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Biological hurdles as modern strategy for extending shelf life of cooked sausages

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Abstract

Introduction. This review analyses current studies on biological preservation strategies for meat products, with particular emphasis on cooked sausages, and examines the key factors influencing the safety and shelf life of sausage products.

Material and methods. A comprehensive literature search was conducted using international scientific databases, including Scopus, Web of Science, and PubMed, as well as open-access sources such as Google Scholar and ResearchGate.

Results and discussion. Extending the shelf life of cooked sausages through the implementation of biological protective hurdles reduces the proportion of spoiled products, thereby lowering economic losses for producers and supporting the principles of sustainable development. The incorporation of lactic acid bacteria (LAB) into cooked sausage production represents an effective preservation strategy, as these microorganisms exhibit antagonistic activity against pathogenic, opportunistic, and spoilage microflora, improving microbiological safety and prolonging shelf life. Through their natural mechanisms of action, LAB establish a protective barrier by competing for nutrients, producing organic acids, antimicrobial peptides, and other bioactive metabolites, and by regulating microbial interactions via quorum sensing.

Food culture manufacturers face the challenge of selecting LAB strains characterized by low acidification capacity, strong antagonistic activity against undesirable microflora, and the ability to preserve acceptable sensory properties of the final product. For cooked sausage producers, difficulties extend beyond strain selection to include the development of effective methods for LAB application, particularly during post-heat-treatment stages such as vacuum packaging or modified-atmosphere packaging. The use of LAB therefore requires thorough investigation and experimental validation to confirm their efficacy in inhibiting pathogenic and spoilage microorganisms under real industrial conditions and within specific product formulations.

The implementation of effective biological hurdles is consistent with current trends in the food industry and growing consumer demand for healthier products, as it enables the partial or complete replacement and significant reduction of additives, particularly sodium nitrite and certain preservatives.

Conclusions. Biological protection using lactic acid bacteria is an effective strategy to enhance the safety and shelf life of cooked sausages. Future research should focus on the development of biological protection as an additional "barrier" for the preservation of meat products.

Introduction

Sausage products are among the most widely consumed categories of processed meat, constituting a significant part of the human diet. Cooked sausages represent the predominant type globally, owing to their relatively high nutritional value, which is associated with the use of high-quality raw materials and advanced processing technologies (Serikkyzy et al., 2022; Stabnikova et al., 2022).

The growing demand for "clean label" products and the refusal to use chemical preservatives pose significant challenges for the food industry, particularly in the production of meat products (Kim et at., 2025). Traditional preservation methods, such as heat sterilization and use of additives, although effective, often deteriorate sensory properties, reduce nutritional value, and can have negative environmental and economic impacts (Nawaz et al., 2025). This trend stimulates the search for safe and environmentally friendly solutions capable of ensuring microbiological stability without the use of synthetic additives (European Commission, 2020).

One promising approach is hurdle technology, which is based on the combined action of several factors that inhibit the growth of pathogenic and spoilage microflora (Leistner and Gould, 2002). Biological hurdles are especially noteworthy, since they are based on natural defence mechanisms that utilise antagonistic microorganisms, bacteriocins, enzymes, and biopolymers with antimicrobial properties (Bertrand et al., 2024; Rahman, 2015). Their application not only enhances product safety but also helps to preserve product quality, aligns with modern simplified label requirements, and contributes to reducing environmental impact.

Recent studies in collaboration with food manufacturers have explored replacing synthetic ingredients with biologically safe alternatives (Paredes-López et al., 2022). In this context, the antimicrobial properties of natural compounds capable of inhibiting pathogenic and opportunistic microorganisms have been investigated (Bertrand et al., 2024). Research has also focused on the use of selected lactic acid bacteria (LAB) strains and their consortia in various food products to ensure microbiological safety, preserve quality, extend shelf life, and optimize raw material utilization (Kumar et al., 2025).

Considering the substantial global losses of meat and meat products attributable to microbiological spoilage (reaching up to 21% in Europe and North America) (Höll et al., 2016), the implementation of biological solutions could represent a crucial measure for reducing food waste and enhancing the economic efficiency of production. Consequently, the use of biological hurdles is justified and highly relevant in the production of cooked sausages.

The aim of this review is to describe the fundamental principles of biological hurdles, summarise current scientific findings on the use of biological protection in meat product manufacture, and evaluate the potential LAB in the production of ready-to-eat products, particularly cooked sausages.

Material and methods

This review is based on an analysis of scientific articles and publications concerning the effects of microorganisms and their metabolic products on the suppression of undesirable microflora adversely affecting food quality and safety, identified through searches in the Web of Science, Scopus, Google Scholar, and PubMed databases.

Results and discussion

Theory of hurdle technologies

The theory of hurdle technologies is based on the use of several technological factors to preserve product quality, which inhibit the growth of undesirable microorganisms (Food Safety Authority of Ireland, 2022).

The concept of "hurdles" determining the safety and quality of food products was first introduced by Leistner (2017). In his work, he repeatedly emphasized that the broad practical implementation of hurdle theory is possible only when research is carried out to define the quantitative parameters of these hurdles within the technological processes used for specific product types.

Initially, hurdle technologies were based on the application of a limited number of hurdles that can inhibit the development of undesirable microorganisms in meat products.

These hurdles primarily include:

- high temperature (sterilisation and pasteurisation),
- low temperature (processing and storage under chilled and frozen conditions)
- decreasing pH value;
- decreasing water activity a_w;
- decreasing redox potential (Eh);
- presence of preservatives;
- presence of competing microflora.

According to Leistner (2017), when the intensity of a particular hurdle is insufficient, it should be strengthened, whereas if an excessively strong hurdle exerts a detrimental effect on the overall quality of the product, its intensity should be reduced. Following such adjustments, all hurdles should be combined in an optimal value that guarantees effective inhibition of microorganisms while preserving the remaining quality parameters of the product (Abdullahi et al., 2021).

Modern hurdle technologies are designed to ensure high product quality and enhanced food safety, while also supporting environmental sustainability and providing economic benefits by reducing the extent of physical and chemical interventions in industrial processing (Rahman, 2015). In recent years, the range of known and potential physical, chemical, and biological hurdles used to inhibit the development of undesirable microorganisms has increased substantially. Among these approaches, notable examples include irradiation (Jia et al., 2025), ultrasound (Guo et al., 2024), ozone treatment (Ayranci et al., 2020), high-pressure processing (Nguyen et al., 2024), and cold plasma (Zhang et al., 2025). Hurdle technologies in meat production are applied sequentially, beginning at the slaughter stage and continuing through processing, packaging, and storage. The implementation of multiple hurdles at different stages of production is a critical factor in ensuring microbiological safety and preserving the quality of meat and meat products (Gragg et al., 2024). The combined effect of multiple hurdles on the inhibition of undesirable microflora in meat products is shown in Figure 1 (Abdullahi, 2021).

The combination of different barriers, such as reduction of water activity, low- and high-temperature treatments, pH adjustment, and others, provides a synergistic effect that forms the basis of the so-called hurdle technology for food preservation (Rahman et al., 2016). A well-structured combination of several preservation methods creates conditions that inhibit the growth of spoilage microorganisms, thereby enhancing the overall effectiveness of food preservation (Edo et al., 2025). Hurdle technology is an example of a synergistic effect achieved by integrating different preservation methods, which provides an increased level of safety and extends the shelf life of food products (Putnik et al., 2020).

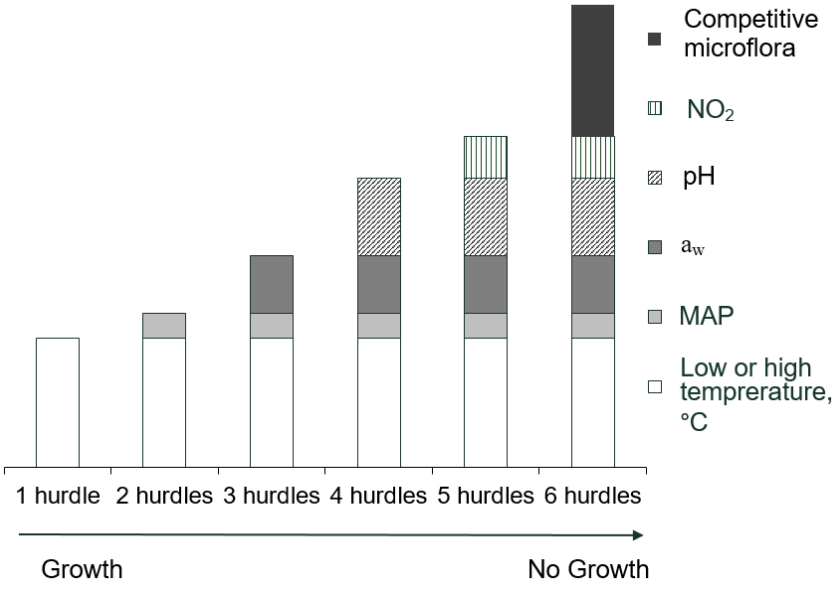

Figure 1. The combined effect of multiple hurdles on the inhibition of undesirable microflora in meat products (adapted from Abdullahi et al., 2021)

Although the total number of potential hurdles may be considerably higher, only a limited subset receives the most attention. Ensuring food safety and microbiological stability in both traditional and novel products requires a synergistic combination of intrinsic and extrinsic factors that function as hurdles (Mafe et al., 2024). An example of how hurdles can be applied to slow down or inhibit the growth of the pathogenic microorganism *Listeria monocytogenes* in packaged cooked ham (Figure 2).

