0 .512
	9.2.FWA	0.753		
	9.3.FWA	0.668		

*Amount of Food Waste =AFW; Food Quantity=FQ; Food Durability=FD; Food Attributes=FA; Food Purchase Frequency=FPF; Food Peeling=FPE; Food Purchasing=FPU; Price=PRI; Food Waste Attitude=FWA

According to Hair et al. (2022), was ≥ 0.70 for the factor loadings. According to the authors, items with factor loading less than 0.40 should be deleted from the measurement model, whereas items with factor loading range between 0.40 and 0.70 should be deleted when AVE or CR of the same variable is less than the required benchmark.

The indicator for Food Quantity (Item 4) was removed from the measurement model due to having a factor loading below 0.40. Factor loadings for Food Quality Items 4, 5, and 6; Food Purchasing Item 1; and Food Waste Attitude Item 3 were calculated between 0.40 and 0.70. Since the AVE coefficient for Food Quality Items 4, 5, and 6 was below the threshold, they were excluded from the measurement model. Similarly, Price Items 2 and Food Quantity Item 3 were removed from the measurement model because their AVE coefficients were below the threshold.

Given that the CR coefficients of the constructs ranged from 0.758 to 0.924, internal consistency reliability can be considered adequate. The constructs achieved convergent validity with factor loadings ranging from 0.662 to 1.000 and AVE values ranging from 0.512 to 0.859.

The HTMT criterion, proposed by Henseler et al., (2015), has been used to assess discriminant validity. The HTMT coefficients are also presented in Table 3.

Table 3

Discriminant validity results (HTMT criterion)

Indicator	Price	Food Durability	Food Peeling	Food Attributes	Amount of Food Waste	Food Waste Attitude	Food Quantity	Food Purchasing
Price								
Food Durability	0.150							
Food Peeling	0.123	0 .322						
Food Attributes	0.298	0 .475	0 .119					
Amount of Food Waste	0.087	0 .081	0 .106	0 .170				
Food Waste Attitude	0.443	0 .153	0 .117	0 .223	0 .150			
Food Quantity	0.197	0 .124	0 .080	0 .086	0 .083	0 .256		
Food Purchasing	0.653	0 .389	0 .310	0 .354	0 .077	0 .470	0 .144	
Food Purchase Frequency	0.052	0 .000	0 .083	0 .027	0 .062	0 .100	0 .188	0 .142

The indicator developed by Henseler et al., (2015), specifies the HTMT as the ratio of the mean of the correlations between items of all variables related to the study to the geometric mean of the correlations between items regarding the same variable. The authors also argue that the HTMT value should be (less than 0.90) for related constructs, and (less than 0.85) for distinct constructs. From the result in Table 4, the HTMT coefficients between HTMT are below the threshold value. Based on the HTMT criterion (Henseler et al., 2015), we can confidently say that discriminant validity is established.

Testing the research model

The structural equation model created to test the hypotheses of the study is shown in Figure 2.

The research model was analyzed using Partial Least Squares (PLS) Structural Equation Modeling (PLS-SEM). The analysis has been done by using SmartPLS 4 Statistical software (Ringle, Wende and Becker, 2022; Yildiz, 2021). The PLS Algorithm was used to check linearity, path coefficients, and R2 value in the analysis of the research model. T-tests of the PLS path coefficients were calculated and the results were bootstrapped using 10,000 subsamples from the data set. Results including VIF and R-squared, and direct effect coefficients are shown in Table (4).