Therefore, to ensure freshness and extend the shelf life of food products, it is essential to create specific conditions that slow down or completely inhibit the growth of pathogenic microorganisms (Khama, 2024). Regulatory criteria aimed at inhibiting the growth of *Listeria monocytogenes* have been incorporated into the legislative frameworks of EU Member States (Begins et al., 2018), the United States, and several other countries (Zhang et al., 2021). Similar requirements are also reflected in Ukrainian food legislation (Ministry of Health, Order No. 548 of 19 July 2012):

- pH ≤4.4;
- water activity (a_w) ≤0.92;
- pH ≤5.0 and a_w ≤0.94;
- freezing temperature.

The Food Safety Authority of Ireland (2022) notes that not all hurdles are equally effective in influencing the ability of pathogens to survive and multiply, or in determining the shelf life of food. However, because each hurdle contributes to risk reduction, they may be regarded as critical control points and should be monitored accordingly.

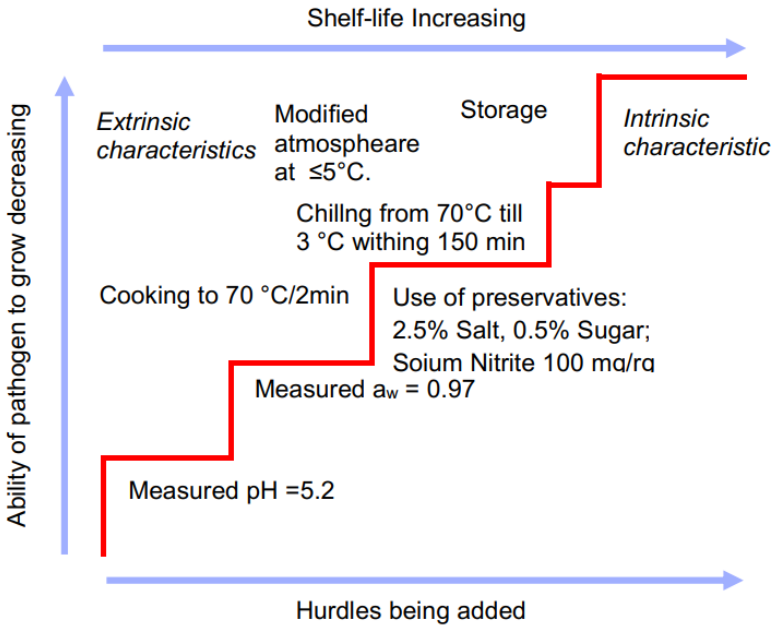

Figure 2. Hurdles applied to inhibit pathogenic microorganisms in cooked sausages (adapted from Food Safety Authority of Ireland, 2022)

Pathogenic microorganisms present in food products are characterized by minimum, maximum, and optimal values that determine their survival and growth (US Food and Drug Administration, 2021). Analysing the intrinsic properties of food products, as well as the extrinsic factors associated with their production and storage, allows for the identification of microorganisms capable of surviving and multiplying in these products, particularly those that pose a risk to human health (Shetty and Singh, 2025).

Factors affecting the microbiological safety and shelf life of foods

All food products possess characteristics that can be classified as intrinsic or extrinsic factors (Table 1). Intrinsic factors are properties inherent to the composition of the food product. Extrinsic factors are properties related to the external production and storage environment that influence the product, such as storage temperature and packaging (Food Safety Authority of Ireland, 2022).

pH value is an important parameter for assessing the suitability of raw materials for further processing. However, the shelf life of meat products cannot be extended solely by lowering pH, as many pathogenic microorganisms exhibit high acid resistance. The optimal pH range for yeast growth is 4.00–6.00 (Riesute et al., 2021), but some yeasts, such as *Saccharomyces cerevisiae*, can grow within a pH range of 2.5–8.5, representatives of the genus *Candida* at pH values below 2.0 (Rane et al., 2019), and mould such as *Aspergillus niger* at pH values from 1.5 to 9.8 (Li et al., 2020).

Lowering the pH of a product can significantly limit the growth of spoilage bacteria. Nevertheless, for meat and meat products, pH can be modified only within approximately from 4.5 to7.5. Products with lower pH values are rarely produced, mainly due to technological constraints and predominant sensory and taste preferences among consumers (Toupchi et al., 2025).

Water activity (a_w) is a key characteristic of a product that indicates the free water that can be utilized by microorganisms for their metabolic activities. A reduction in water activity inhibits microbial proliferation, disrupts intracellular metabolic processes, and adversely affects microbial survival. In meat products, lower water activity values are associated with extended shelf life (Figura et al., 2023). Intrinsic and extrinsic characteristics affecting the microbiological quality and shelf life of food products are shown in Table 1.

Table 1

Intrinsic and extrinsic characteristics affecting the microbiological quality and shelf life of food products*

Extrinsic	Intrinsic
– Temperature (during production, storage, distribution, and display) – Packaging – Gas atmosphere – Relative humidity – Food processing – Historical factors (my also relate to intrinsic characteristics depending on the nature of the data) – Good manufacturing and hygiene practices – Storage and distribution – Consumer practices	– pH and type of acid present – Water activity (a_w) – Redox potential (Eh) – Natural barriers – Nutritional content of food and availability – Antimicrobial substances – Microflora – Microbiological quality of ingredients – Food formulation and composition – Food assembly and structure

*Adapted from Food Safety Authority of Ireland, 2022

Table 2 provides a summary of the effects of water activity on microorganisms.

Table 2

Dependence of microbial growth on water activity (a_w)*

Value of a_w	Impact on microbial growth
< 0.90	Most bacteria cease to multiply
< 0.88	Most yeasts cease to multiply
< 0.80	Most mold cease to multiply
0.60–0.75	Halophilic bacteria, xerophilic mold, osmophilic yeasts can be active

* Adapted from Figura et al., 2023

Redox potential (E_h) which depends primarily on the amount of oxygen incorporated during the preparation of minced meat and on conditions that promote its reduction, has an inhibitory effect on aerobic microorganisms that tolerate the presence of sodium nitrite and favours the selection of lactic acid bacteria. Microorganisms differ in their requirements and

tolerance for oxygen and redox potential, as follows (Food Safety Authority of Ireland, 2022):

- aerobes require oxygen for growth and can grow at an oxidation–reduction potential of approximately +300 to +500 mV
- facultative anaerobes can grow in the presence or absence of oxygen and are able to grow at an oxidation–reduction potential of approximately +300 to −100 mV
- anaerobes do not require oxygen for growth and can grow at an oxidation–reduction potential of approximately +100 to ≤−250 mV.

The redox potential values for selected products are presented in Table 3.

Table 3

Redox potential of selected food matrices*

Product	Eh, mV	pH
Raw meat (post-rigor)	–200	5.7
Chilled minced meat	+225	5.9
Cooked sausages and canned ham	–20 to –150	≈6.5
Wheat (whole grain)	–320 to –360	6.0
Barley (ground grain)	+225	7.0
Potatoes	≈ –150	≈ 6.0

*Adapter from Adams et al., 2024

Sodium chloride and curing salt. Salt plays a key role in flavour development, modifies the microstructure of minced meat, and affects its texture (Shevchenko et al., 2021). When dissolved in the water present in meat raw materials or mince, salt lowers water activity (a_w), thereby inhibiting or slowing microbial growth and promoting the selective development of particular microbial groups (Food Safety Authority of Ireland, 2022). Furthermore, regulatory authorities recommend the addition of nitrate and nitrite curing salts to impart the characteristic reddish colour to cured meat products and to inhibit the growth of vegetative cells of *Clostridium botulinum* (Lemos et al., 2024).

At the same time, certain microorganisms (e.g., *Staphylococcus* spp.) (Table 4) are able to tolerate high concentrations of sodium chloride in aqueous systems. Therefore, only the combined action of multiple hurdles can ensure prolonged preservation of finished products (Gragg et al., 2024). According to Commission Regulation (EU) No. 1129/2011 (European Commission, 2011), the use of nitrates and nitrites as food additives is permitted in the European Union, specifically sodium nitrate (E251), potassium nitrate (E252), sodium nitrite (E250), and potassium nitrite (E249). This regulation, which amends Annex II to Regulation (EC) No. 1333/2008, establishes maximum levels of nitrates and nitrites in meat products that vary according to product type. Non-heat-treated meat products may contain up to 150 ppm of nitrate or sodium or potassium nitrite, whereas heat-treated meat products (with the exception of sterilized products) may contain up to 150 ppm of sodium or potassium nitrite. Certain traditional smoked meat products are exempt from these general limits and may be permitted higher maximum levels (Melios et al., 2024). Growth characteristics of common foodborne bacterial pathogens are shown in Table 4.

Table 4

Growth characteristics of common foodborne bacterial pathogens

Pathogens	Temperature, °C	pH	Water activity (a_w)	Salt, %
	Min (Optimum) Max *Allowing Growth*		Min *Allowing Growth*	Max *Allowing Growth*
Salmonella spp.[1]	5 (35–43) 47	3.8 (7–7.5) 9.5	0.94	4
Clostridium botulinum[2] (proteolytic)	10 (35–40) 42	<4.6 (7) 8	>0.94	10
Clostridium botulinum[2] (non-proteolytic)	3 (28–30) 35	<5.0 (7) 8	>0.97	5
Staphylococcus aureus[3]	10 (40–45) 48	4 (7–8) 9.6	0.83	10
Campylobacter spp.[4]	32 (42–43) 45	4.9 (6.5–7.5) 9	>0.98	1.5
Yersinia enterocolitica[5]	–1.3 (25–37) 42	4.2 (7.2) 9.6	0.94	7
Listeria monocytogenes[6]	–1.5 (30–37) 45	4.2 (7) 9.5	0.92	12
Clostridium perfringens[7]	10 (43–47) 50	5.5 (7.2) 9	0.93	6
Shiga toxin (*STEC*) or Verocytotoxin (*VTEC*) producing *Escherichia coli*[8]	6.5 (30–40) 45	3.6 (6–7) 9	0.95	>6.5

Source: Food Safety Authority of Ireland (2022). [1]Egg, meat; [2]Foods which are canned, vacuum packed, modified atmosphere packed, jarred (i.e. low oxygen environments); [3]Eggs, poultry, meats, salads, sandwiches; [4]Poultry meat, unpasteurised/raw drinking milk and dairy products (e.g. cheese, butter); [5]Fresh meats (pork in particular) and unpasteurised/raw drinking milk and dairy products (e.g. cheese, butter); [6]Chilled, ready-to-eat foods (e.g. smoked salmon, sliced cooked meats, coleslaw); [7] Cooked meats; [8]Meat, poultry, unpasteurised/raw dairy products and apple juice, sprouted seeds, salad vegetables, untreated drinking water (e.g. from a well) etc.

Commission Regulation (EU) 2023/2108 reduced the maximum permitted levels of sodium and potassium nitrite to 55-80 ppm (expressed as NO_2 ion) and sodium and potassium nitrate to 90 ppm (expressed as NO_3 ion), with certain exceptions maintained for traditionally cured products (European Commission, 2023). In this most recent legislation, the input quantities are expressed in terms of ions rather than salts; consequently, the actual reduction may not be as substantial as it initially appears.

A major drawback associated with the use of nitrites and nitrates in meat products is the formation of nitrosamines during processing, compounds that may adversely affect human health (Habermeyer et al., 2019). In response to these concerns, the European Parliament's Committee on the Environment, Public Health and Food Safety has recently submitted a motion for a resolution proposing a complete ban on the addition of nitrates and nitrites to smoked meat products (Melios et al., 2024).

Table 4 presents key characteristics of the growth conditions of common foodborne bacterial pathogens that pose a significant risk to consumer health. Pathogenic microorganisms exhibit different requirements for the gaseous composition of their environment, which determines their growth potential (Table 5).

Table 5

Oxygen-related growth requirements of pathogenic microorganisms

Pathogens	Relationship to oxygen
Salmonella spp.	Facultative
Clostridium Botulinum (proteolytic)	Anaerobic
Clostridium botulinum (non-proteolytic)	Anaerobic
Staphylococcus aureus	Facultative
Campylobacter spp.	Microaerophilic
Yersinia enterocolitica	Facultative
Listeria monocytogenes	Facultative
Clostridium perfringens	Anaerobic
Shiga toxin (*STEC*) or Verocytotoxin (*VTEC*) producing *Escherichia coli*	Facultative

Source: Food Safety Authority of Ireland (2022)

The values presented in Table 4 and Table 5 are approximate and apply under optimal conditions; therefore, they should be used for guidance only. Pathogenic microorganisms may be capable of growth outside these ranges (Food Safety Authority of Ireland, 2022).

Cooking process is a sequence of complex physicochemical transformations in the meat matrix aimed at fixing the product's shape and structure, inactivating vegetative microflora, developing desirable sensory properties, and enhancing resistance to mould growth and microbiological spoilage during storage (Andrade et al., 2025).

Non-optimized or uncontrolled heat treatment regimes may result in souring of minced meat and the reduction of nitrite to molecular nitrogen. Consequently, grey discolorations can appear on the cut surface of sausage batons, porosity may develop due to gas production by heterofermentative microflora, and microbiological spoilage of the finished products can occur during storage (Andrade et al., 2025).

Packaging process. Cooked sausages are typically packaged and marketed under vacuum. During storage, it is necessary to consider process-related factors such as synergistic microbial interactions and the proliferation of rod-shaped, slime-producing bacteria (e.g., *Bacillus* spp.), which are among the main causes of defects in vacuum-packed meat products. Deterioration in the appearance and colour of products can adversely affect consumer perception and may lead to rejection of the product before the end of its shelf life (de Lima et al., 2022).

Blowing of the packaging and the presence of a milky-coloured exudate in vacuum-packed cooked sausages, irrespective of the formulation, are associated in approximately 50% of cases with contamination by bacteria of the genus *Leuconostoc*. Substantial batch-to-batch variation in bacterial counts and initial contamination levels is likely related to cross-contamination under industrial production conditions. Experimental findings indicate that the reduction in the shelf life of cooked hams is specifically linked to cross-contamination occurring during slicing (Blanco–Lizarazo, 2022).

If no additional processing is applied at the packaging stage, the only hurdle capable of significantly reducing initial microbial contamination is the heat treatment of the product. External hurdles, typically applied in the form of preservatives, exert primarily a bacteriostatic rather than a bactericidal effect (Rogovski et al., 2021).

Sources of contamination in cooked sausages

Raw meat is an excellent medium for bacterial proliferation because of its rich nutrient composition, high a_w, and slightly acidic pH (Cauchie et al., 2020). In combination with the action of endogenous autolytic enzymes, these conditions accelerate the progression of meat spoilage (Shao et al., 2021). Meat spoilage is primarily caused by bacterial activity and is influenced by multiple factors, including product composition and the microbiological status of the carcass at slaughter. Additional parameters include the nature of surface treatments, subsequent processing steps, hygienic conditions during production, storage temperature, and the type of packaging use (Mafe et al., 2024). Moreover, the processing environment itself may act as a source of contamination with pathogenic and spoilage microorganisms, thereby compromising the quality and safety of meat and meat products, including cooked sausages (Barcenilla et al., 2024). Source of contamination of cooked sausages are shown in Table 6.

Table 6

Source of contamination of cooked sausages

Process	Source of contamination	Reference
Slaughter and primary processing	Equipment, knifes, surfaces	Salama et al., 2024
Production environment	Air, water, dust	Barcenilla et al., 2024
Cutting and mixing	Surface of equipment	Salama et al., 2024
Packaging (MAP/vacuum)	Packaging material, packaging equipment	Pellissery et al., 2020
Storage and logistics	Temperature, duration of storage	Park et al., 2024

The level of contamination in meat raw materials is influenced by their temperature prior to processing (fresh, chilled, or frozen) and by the sanitary conditions within the production environment. The use of thawed meat, combined with non-compliance with prescribed temperature requirements in processing and cutting areas, leads to an elevated microbiological load (Park et al., 2024; Shevchenko and Tunik, 2024).

Contamination originating from slaughter equipment, air, water, soil, and workers' hands can facilitate the growth of spoilage bacteria, thereby degrading the quality of meat components (Holman et al., 2018).

The natural microbiota of meat comprises a diverse range of spoilage microorganisms, including Gram-positive and Gram-negative bacteria, filamentous fungi, and yeasts belonging to the genera *Trichosporon* and *Candida* (Lemos, 2024).

Meat and meat products are recognized as common sources of major foodborne pathogens (Ali et al., 2022), including members of the *Enterobacteriaceae* family (Pellissery et al., 2020). Significant quality deterioration and alterations in the physicochemical and sensory properties of meat can result from putrefactive microorganisms present in raw materials, such as *Pseudomonas* spp. and *Brochothrix thermosphacta* (Pellissery et al., 2020). Proteolytic bacteria produce sulfur compounds, ammonia, and amines, which contribute to putrid and unpleasant odors (Wang et al., 2023). Additionally, bacteria such as *Pseudomonas* spp. synthesize pigments that cause green, grey, or brown discoloration (Wang et al., 2023).

In addition, meat spoilage can be caused by representatives of the genera *Acinetobacter, Bacillus, Shewanella, Aeromonas*, and *Clostridium*, as well as by lactic acid bacteria belonging to the genera *Weissella, Leuconostoc, Carnobacterium, Lactococcus*, and *Lactobacillus* (Poirier, 2018; Shao et al., 2021).

Microorganisms present in meat raw materials can contaminate the internal matrix of the sausage during production (Salama and Chennaoui, 2024). Thermal processing remains a key factor in ensuring the microbiological safety of cooked sausages. During cooking, when the core temperature of the product reaches 72 °C, up to 99% of vegetative bacterial cells are inactivated; however, spore-forming microorganisms, such as clostridia, may survive. Among them, *Clostridium botulinum*, the causative agent of botulism, is considered the most critical pathogen (Devine et al., 2023). Microorganisms contaminating the surface of sausages can also contribute to product spoilage (Tian et al., 2025). Cooked sausages produced from raw meat materials with a high initial microbial load, such as meat trimmings, heavily processed trimmings, and offal, exhibit the lowest storage stability. In addition, cooked sausages generally have a higher moisture content than semi-dry and dry products (e.g., cooked-smoked, semi-smoked, and fermented sausages), which consequently leads to a reduced shelf life (Halagarda and Wójciak, 2022фдцфны.