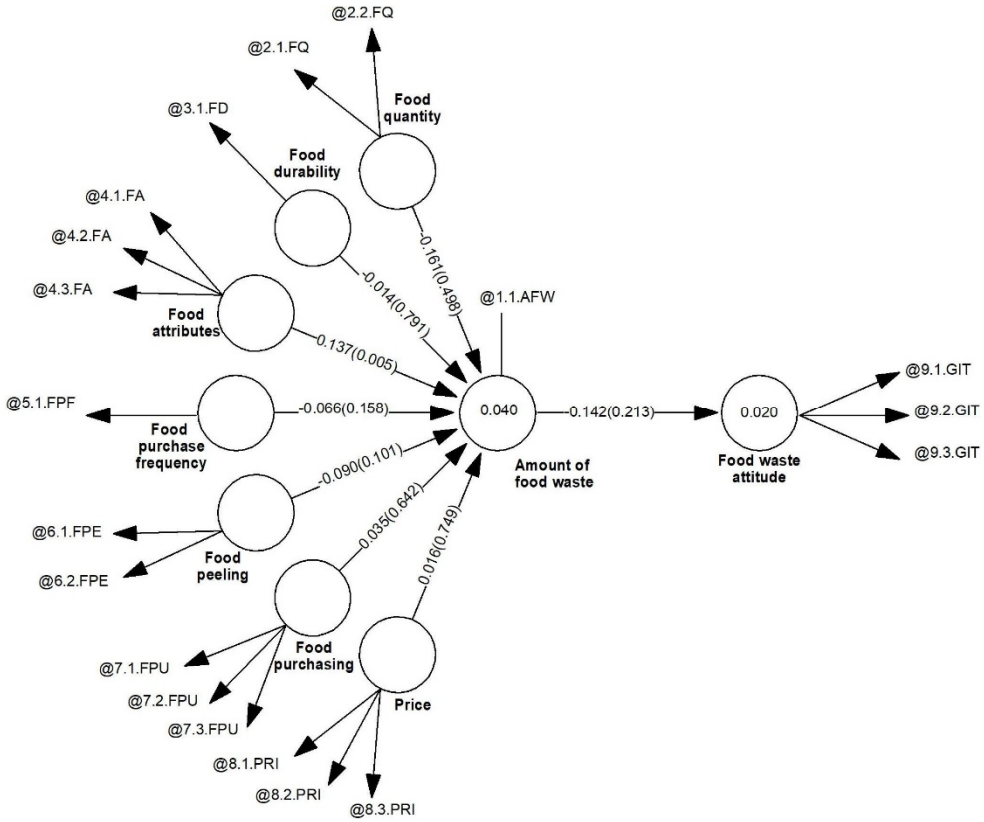

Figure 2. Measurement model with path coefficients

Table 4

Research model coefficients

Food Quantity	Amount of Food Waste	1.025	0.040
Food Durability		1.358	
Food Attributes		1.238	
Food Purchase Frequency		1.026	
Food Peeling		1.125	
Food Purchasing		1.236	
Price		1.126	
Amount of Food Waste	Food Waste Attitude	1.000	0.020

According to Hair et al. (2022), while VIF values lower than 5 imply there are no multicollinearity issues among the variables. In Table 3, It is seen that all the VIF values of the coefficients is less than the threshold of 5. In accordance with these results, it can be said no multicollinearity problems are present in the variables used in the study. After checking for the R^2 values of the model, I found that your model explains 4% of the food waste quantity, and 2% of the food waste attitude.

Table 5 shows a structural equation model (SEM) analysis in which the factors affecting food waste are statistically tested. The effect of each factor on food waste (β coefficient) is presented along with its standard deviation, t-value, p-value and hypothesis result.

Table 5

Research model direct effect coefficients

Path		β	Standard Deviation	t-value	p	Hypothesis Conclusion
Food quantity	Amount of Food Waste	-0.061	0.090	0.678	0.498	Rejection
Food durability		-0.014	0.054	0.265	0.791	Rejection
Food attributes		0.137	0.049	2.795	0.005	Acceptance
Food purchase frequency		-0.066	0.047	1.412	0.158	Rejection
Food peeling		-0.090	0.055	1.640	0.101	Rejection
Food purchasing		0.035	0.075	0.466	0.642	Rejection
Fiyat		0.016	0.051	0.320	0.749	Rejection
Amount of food waste	Food Waste Attitud	-0.142	0.114	1.245	0.213	Rejection

The table provides information about the effects of each variable on one another, the statistical significance of these effects, and whether the hypotheses are accepted or not. The analysis results show that food quantity, durability, purchasing frequency, peeling habit, price and food purchasing behaviors do not have a significant effect on food waste. According to the analysis results, the only variable that has a significant effect on food waste was determined as food attributes (β=0,137, p=0,005<0,05). The only significant relationship found is between food quality and food waste quantity. This suggests that consumers' emphasis on physical attributes of food, such as its appearance, freshness and packaging, increases food waste. Therefore, the findings suggest support for hypothesis H3, while hypotheses H1, H2, H4, H5, H6, H7, and H8 are not supported. Food waste quantity is not directly associated with food quantity, durability, purchasing frequency, preparation, or price.