Sausage casings have an impact on overall quality of cooked sausages. Both natural casings (e.g., hog, sheep, and beef intestines) and artificial casings (e.g., cellulose, collagen, and plastic) are used in production, but artificial casings generally provide better sanitary conditions and protection. Because natural casings are obtained from different regions around the world, their microbiological quality can be highly variable (Liu et al., 2023). They contain protein, water, and often a fat layer, which together create favourable conditions for microbial growth. If natural casings are not properly cleaned, they may develop a musty odour and carry large numbers of spoilage microorganisms, especially bacteria of the genera *Proteus* and *Clostridium*, which can cause sausage spoilage during storage (de Araújo et al., 2022).

When the microbial load reaches 10^7–10^8 CFU/cm², it leads to significant sensory changes in the muscle tissue (Ndoye et al., 2025). Meat exhibiting a high level of microbiological contamination by mesophilic, aerobic, and facultative anaerobic microorganisms, despite retaining an acceptable visual appearance, can result in technological defects and a decline in the overall quality of the final product (Tian et al., 2025). Meat raw materials with an approximate total contamination level of 1×10^7 CFU/g exhibit all characteristic of spoilage, including greening, persistent off-flavor, surface slime, rancidity of fat (Cauchie et al., 2020; Shevchenko et al., 2020).

The total number of viable microorganisms in the finished product depends on the initial microbial load of the raw materials (Cauchie et al., 2020). Consequently, a low initial level of microbial contamination in meat raw materials represents the first critical hurdle and has a significant impact on the shelf life of the final product (Salama and Chennaoui, 2024).

A modern view of bioprotection for cooked sausages

A review of publications in the Scopus database (www.scopus.com) conducted in November 2024 using the keywords “bacteriocins” and “meat” identified 691 publications, including 94 review articles, with more than 60% published within the last decade. This trend reflects the strong and growing interest of both the scientific community and the food industry in developing strategies to partially or entirely replace chemical preservatives in food products. Bioprotection was initially defined as an approach to control specific pathogens in food systems. However, in recent years, it has acquired broader significance and is now recognized as a key strategy for inhibiting spoilage-associated microflora, extending shelf life, and improving the overall stability of food systems. Within this framework, bacteriocin-producing cultures and their metabolites are increasingly being explored as promising bioprotective agents for application in meat products (Souza et al., 2022).

During extended storage of food products, spoilage is a complex process often driven by consortia of microorganisms from various species. Consequently, starter cultures must exhibit a broad spectrum of inhibitory activity (Alessandria et al., 2023). The use of bacteriocinogenic strains (mainly lactic acid bacteria) is described in the works (Azevedo et al., 2024; Urso et al., 2006). To identify suitable strains capable of producing bacteriocins, it is necessary to adapt and reproduce the conditions under which the suppression of consortia of microorganisms that spoil food products will occur. Some studies show that it is more promising to search for suitable strains in fresh products while the microbiological characteristics are still satisfactory and autochthonous populations can be used to protect them (Cocolin, 2025). Since microorganisms responsible for food spoilage are not necessarily pathogenic, recent research has focused on developing novel preservation strategies, while the underlying principle remains the same: employing natural or microbially derived agents to inhibit undesirable microflora and maintain product quality. By inhibiting microorganisms or bacterial groups responsible for spoilage, it is possible to extend the shelf life of food products and improve the stability of food systems. Methods for identifying specific spoilage organisms (SSO) were described by Gram et al. (1987).

The bioprotection strategy is comparable to that employed for controlling foodborne pathogens but is applied to any microorganism responsible for spoilage (Cocolin, 2025). It involves conducting microbiological analyses of ready-to-eat products, such as cooked sausages, both at the beginning and at the end of their shelf life to monitor microbial dynamics and ensure product safety and quality.

At the beginning of shelf life, the food ecosystem is characterized by a balanced microflora that reflects both the intrinsic properties of the product and the conditions under which it was produced. However, during storage, shifts in the microbial population may occur, adversely affecting quality attributes such as color, taste, and aroma and ultimately leading to product spoilage (Cocolin, 2025). Microbial colonies isolated at the beginning of shelf life and representing different microbial groups, cultivated on selective and non-selective media (de Man, Rogosa and Sharpe (MRS) agar for lactic acid bacteria and Brain Heart Infusion (BHI) agar for total microflora), can be evaluated for their inhibitory activity against spoilage consortia that develop at the end of shelf life. This approach enables the identification of spoilage-associated microorganisms and their isolation in pure culture, allowing their subsequent use as indicator strains for screening bacteriocin-producing bacteria by means of agar well diffusion assays (Cocolin, 2025).

A comparable strategy has been successfully employed to evaluate the effectiveness of starter cultures in sliced cooked ham. In that study, nine lactic acid bacteria strains were selected from 140 isolates based on their capacity to inhibit a broad spectrum of spoilage microorganisms through bacteriocin production at 4 °C (Alessandria et al., 2023).

Biological protection mode of action

Bacteriocins are antimicrobial peptides synthesized by bacteria that exhibit activity against other microorganisms. Their production confers a competitive advantage on the producer strains in shared ecosystems by inhibiting the growth of undesirable or pathogenic microflora. Notably, bacteriocins are generally considered as non-toxic, can display either narrow- or broad-spectrum antimicrobial activity, and are effective at low concentrations. These properties make them promising candidates for use as natural biopreservatives in meat products and other perishable foods (Arbulu et al., 2022) and stimulated considerable scientific interest in bacteriocins as potential natural alternatives to conventional preservatives for use in food products.

Particular attention is given to bacteriocins produced by lactic acid bacteria, as these microorganisms are already widely employed in the food industry (Stabnikov et al., 2025). Such cultures can enhance the safety and quality of products through active biological interactions with both the food matrix and the product microbiota (Nascimento et al., 2025). The mode of action of biological hurdles is shown in Figure 3.

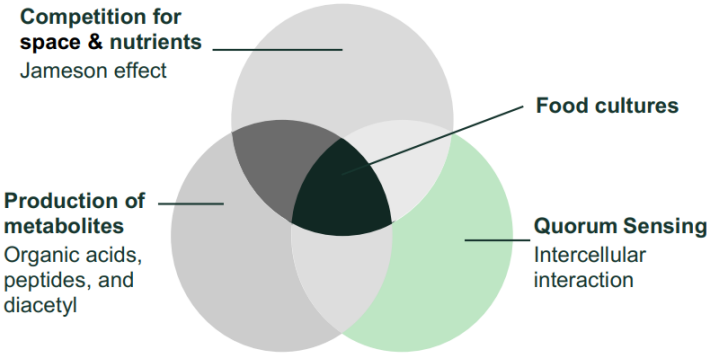

Figure 3. Mode of action of biological hurdles
Adapted from Mgomi et al., 2023

Currently, the concept of the microbiome refers to the microbial community of a defined environment, occupying a specific niche with characteristic physicochemical conditions, and encompasses not only the microorganisms present but also their interactions, forming an integrated ecosystem (Berg et al., 2020).

Scientific research on the role and application of the microbiome in the food industry has intensified in recent years. Beyond the field of human health, these studies have increasingly focused on biotechnological processes based on microbial fermentation of food systems, aiming to improve technological solutions (Zhao et al., 2025).

In the context of microbiome functioning, particularly the mechanisms of bacterial interaction, increasing attention is being paid to the concept of microbial competition as a potential tool for improving food safety and quality. It has been demonstrated that microorganisms can establish complex relationships based on positive and negative interactions, thereby shaping population equilibrium and suppressing other microbial groups (Raimondi et al., 2019).

It has been established that LAB can employ various competitive strategies, ultimately enabling them to dominate specific ecosystems. The most common mechanisms include the production of organic acids, hydrogen peroxide, diacetyl, antimicrobial compounds (often referred to as “killer factors”), as well as competition for space and nutrient resources (Raimondi et al., 2019).

Natural antimicrobial metabolites also include bacteriocins – peptides whose production provides the producing strains with a competitive advantage over other microorganisms occupying the same ecological niche (Smaoui et al., 2023). Lactic acid bacteria have considerable potential for application in bioprotection technologies, and are naturally capable of dominating the microbiota of many products during storage (Zapaśnik et al., 2022).

In raw meat products stored at low temperatures under vacuum or in an atmosphere with elevated CO_2 levels, lactic acid bacteria become the dominant microbial group and contribute

to preservation of the raw material through their "hidden" metabolism. A similar phenomenon is observed in ready-to-eat meat products when these bacteria partially survive heat treatment or are reintroduced after processing (Cocolin, 2025).

In recent years, this strategy has been increasingly adopted in industrial production as an additional hurdle for biological protection during food storage. Notably, Novonesis A/S (Denmark) has reported substantial progress in utilizing starter cultures as a source of natural microbial biopreservation. These commercial starter cultures are now widely incorporated into meat and other perishable products to enhance safety and extend shelf life without exclusive reliance on preservatives.

Antimicrobial activity and classification of bacteriocins

According to the classification proposed by Smaoui et al. (2023), bacteriocins are categorized into three classes: Class I (lantibiotics), they are low-molecular-weight peptides (<5 kDa), thermostable, resistant to proteolytic degradation, and post-translationally modified; Class II (non-lantibiotics), they are low-molecular-weight peptides (<10 kDa), thermostable, stable across a wide pH range, and not post-translationally modified; and Class III, comprising high-molecular-weight proteins (>30 kDa) that are thermolabile (Fig. 2). It is well established that bacteriocins can be produced by both Gram-positive and Gram-negative microorganisms (Ageitos et al., 2017), as reflected in the classification proposed by Simons et al. (2020).

Class I lantibiotics comprise small bacteriocins (<5 kDa) that undergo post-translational modifications. A distinctive feature of this group is the presence of unusual amino acids, including dehydrated residues, lanthionine, and 3-methyllanthionine, which form ring structures and confer structural stability and heat resistance.

This class is typically associated with the inhibition of Gram-positive bacteria, including foodborne pathogens (He and Deber, 2024). It also encompasses lantibiotics such as lacticin, cytolysin, and salivaricin, whose antimicrobial activity is linked to the inhibition of specific enzymes that are essential for the survival of target bacteria.

Class II bacteriocins, or non-lantibiotics, do not contain unusual amino acids in their structure (Yang et al., 2024), and post-translational modification is limited to the formation of disulfide bonds in only a few members (e.g., pediocin PA-1, pediocin AcH). Similar to class I bacteriocins, these peptides are thermostable and relatively small (<10 kDa). Their antimicrobial activity is mainly exerted through destabilization and increased permeability of bacterial membranes or via pore formation in the membrane (Marr et al., 2006; Pontes et al., 2022). This group can be further divided into four subclasses. The first subclass comprises bacteriocins with a linear structure stabilized by disulfide bonds and exhibiting broad antilisterial activity; therefore, they are commonly referred to as antilisterial bacteriocins (e.g., leucocin A, acidocin A, pediocin PA-1) (Batoni et al., 2011; Zasloff et al., 2002).

The second subclass consists of two-peptide (α/β) bacteriocins, in which both peptides are produced and are required for full antimicrobial activity (e.g., lactococcin G, lactococcin Q, and plantaricin NC8) (Fontanot et al., 2020). The third subclass includes small bacteriocins associated with a signal sequence and containing one or two cysteine residues in their structure; these are referred to as cystibiotics and thiolbiotics, respectively. This subgroup comprises several molecules, such as lactococcin A, divercin A, and acidocin B (Marr et al., 2006). The fourth subclass encompasses all remaining class II bacteriocins that do not fall into any of the aforementioned categories.

Unlike Class I and Class II bacteriocins, Class III bacteriocins are large peptides (>30 kDa) that are generally thermolabile and may or may not induce cell lysis (Beis et al., 2019; Kapil et al., 2020). This class includes bacteriocins such as zoocin A, lysostaphin, and

helveticins J and V (Kapil et al., 2020). Their antibacterial activity is primarily associated with enzymatic functions (e.g., endopeptidase activity) that lead to degradation of the bacterial cell wall.

Class IV bacteriocins are distinguished by a structure incorporating lipid or carbohydrate moieties (He and Deber, 2024), as exemplified by plantaricin S (Kuma et al., 2018) and leuconocin S, which disrupt bacterial cell membranes. This structural characteristic renders these molecules susceptible to degradation by various enzymes, including glycolytic and lipolytic enzymes.

The antimicrobial activity of bacteriocins is primarily associated with their ability to disrupt the cell membrane of Gram-positive bacteria by forming pores, which trigger the passive leakage of essential intracellular components and ultimately lead to cell death. Biological protection in food products is mainly achieved either by adding starter cultures (predominantly lactic acid bacteria) capable of producing bacteriocins in *situ*, or by incorporating purified bacteriocins (Stabnikov et al., 2025). Bacteriocins are effective against foodborne pathogens as well as a wide range of spoilage microorganisms (Chen et al., 2020). The overall classification scheme of bacteriocins produced by lactic acid bacteria and their main characteristics (Smaoui et al., 2024) is presented in Figure 4. In a study by Dal Bello et al. (2010), approximately 1,000 autochthonous LAB strains isolated from artisanal fermented products were examined, among which 98 bacteriocin-producing cultures (around 10%) were identified. The bacteriocins synthesized by these strains exhibited antimicrobial activity against various spoilage microorganisms and inhibited the growth of pathogenic and opportunistic bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium tyrobutyricum*, and *Brochothrix thermosphacta.*

It has been demonstrated that bacteria belonging to the genera *Lactococcus* and *Enterococcus* are effective against several microorganisms responsible for meat product spoilage and are also capable of inhibiting pathogenic bacteria (Smaoui et al., 2024). This is evidence that bacteriocin-producing strains are frequently present in competitive ecosystems, such as fermented foods, where the ability to synthesize antimicrobial compounds confers an ecological advantage and promotes their dominance within the microbiota (Cocolin, 2025). The classification scheme of bacteriocins produced by lactic acid bacteria, along with their main characteristics, is shown in Figure 4.

In accordance with the regulatory status of food ingredients, microorganisms recognized as safe for consumption (GRAS) by the U.S. FDA and granted Qualified Presumption of Safety (QPS) status in Europe (EFSA, 2020) can be used under specified conditions, but their application must still comply with relevant food safety regulations and labelling requirements. However, the incorporation of purified or partially purified bacteriocins is not yet fully supported at the regulatory level, and their use depends on national authorities and intended applications (Stabnikova et al., 2023; Verma et al., 2022). For instance, nisin (E234) is the best-known and most extensively studied bacteriocin and remains the only substance approved as a food additive for some type of products in the European Union (European Commission, 2011).

Barcenilla et al. (2022) investigated the potential application of LAB and bacteriocins in meat products to enhance safety, including their possible use as functional components in active packaging.

However, manufacturers face emerging challenges related to improving technological processes and implementing biological methods for food system protection, which would contribute to increased product safety and extended shelf life.

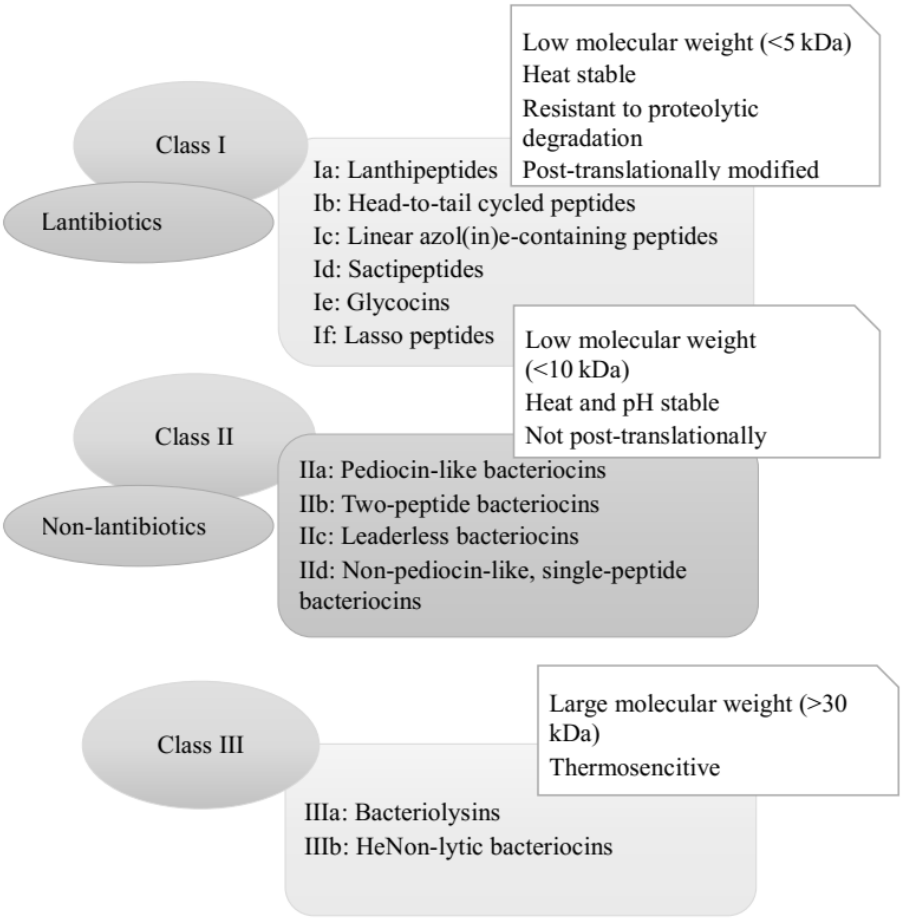

Figure 4. Classification scheme of bacteriocins produced by lactic acid bacteria and their main characteristics
Adapted from Smaoui et al., 2024

Bacteriocin production by bacteriocinogenic strains in cooked sausage manufacturing

Interest in bioprotection and lactic acid bacteria strains capable of producing bacteriocins has increased substantially in recent years, accompanied by numerous studies on their detection and characterization (Cocolin, 2025). However, research specifically addressing the effective release of bacteriocins during food processing remains limited. This limitation is largely due to the difficulty of assessing bacteriocin relising in the specific food matrix in which they reside, although this parameter is critical for selecting a strain as a bioprotective component (Cocolin, 2025).

Several conditions encountered by microorganisms during food production can influence bacteriocin synthesis. Factors such as salt concentration, pH range, heat treatment, microbial competition, and the presence of preservatives significantly affect the protective efficacy of bacteriocin-producing LAB strains (Todorov et al., 2022). One approach to demonstrate bacteriocin synthesis in food products is to monitor the expression of the gene encoding the bacteriocin and correlate this expression with evidence of active bacteriocin production by the protective culture during processing (Cocolin, 2025). Such confirmation typically involves combining molecular techniques, such as quantitative PCR or transcriptomic analysis, with biochemical assays that detect the presence and activity of the bacteriocin in the food matrix.