The purpose of this case study research drawing from Azerbaijan was aimed at establishing consumer awareness of food waste and the ration of leading factors to food waste economically. The food waste issue is not significantly predictable from buying the right amount of food, Food Quantity (H1). Participants in the study felt that sale packages in large quantities (G2_2), the number of meals planned to be cooked (G2_4), using uneaten food scraps (G2_3), and the amount of meal ended up being a surplus compared to the number of

people (G2_1) do not affect the purchase of the right amount of food. Which indicates at this stage of the game, complete ignorance of food wastage. The other studies made similar observations, suggesting that consumers are not largely cognant of food waste (Giordano et al., 2023; Hamilton et al., 2005; Qi and Roe, 2016).

With regards to food durability, As shown in H2, there is no significant relationship between food durability and the amount of wasted food. Before purchasing food, consumers all want to know how long it will last or what does the expiration or use-by date is. These findings differ from those of Fan et al. (2022) and Thompson et al., (2020) concludes that problems with expiry date knowledge contribute to the consumer waste based on the interaction between food durability and food waste.

The perceived food quality (H3) was another food characteristic that influences the quantity of food waste (Food Quality; Foods to purchase are fresh (G4_2), well packaged (G4_6), tasty (G4_5) and visually attractive (G4_4). This result is consistent with Richter and Bokelmann (2017) and Veselá et al., (2023). Likewise, related studies have also recognized food taste effect to the waste (Coskun and Özbük 2020; Szymkowiak et al. 2022). However, Do Carmo Stangherlin et al. (2020) found that defective food items did not increase food waste rates, contradicting this study's findings, indicating variability depending on consumer attitudes. Some individuals adopting zero-waste practices may tend to prefer these food items.

Purchasing frequency (Purchasing Frequency, H4) does not affect the amount of food waste. This finding is consistent with Giordano et al. (2019), where the frequency or quantity of food purchases did not influence food waste. Similarly, Veselá et al. (2023) found that purchasing frequency did not affect food quantity. Lack of planning for the amount of food to be used may be one reason for the increase in food waste.

The act to peel fruit or hartista vegetables had no registered influence on the food waste activity, and as a consequence, it is not an important factor in order to gap more food waste (Peeling of Food, H5) The results of this study are opposite to what Derqui and Fernandez (2017) and Richter and Bokelmann (2017) state on fruit and vegetable activity.

The study found that the price variable was not statistically significant. Accordingly, the hypothesis that price affects the amount of food waste (Price, H7) was not accepted. Participants do not consider food price to be a significant factor in food waste, supported by Giordano et al. (2019). Conversely, Van Geffen et al. (2020) found that price is a determinant of food waste.

Lastly, food waste quantity (Food Waste Quantity, H8) is not influenced by consumers' attitudes towards food waste. It is crucial to recognize this, as participants may not perceive food waste, thereby neglecting to evaluate food waste at home. Participants are unaware of their food management at home and do not realize they are contributing to food waste. Studies detecting food waste contradict this research (Parizeau et al., 2015; Stancu et al., 2016; Veselá et al., 2023), indicating that awareness of this issue increases with awareness of its urgency, shaping attitudes towards and awareness of food waste. Consequently, deficiencies in food management awareness may increase based on attitudes and awareness, depending on individual recognition of food waste.

Conclusion

1. Azerbaijan ranks high in the West Asia region in terms of annual food waste per capita, but this issue does not receive sufficient attention and academic research remains limited.