For example, Urso et al. (2006) demonstrated that during sausage production with *Latilactibacillus sakei* I151, the strain was capable of producing sakacin P. The approach involved manufacturing sausages using *L. sakei* I151 as a starter culture and collecting samples at regular intervals throughout fermentation. A representative sample was subjected to RNA extraction, followed by membrane transfer and hybridization using a *sakP* gene-specific probe. Gene expression *in vitro* peaked after approximately 15 hours and persisted until the end of the experiment, which lasted nearly five days.

This hypothesis was further supported by agar activity tests using the agar diffusion method. Aliquots of fermented sausage homogenate, previously neutralized in physiological solution, were distributed into wells of an agar layer inoculated with *Listeria monocytogenes*. Clear inhibition zones were observed around the wells on days 3, 7, and 14 of fermentation (Cocolin, 2025). These results confirmed antimicrobial activity associated with the secretion of sakacin P, which correlated perfectly with the expression of the *sakP* gene. Further evidence was provided by the sustained expression of the *sakP* gene, which remained active even after 14 days of fermentation. This finding indicates the ability of *Latilactibacillus sakei* I151 to maintain bioprotective activity against *Listeria monocytogenes* throughout the production process by utilizing a biological mechanism (Cocolin, 2025).

García-López et al. (2023) demonstrated the feasibility of incorporating two autochthonous bacteriocin-producing strains during salami production. For comparison, a control batch underwent spontaneous fermentation with the selected strains, while another batch used a commercial starter culture. The results showed that inhibition of *Listeria monocytogenes* was equally effective in salami produced with either the autochthonous strains or the commercial starter. These findings underscore the ability of native LAB to produce bacteriocins within real food matrices, such as cooked sausages.

Competition for nutrients

The synthesis of antimicrobial compounds is among the most extensively studied mechanisms for inhibiting both pathogenic and spoilage microorganisms. However, because the concentrations of these compounds may be insufficient to suppress certain species, additional mechanisms are likely involved (Mgomi et al., 2023). One such mechanism is nutrient competition: spoilage bacteria and LAB compete for available resources, often in combination with the action of bioactive compounds. By efficiently utilizing nutrients and limiting or completely depleting them in the environment, lactic acid bacteria can effectively inhibit the growth of spoilage organisms (Siedler et al., 2019).

However, the competitive exclusion of microorganisms in different food matrices remains insufficiently studied, and its role within the overall bioprotective mechanism is still unclear. It has been shown that three strains of *Lactobacillus paracasei* utilized metabolites to varying degrees, revealing an inverse correlation between glucose and glutamine

consumption and the inhibition of spoilage mold growth: more intensive nutrient utilization by the bacteria was associated with reduced mold development (Honoré et al., 2016). In the human body, competitive exclusion is described as "nutritional immunity," in which limited availability of iron and zinc in specific tissues suppresses the growth of pathogenic microorganisms. Although this mechanism may not apply to lactic acid bacteria, competition for other nutrients in nutrient-rich environments cannot be excluded (Gerwien et al., 2018).

The presence of oxygen in contact with food products can significantly deteriorate quality by stimulating mold growth and promoting lipid oxidation, which reduces shelf life (Marin et al., 2021). Molds and yeasts require water, minerals, and carbon and nitrogen sources for growth and reproduction. They exhibit high resistance to environmental stress, such as elevated salt or sugar concentrations, due to the structural properties of their cell walls, which contain chitin and cellulose. For example, yeasts belonging to the genera *Candida*, *Saccharomyces*, *Zygosaccharomyces*, *Pichia*, *Meyerozyma*, *Metschnikowia*, and *Wickerhamomyces* are capable of growing in products with high sugar concentrations and low moisture content (15–50%) (Hu, 2016; Tarlak, 2023).

Molds responsible for food spoilage, including those affecting cooked sausages, are generally strict aerobes (Walker et al., 2017). Therefore, limiting oxygen availability is an effective strategy to inhibit their growth. However, certain molds, such as *Penicillium roqueforti*, *Mucor plumbeus*, and some *Fusarium* species, can grow at oxygen concentrations as low as 0.5–2%. In addition, molds are capable of utilizing oxygen present within the food matrix itself, not solely from the surrounding atmosphere. Consequently, reducing atmospheric oxygen in packaged foods should be combined with additional inhibitory measures, such as the application of carbon dioxide or inert gases (Dangas et al., 2013).

The presence of 5% O_2 in a modified atmosphere delayed the growth of *A. niger*, *Eurotium amstelodami*, *P. chrysogenum*, and *Fusarium oxysporum*. Alternatively, anaerobic conditions combined with CO_2 resulted in complete inhibition of mold growth (Dagnas and Membré, 2013).

Traditional methods for preserving meat and meat products include air-permeable packaging, vacuum packaging, and modified atmosphere packaging (Gómez et al., 2020; Pasichnyi et al., 2022). Reducing residual oxygen in a modified atmosphere helps minimize lipid oxidation, inhibit undesirable microorganisms, stabilize color, and maintain nutritional quality (Niazmand and Yeganehzad, 2020). However, complete removal of oxygen during packaging is not feasible due to the limited efficiency of inert gas flushing or vacuuming. Furthermore, oxygen can permeate packaging materials during storage, leading to deterioration of product quality (Röcker et al., 2021). The residual oxygen level in vacuum-packaged cooked pork products typically ranges from 0.11–0.15%, while in modified atmosphere packaging it is approximately 0.9–1.1% (Smiddy et al., 2002). Since many foods release oxygen after packaging, residual oxygen levels of 0.5–5% may remain in the headspace of modified atmosphere packages. This can result from leakage through the packaging material, improper sealing, or oxygen released from the product itself (Sängerlaub et al., 2021).

Studies conducted over several decades have demonstrated that cooked-smoked ham undergoes color changes under the combined influence of oxygen and light (Andersen et al., 1988). Even residual oxygen levels as low as 0.1% in the packaging can induce photooxidation of nitrosomyoglobin and nitrohemoglobin, resulting in the formation of grey-brown metmyoglobin (Juncher et al., 2003; Møller et al., 2000, 2002).

Chemical oxygen scavengers can be applied to reduce oxygen levels in MAP. Modern packaging technologies employ one or more of the following approaches: oxidation of divalent iron powder, oxidation of ascorbic acid, oxidation of photosensitive dyes, enzymatic

oxidation (e.g., glucose oxidase and alcohol oxidase), unsaturated fatty acids (e.g., oleic or linolenic acid), or immobilized yeast on solid substrates (Kordjazi and Ajji, 2022). Most commercially available oxygen absorbers rely on iron powder oxidation (Jain et al., 2025), and the use of oxygen-absorbing materials is typically indicated on product labels (Gupta et al., 2024). The main disadvantages of these solutions are their relatively low efficiency and the prolonged activation time required the system to become effective. Additionally, food legislation in certain regions restricts or prohibits the use of chemical oxygen absorbers. Consequently, alternative or complementary strategies are needed to reduce residual oxygen in packaged products. Such methods must comply with regulatory requirements for safety and quality while meeting the expectations of increasingly demanding consumers (Jain et al., 2025).

The potential of microorganisms to reduce residual oxygen in MAP has been demonstrated and described in patent WO 2007/057026 A1. The microbial oxygen "absorber" consists of microorganisms that consume oxygen while producing minimal or no metabolic by-products such as carbon dioxide or organic acids. According to this invention, such microorganisms can be applied in packaged food products.

An example is the *Lactococcus lactis* strain with a defect in the pyruvate dehydrogenase and/or lactate dehydrogenase complex, as detailed in patents EP 0937774 and EP 0928333. This strain exhibits oxygen absorption due to a natural mutation that alters its metabolism, resulting in the loss of lactate dehydrogenase activity and enabling active oxygen uptake without lactic acid formation.

Company Novonesis has developed and commercially implemented a starter culture capable of absorbing residual oxygen in the packaging of meat products and other food items. This innovation enhances the preservation of a wide range of products, particularly ready-to-eat meat products and prepared meals, including those produced using sous-vide technology.

By employing diverse strategies to minimize residual oxygen in packaging, meat product manufacturers can enhance the microbiological safety and shelf life of ready-to-eat products (Gupta, 2024).

Quorum Sensing: recent insights and evolutionary aspects

Quorum sensing (QS) is a bacterial cell-to-cell communication mechanism that relies on the synthesis and detection of small signalling molecules known as autoinducers. This system enables microorganisms to monitor population density and coordinate the expression of genes that confer collective benefits only when a sufficient number of cells are present. Such QS-regulated processes include biofilm development, production of virulence factors, sporulation, and genetic transformation (Miller and Bassler, 2001; Waters and Bassler, 2005).

However, recent studies challenge the universality of this paradigm, emphasizing that autoinducer concentrations are strongly influenced by environmental factors, rendering population density estimates unreliable (West et al., 2012). An alternative perspective proposes quorum sensing as a mechanism of collective environmental sensing, wherein bacteria exchange information about surrounding conditions rather than merely assessing cell numbers. Moreno-Gámez et al. (2003) developed a theoretical model demonstrating that QS can function as a distributed system for environmental perception, enabling microbial communities to integrate and disseminate information at appropriate spatial scales through a form of "collective intelligence". This approach explains why QS regulates not only shared resources (exoenzymes) but also individual functions that are useful to a single cell regardless of the behavior of neighboring cells (Schuster et al., 2017). Evolutionary models suggest that the development of collective sensing is facilitated by local cell interactions and limited

signal diffusion. Spatial organization at the micron scale promotes the formation of bacterial clusters, thereby enhancing the efficiency of signal exchange and coordinated responses. Conversely, excessive diffusion or pronounced environmental heterogeneity reduces the adaptive significance of QS, as signalling molecules become less informative and collective coordination is weakened (Moreno-Gámez et al., 2023).