2. This study aims to fill this gap and increase awareness of food waste. At the same time, it contributes to national and international comparisons of food waste causes and consumer behavior in Azerbaijan.
3. The hypotheses tested in the study were analyzed using the PLS-SEM (Structural Equation Modeling) method and only one hypothesis was found to be statistically supported.
4. The physical properties of food variable was found to have a significant and positive effect on food waste ($\beta = 0.372$, $p = 0.005$).
5. Food price ($\beta = -0.04$, $p = 0.312$), purchasing method ($\beta = 0.032$, $p = 0.541$), purchasing frequency ($\beta = -0.021$, $p = 0.429$), peeling habits ($\beta = -0.054$, $p = 0.278$), durability ($\beta = 0.015$, $p = 0.692$) and quantity ($\beta = -0.038$, $p = 0.358$) variables were not found to have a statistically significant effect on food waste.
6. These results show that Azerbaijani consumers have low awareness of food waste and that waste is caused by the physical properties of food rather than economic or behavioral reasons.
7. The findings support some literature studies (Ilakovac et al., 2020; Richter and Bokelmann, 2017; Setti et al., 2018; Veselá et al., 2023) while contradicting some studies (Russell et al., 2017; Wilson et al., 2017). In particular, the conclusion that the price factor is not a determinant is not consistent with some previous studies.
8. The research can serve as an example for initiatives targeting behavioural change by addressing attitudes towards food waste. It also includes factors required for food management at the household level.
9. These findings are of great importance to practitioners and future researchers. It would be beneficial, especially for practitioners, to organize training and awareness activities that will encourage positive habits.
10. A limitation of the study is that the sample size is moderate (455 participants). Therefore, it is recommended that comparative analyses be conducted with more participants in future studies.
11. Reducing food waste continues to be an important issue on a global scale today and will continue to be so in the future.

References

Ananda J., Karunasena G.G., Mitsis A., Kansal M., Pearson D. (2021), Analysing behavioural and socio-demographic factors and practices influencing Australian household food waste, *Journal of Cleaner Production*, 306, 127280, https://doi.org/10.1016/j.jclepro.2021.127280

Aschemann J.W., De Hooge I., Amani P., Bech-Larsen T., Oostindjer M. (2015), Consumer-related food waste: Causes and potential for action, *Sustainability*, *7*(6), pp. 6457–6477.

Cohen L., Manion L., Morrison K. (2018), *Research methods in education*, Routledge.

Coşkun A., Özbük R. M. Y. (2020), What influences consumer food waste behavior in restaurants? An application of the extended theory of planned behavior, *Waste Management*, 117, pp. 170–178.

Derqui B., Fernandez V. (2017), The opportunity of tracking food waste in school canteens: Guidelines for self-assessment, *Waste Management*, 69, pp. 431–444, https://doi.org/10.1016/j.wasman.2017.07.030

Do Carmo S., I., De Barcellos M.D., Basso K. (2020), The impact of social norms on suboptimal food consumption: a solution for food waste, *Journal of International Food and Agribusiness Marketing*, *32*(1), pp. 30–53. https://doi.org/10.1080/08974438.2018.1533511

Fan L., Ellison B., Wilson N. L. (2022), What Food waste solutions do people support? *Journal of Cleaner Production*, 330, 129907, https://doi.org/10.1016/j.jclepro.2021.129907

FAO (2023), *The state of food security and nutrition in the world 2023. Urbanization, agrifood systems transformation and healthy diets across the rural–urban continuum,* https://doi.org/10.4060/cc3017en

FAO (2024), *Food Loss and Waste Database*. FoodLossWaste, Available at: https://www.fao.org/platform-food-loss-waste/flw-data

Freud S. (1923), *A Psychoanalytic Study. Stavrogin's Confession. Translated by Virginia Woolf and SS Koteliansky*, New York.

Giordano C., Alboni F., Cicatiello C., Falasconi, L. (2019), Do discounted food products end up in the bin? An investigation into the link between deal-prone shopping behaviour and quantities of household food waste, *International Journal of Consumer Studies*, *43*(2), pp. 199-209, https://doi.org/10.1111/ijcs.12499

Giordano C., Di Fiore G., Alboni F., Carloni, E., Rivaroli, S., Falasconi, L. (2023), Household Food Waste Awareness in Relation to Motivations, *Sustainability*, 15(15), 11582, https://doi.org/10.3390/su151511582

Graham E. R., Jessop D. C., Sparks P. (2014), Identifying Motivations and barriers to minimising household food waste, *Resources, Conservation and Recycling*, 84, pp. 15-23.

Grainger M.J., Aramyan, L., Logatcheva, K., Piras, S., Righi, S., Setti, M., Vittuari, M., Stewart, G.B. (2018), The use of systems models to identify food waste drivers, *Global food security*, 16, pp. 1–8.

Griffin M., Sobal J., Lyson, T. A. (2009), An analysis of a community food waste stream. *Agriculture and Human Values*, 26, pp. 67–81.

Hair J.F., Black W.C., Babin B.J., Anderson R.E., Tatham R.L. (2019), *Multivariate Data Analysis.* (8th Ed), Pearson Prentice Hall.

Hair J.F., Hult G.T.M., Ringle C.M., Sarstedt M. (2022), *A primer on partial least squares structural equation modeling (PLS-SEM)* (3rd ed), Sage.

Hamilton C., Denniss, R., Baker D. G. (2005), *Wasteful Consumption in Australia*. Australia Institute Canberra, Australia, Available at: https://australiainstitute.org.au/wp-content/uploads/2020/12/DP77_8.pdf

Hebrok M., Boks C. (2017), Household food waste: drivers and potential intervention points for design–an extensive review, *Journal of Cleaner Production*, 151, pp. 380–392.

Henseler J., Ringle C. M., Sarstedt M. (2015), A new criterion for assessing discriminant validity in variance-based structural equation modeling. *Journal of the Academy of Marketing Science*, 43(1), pp. 115–135. https://doi.org/10.1007/s11747-014-0403-8

Ilakovac B., Voca N., Pezo, L., Cerjak M. (2020), Quantification and determination of household food waste and its relation to sociodemographic characteristics in Croatia. *Waste Management*, 102, pp. 231–240, https://doi.org/10.1016/j.wasman.2019.10.042

Kantor L. S., Lipton K., Manchester A., Oliveira V. (1997), Estimating and Addressing America's Food Losses, *Food Review/National Food Review*, 20(1), pp. 2–12.

Katsarova I. (2016), Katsarova: tackling food waste: the eu's contribution to a global issue, *EPRS European Parliamentary Research Service*, Available at: https://policycommons.net/artifacts/1339663/tackling-food-waste/1949563

Lipinski B., Hanson C., Waite R., Searchinger T., Lomax J. (2013), Reducing food loss and waste, *World Resources Institute Working Paper*, pp. 1–38.

Marshall A. (1890), *Principles of Economics, 8th edn.,* Mcmillan, London.

Mnerie D., Gaceu L., Gubenia O., Shamtsyan M., Birca A., Mnerie G. (2016), Comparative study on the evolution of the food labeling quality in some countries from the black sea region, *Journal of Hygienic Engineering and Design*, 14, pp. 60–65.

Parfitt J., Barthel M., Macnaughton S. (2010), Food waste within food supply chains: quantification and potential for change to 2050, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1554), pp. 3065–3081, https://doi.org/10.1098/rstb.2010.0126

Parizeau K., Von Massow M., Martin R. (2015), Household-level dynamics of food waste production and related beliefs, attitudes, and behaviours in Guelph, Ontario, *Waste Management*, 35, pp. 207–217.

Pavlov P. I. (2010), Conditioned reflexes: an investigation of the physiological activity of the cerebral cortex, *Annals of Neurosciences*, 17(3), 136.

Qi D., Roe B. E. (2016), Household food waste: Multivariate regression and principal components analyses of awareness and attitudes among US consumers, *PloS one*, 11(7), e0159250, https://doi.org/10.1371/journal.pone.0159250

Quested T., Johnson H. (2009), Household Food and Drink Waste in the Uk: A Report containing quantification of the amount and types of household food and drink waste in the Uk. Report Prepared by WRAP (Waste and Resources Action Programme), Banbury.