Biological hurdles and color retention of meat products

Studies investigating the microbiota of packaged meat products have revealed a correlation between microbial activity and color changes during storage. Li et al. (2019) reported noticeable discoloration in vacuum-packed bacon after seven days of refrigerated storage and identified *Leuconostoc* and *Lactobacillus* as the dominant spoilage-associated genera.

Peirson et al. (2003) reported visible greening in slices of Bologna sausage, with the degree of discoloration linked to the presence of *Weissella viridescens*, *Aerococcus viridans*, and *Carnobacterium viridans*. Furthermore, recent literature highlights that heat-resistant spore-forming bacteria, particularly *Bacillus subtilis*, adversely affect the color stability of vacuum-packed cooked sausages, posing a significant challenge to product quality during storage (Guerra et al., 2023).

The growing scientific interest in the bacterial ecosystem of meat products (Wu et al., 2024) was stimulated by an unexpected observation: certain cooked hams retained their characteristic color throughout their shelf life, even under conditions of microbiological spoilage (Wu et al., 2023). Remarkably, the color remained stable and unaffected by light exposure, consistently exhibiting a pink hue. Pigment analysis revealed the simultaneous presence of Zn-protoporphyrin IX (ZnPP) and nitrosomyoglobin. Notably, ZnPP – formed by the substitution of iron in the heme group with zinc – demonstrated exceptional resistance to light and heat, distinguishing it from other known myoglobin derivatives (Wakamatsu, 2022).

Numerous studies have examined the mechanism of ZnPP formation in nitrite-free hams, as evidence indicates that nitrite and nitrate inhibit its synthesis (Higuero et al., 2020). Furthermore, Asaduzzaman et al. (2020) identified bacterial strains capable of promoting high levels of ZnPP in minced meat homogenates, resulting in the preservation of a brighter red color for up to 14 days. Even after thermal treatment at 75 °C for 15 min, the color of cooked meat remained relatively stable, with residual ZnPP concentrations significantly higher than those in uninoculated controls. Despite these findings, the biochemical pathway underlying ZnPP formation remains incompletely understood. The potential involvement of endogenous and bacterial ferrochelatases (FECH), along with non-enzymatic reactions influencing iron and zinc incorporation into the porphyrin ring, has yet to be fully elucidated (Wakamatsu, 2022).

Therefore, Wu et al. (2024) proposed three conceptual models for ZnPP formation: (a) non-enzymatic model: ZnPP forms through a progressive reaction between Zn(II) ions and protoporphyrin IX during processing and storage; (b) endogenous enzymatic model: ZnPP formation occurs via an enzymatic pathway prior to complete thermal processing, likely resulting from structural modifications of endogenous ferrochelatase (FECH) that alter its catalytic activity; (c) combined enzymatic–microbial model: Certain spoilage-associated microorganisms exhibit elevated FECH activity, facilitating zinc incorporation into protoporphyrin IX during ham deterioration.

The studies conducted by Wu et al. (2023, 2024), which aimed to investigate storage conditions and quality changes in cooked hams, as well as identify key factors influencing

pigment transformation associated with the substitution of iron by zinc in heme, do not fully elucidate the mechanisms linking ZnPP formation to variations in nitrosomyoglobin content. Moreover, aspects related to lipid and protein oxidation, along with shifts in the product's microbiota, remain insufficiently explored. Consequently, future research should focus on elucidating ZnPP formation in cooked meat products containing nitrite, determining factors that promote ZnPP synthesis under predominantly non-enzymatic conditions, and clarifying the dominant role of microorganisms and their contribution to ZnPP formation.

Conclusions

Bioprotection is key modern strategy that contribute to the sustainable production of food. Harnessing microbial competition mechanisms inherent to natural microbial ecosystems offers additional opportunities for the development of safe products without the use of chemical preservatives or with their minimal application. This, in turn, promotes the extension of shelf life for substantial volumes of food products, supports the preservation of raw material resources, and has a positive impact on consumer health. The use of microbial competition is not a new approach in food technology, as clearly demonstrated by fermented products. In this context, bacteriocins, which are natural antimicrobial compounds, may serve as an important additional component of the meat product ecosystem when combined with starter cultures that form the dominant microbiota. Lactic acid bacteria are capable of producing a wide range of compounds with antimicrobial activity; however, it should be noted that the concentration of individual metabolites might sometimes be insufficient for effective inhibition of undesirable microflora. Moreover, there is a need for a deeper understanding of the biological characteristics of microorganisms responsible for spoilage of cooked sausages in order to elucidate the mechanisms of action of bioactive antimicrobial compounds. An important aspect is also to clarify the role of peptides with pronounced antifungal activity that are not produced directly by lactic acid bacteria, but may be generated through proteolysis of plant- or animal-derived proteins present in the matrix.

There are several questions remain that require further investigation to enable the effective use of microorganisms as tools of competitive interaction and bioprotection in cooked meat products. In particular, promising directions include the development of new methods for identifying bacteriocinogenic strains and the experimental validation of their efficacy in specific food matrices and under relevant production conditions.

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Effect of ultrasound-assisted modes on cholesterol esterase inhibition activity and functional properties of whiteleg shrimp (*Litopenaeus vannamei*) head protein hydrolysate

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Abstract

Introduction. This study investigated three ultrasound-assisted modes (ultrasound pretreatment, ultrasound-simultaneous treatment, and ultrasound post-treatment) to enhance cholesterol esterase inhibitory activity and functional properties of protein hydrolysates obtained from whiteleg shrimp (*Litopenaeus vannamei*) heads.

Materials and methods. Whiteleg shrimp heads were hydrolysed using alcalase to produce protein hydrolysates. For each ultrasound-assisted mode, ultrasonic amplitude and processing time were optimized to obtain hydrolysates with the highest cholesterol esterase inhibitory activity. The resulting hydrolysates were subsequently characterized in terms of degree of hydrolysis, half-maximal inhibitory concentration (IC_{50}) for cholesterol esterase inhibition, and techno-functional properties, including solubility, thermal stability, water-holding capacity, and oil-holding capacity.

Results and discussion. The highest cholesterol esterase inhibitory activity of the hydrolysates was obtained at an ultrasonic amplitude of 60% and a treatment time of 25 min for both ultrasound pretreatment and ultrasound-simultaneous treatment modes, while the same conditions (60% amplitude and 25 min) also resulted in the greatest inhibitory activity for the ultrasound post-treatment mode. Under these conditions, the cholesterol esterase inhibition activities of the ultrasound pretreatment, ultrasound-simultaneous treatment, and ultrasound post-treatment hydrolysates were 77.74±1.46%, 67.21±0.60%, and 64.67±0.71%, respectively, representing increases of 1.34-, 1.16-, and 1.12-fold compared with the unsonicated hydrolysate. The IC_{50} values of the ultrasound-treated hydrolysates ranged from 0.68 to 0.78 mg/mL and were significantly higher than that of the reference drug simvastatin (IC_{50} = 0.08324 μg/mL). Across a pH range of 3–8, all ultrasound-treated whiteleg shrimp head protein hydrolysates exhibited high solubility, exceeding 74%, even after thermal treatments at 63 °C for 30 min or 93 °C for 30 s. Regarding fluid-holding capacity, all three ultrasound-assisted modes markedly enhanced oil-holding capacity while reducing water-holding capacity of the hydrolysates.

Conclusions. These findings demonstrate the efficacy of ultrasound treatment in enhancing cholesterol esterase inhibitory activity and other functional properties of whiteleg shrimp head hydrolysate, suggesting its potential use as a natural supportive agent for hypercholesterolemia management and as a green emulsifier to improve food structure.

Introduction

The Vietnam Association of Seafood Exporters and Producers reported that Vietnam's shrimp exports reached approximately USD 4 billion in 2024, with whiteleg shrimp accounting for nearly 70% of this total. During processing, shrimp heads, which constitute 35–45% of the total shrimp weight (Majura et al., 2023), and contain substantial protein content (55.9–57.9% on a dry matter basis) are generated and typically considered waste. Transforming agricultural and industrial wastes into useful products contributes significantly to economic growth while helping to reduce environmental pollution (Stabnikova et al., 2023a, b). Whiteleg shrimp head waste can be valorized to produce protein hydrolysates, which exhibit diverse biological activities, including antioxidant and antimicrobial effects (Rashidian et al., 2021; Vo et al., 2019), and show potential as a source of antidiabetic peptides (Xiang et al., 2021). In addition, these hydrolysates possess functional properties, such as antifreezing and copper-binding capacity (Majura et al., 2023; Vo et al., 2020a).

To date, no studies have been published on cholesterol esterase inhibitory protein hydrolysates derived from whiteleg shrimp heads. Cholesterol esterase liberates free cholesterol from dietary cholesterol esters, accelerating cholesterol absorption. Excessive cholesterol intake may induce excessive cholesterol accumulation in the plasma, increasing the risk of heart disease (Alnuaimi et al., 2023; Jafar et al., 2018). Therefore, inhibiting cholesterol esterase offers a prospective approach to manage hypercholesterolemia. Zhang et al. (2024) reported that compared to simvastatin (a synthetic drug), protein hydrolysates present the advantages of minimal side effects and cost-effectiveness in hypercholesterolemia management.