Richter B., Bokelmann W. (2017), Explorative study about the analysis of storing, purchasing and wasting food by using household diaries, *Resources, Conservation and Recycling*, 125, pp. 181–187. https://doi.org/10.1016/j.resconrec.2017.06.006

Ringle C.M., Wende S., Becker J.M. (2022), *SmartPLS 4*. Boenningstedt: SmartPLS, Available at: https://www.smartpls.com

Russell S. V., Young C. W., Unsworth K. L., Robinson, C. (2017), Bringing habits and emotions into food waste behaviour, *Resources, Conservation and Recycling*, 125, pp. 107–114, https://doi.org/10.1016/j.resconrec.2017.06.007

Setti M., Banchelli F., Falasconi L., Segrè, A., Vittuari M. (2018), Consumers' food cycle and household waste. When behaviors matter, *Journal of Cleaner Production*, 185, pp. 694–706, https://doi.org/10.1016/j.jclepro.2018.03.024

Solomon M. R., Bamossy G. J., Askegaard S. T., Hogg M. K. (2006), *Consumer behaviour: A European perspective*, Prentice Hall, New York.

Stancu V., Haugaard P., Lähteenmäki L. (2016), Determinants of consumer food waste behaviour: Two routes to food waste, *Appetite*, 96, pp. 7–17.

Stenmarck Å., Jensen C., Quested T., Moates G., Buksti M., Cseh B., Juul S., Parr, A., Politano A., Redlingshofer B. (2016), E*stimates of European food waste le*vels. IVL Swedish Environmental Research Institute. Public Wageningen Food & Biobased Research-Report, *2102*, 39.

Szymkowiak A., Borusiak B., Pierański B., Kotyza P., Smutka L. (2022), Household food waste: the meaning of product's attributes and food-related lifestyle, *Frontiers in Environmental Science*, 10, 918485, https://doi.org/10.3389/fenvs.2022.918485

Taş H., Boyacıoğlu E. Z. (2024), Shrinkflasyon algısının tüketici davranişlarina yansimasi, *Nişantaşı Üniversitesi Sosyal Bilimler Dergisi*, 12(Özel Sayı), pp. 377–398.

The World Bank (2024), *Population Estimates and Projections | Data Catalog*. https://datacatalog.worldbank.org/search/dataset/0037655

Thompson B., Toma, L., Barnes, A. P., Revoredo-Giha, C. (2020), Date-label use and the waste of dairy products by consumers, *Journal of Cleaner Production*, 247, 119174, https://doi.org/10.1016/J.JCLEPRO.2019.119174

UN Environment Programme (2024), *Food Waste Index Report 2024*. UNEP - UN Environment Programme, Available at: http://www.unep.org/resources/publication/food-waste-index-report-2024

Van Geffen L., Van Herpen, E., Van Trijp, H. (2020), Household food waste—how to avoid it? An integrative review. In: E.I. Närvänen, N. Mesiranta, M. Mattila A. Heikkinen (Eds.), *Food Waste Management*, pp. 27–55, Springer International Publishing, https://doi.org/10.1007/978-3-030-20561-4_2

Veselá L., Králikov, A., Kubíčková L. (2023), From the shopping basket to the landfill: Drivers of consumer food waste behaviour, *Waste Management*, 169, pp. 157–166. https://doi.org/10.1016/j.wasman.2023.07.002

Wilson N.L., Rickard B.J., Saputo R., Ho S.T. (2017), Food waste: The role of date labels, package size, and product category, *Food Quality and Preference*, *55*, pp. 35–44.

Yildiz E. (2021), *SmartPLS ile Yapısal Eşitlik Modellemesi Reflektif ve Formatif Yapılar* (2. Baskı), Seçkin Yayıncılık.

Zhang H., Duan H., Andric J.M., Song M., Yang B. (2018), Characterization of household food waste and strategies for its reduction: A shenzhen city case study, *Waste Management*, 78, pp. 426–433.

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2 автора	(Kuievda and Bront, 2020)
3 і більше авторів	(Bazopol et al., 2022)

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