Bioactive peptides are generally inactive within their native protein sequences and can be released through protease-catalysed hydrolysis, which may be enhanced by ultrasound post-treatment (Mudgil et al., 2023). However, some intact proteins are resistant to enzymatic hydrolysis because their tertiary and quaternary structures limit enzyme access to cleavage sites (Rao et al., 2024). In such cases, ultrasound, a green, non-thermal, and safe physical processing technology, has been shown to effectively assist enzymatic protein hydrolysis (Hong et al., 2022).

The application of ultrasound can generate mechanical effects (e.g., turbulence, shear forces, shock waves, and micro-jets), thermal effects, and chemical effects (such as sonochemical reactions and sonolysis of water), leading to protein disruption and partial denaturation, as well as enhanced mass transfer, thereby improving enzyme accessibility to substrates (Santos et al., 2024; Wali et al., 2017). This technology has been successfully applied to increase the cholesterol esterase inhibitory activity of protein hydrolysates derived from soybean (Chen et al., 2024), rice bran (Fathi et al., 2021), and millet (Rao et al., 2024).

This study investigated the effects of three ultrasound-assisted modes, ultrasound pretreatment (UPr), ultrasound-simultaneous treatment (US), and ultrasound post-treatment (UPo), on the cholesterol esterase inhibitory activity and functional properties of alcalase-derived protein hydrolysates from whiteleg shrimp heads. For each mode, the ultrasonic amplitude and processing time were optimized to obtain hydrolysates with maximal cholesterol esterase inhibition. The resulting hydrolysates were further evaluated for their degree of hydrolysis and functional properties, including solubility, thermal stability, water-holding capacity, and oil-holding capacity.

Materials and methods

Materials

Whiteleg shrimp heads were obtained from the Vietnam Food Company and transported to the laboratory on dry ice. Upon arrival, the shrimp heads were washed and initially dried at 90 °C for 20 min, followed by further drying at 60 °C until a constant weight was achieved. The dried heads were then ground, packed in sealed polyacrylamide bags, and stored at room temperature. The proximate composition of the shrimp head powder was determined following AOAC (2023) methods and consisted of 5.36 ± 0.12% moisture, 54.5 ± 0.5% protein, 10.5 ± 0.15% lipid, 10.6% carbohydrate, and 19.1 ± 0.23% ash.

Alcalase® 2.5L preparation (2.5 Anson unit/g, optimal performance at pH 7.5 and 55°C) was sourced from Novozymes. All reagents used in this study were of analytical grade, purchased from Sigma-Aldrich, Thermo Scientific Chemicals, and Merck.

Preparation of whiteleg shrimp head protein hydrolysates

Whiteleg shrimp head hydrolysates were prepared following the method of Vo et al. (2025). Shrimp head powder was mixed with distilled water at a 1:6 (w/v) ratio, and the pH of the mixture was adjusted to 7.5 using 1 M HCl or 1 M NaOH. Alcalase® 2.5L was then added at an enzyme-to-substrate ratio of 30 U/g protein, and hydrolysis was carried out at 55 °C for 4 h. After hydrolysis, the mixture was heated at 90 °C for 10 min to inactivate the enzyme, followed by centrifugation to collect the hydrolysate (supernatant). The resulting hydrolysate was lyophilized and stored at −20 °C until further use.

Ultrasound treatment

Ultrasonication was conducted using a probe ultrasonicator (VC505 – Sonics, USA) with a 19 mm diameter probe and a maximum power of 500 W. This device was operated at a fixed frequency of 20 kHz. Amplitude was varied from 30% to 70% in 10% increments, and ultrasonic time ranged from 10 to 30 min with a 5-min interval. A sample-containing beaker was placed on ice during the sonication to maintain low temperature, and the ultrasonicator probe was immersed in the sample at a depth of 2 cm. Three ultrasound-assisted modes were applied in the alcalase hydrolysis: (1) pretreatment (UPr) (ultrasound pretreatment), where the dispersion of whiteleg shrimp head powder in distilled water was sonicated before hydrolysis (before adjusting its pH to 7.5); (2) simultaneous treatment (US) – ultrasonication was applied during hydrolysis (immediately after alcalase addition); and (3) post-treatment (UPo) (ultrasound post-treatment), where ultrasonication was performed after hydrolysis (for the gained Alcalase hydrolysate). The produced hydrolysates were then evaluated for their cholesterol esterase inhibitory activity. For each ultrasound-assisted mode, ultrasonic amplitude and time were investigated to yield the hydrolysates with the highest cholesterol esterase inhibitory activity.

Cholesterol esterase inhibition activity assay

The cholesterol esterase inhibitory activity of whiteleg shrimp head hydrolysates was determined following the method of Alnuaimi et al. (2023) with slight modifications. A sample including 0.25 mL protein hydrolysate (1 mg/mL), 2 mL of sodium phosphate buffer (100 mM, pH 7.2, containing 100 mM NaCl), 0.5 mL of 5 mM p-nitrophenyl butyrate

solution (substrate) and 0.5 mL of cholesterol esterase solution (5 μg/mL) was incubated at 37°C for 30 min. The absorbance at 405 nm of the mixture was then measured. All the protein hydrolysate, substrate, and enzyme solutions were prepared in the sodium phosphate buffer 100 mM, pH 7.2, containing 100 mM NaCl. For the blank, control, and control blank samples, the enzyme solution, protein hydrolysate, and both the components were replaced with the sodium phosphate buffer, respectively.

The cholesterol esterase inhibitory activity (CEIA) of the hydrolysates was then determined using the equation (1):

$$\text{CEIA } (\%) = \left(1 - \frac{C - D}{A - B}\right) \times 100 \quad (1)$$

where A, B, C, and D are the absorbances of the control, control blank, sample, and blank, respectively.

IC50 values of the protein hydrolysates and Simvastatin Stella 20 mg drug (standard) were determined to evaluate their cholesterol esterase inhibitory activity.

Determination of degree of hydrolysis

The formaldehyde titration method described by Vo et al. (2025), was adapted to measure degree of hydrolysis of whiteleg shrimp head hydrolysates. A mixture of protein hydrolysate (5 mL) and distilled water (60 mL) was adjusted to pH 8.2 using 0.05 M NaOH. Subsequently, 10 mL of 36-38% (v/v) formaldehyde solution was added, and the resulting solution was titrated with 0.05 M NaOH solution until its pH reached 9.2. A blank sample (replacing the protein hydrolysate with distilled water) was processed identically. The degree of hydrolysis (DH) of the protein hydrolysate was calculated using the equation (2):

$$\text{DH } (\%) = \frac{(V_1 - V_2) \times C \times M \times V}{m \times P \times 5} \times 100 \quad (2)$$

where V_1 (L) and V_2 (L) depicts volume of 0.05 M NaOH solution used to titrate the sample and the blank sample, respectively; C (mol/L) is the concentration of NaOH solution used for the titration; M (g/mol) is nitrogen's molar mass; V (mL) is the total volume of the protein hydrolysate gained from m (g) of whiteleg shrimp head powder. P (%, w/w) is the total nitrogen content of the whiteleg shrimp head powder; 5 (ml) is the volume of the protein hydrolysate used in this assay.

Determination of solubility and heat stability

The solubility and thermal stability of whiteleg shrimp head hydrolysates were assessed following the method of Vo et al. (2025). Lyophilized hydrolysate powders were re-dissolved in distilled water at a concentration of 10 mg/mL, and the pH of each solution was adjusted to 3, 4, 5, 6, 7, or 8 using 1 M HCl or 1 M NaOH. For solubility determination, the solution underwent a 30-min shake at ambient temperature, then centrifuged (3000 g, 15 min, room temperature) to yield supernatants.

To assess heat stability, the solution was subjected to a heat treatment at either 63°C for 30 min or 93°C for 30s. They were then cooled in an ice-water bath for 10 min, centrifuged (3000 g, 15 min, room temperature) to gain supernatants. The supernatants were then evaluated for their soluble protein content using the Lowry method.

Heat stability was expressed via solubility, which was calculated using the equation (3):

$$\text{Solubility } (\%) = \frac{\text{Protein content in supernatant}}{\text{Total protein content in sample}} \times 100 \quad (3)$$

Total protein content in the sample was the soluble protein content of the solution prepared by dissolving 100 mg of hydrolysate powder in 10 mL of 0.5 M NaOH solution at room temperature.

Determination of oil-holding capacity and water-holding capacity

Procedures detailed in our previous study were employed to estimate the oil-holding capacity (OHC) and water-holding capacity (WHC) of whiteleg shrimp head hydrolysates (Vo et al., 2025).

For the oil-holding capacity assessment, a 50 mL centrifuge tube containing hydrolysate powder (0.5 g) and vegetable oil (10 mL) was placed at 25±1°C. The tube underwent three 30-s agitation cycles, with 10 min intervals between each cycle. After that, the tube was centrifuged (3000 g, 15min, room temperature) and the supernatant volume was recorded.

The water-holding capacity was determined by adding distilled water (20 mL) to hydrolysate powder (0.5 g) in a 50 mL centrifuge tube. The tube was shaken for 30s, then stood at ambient temperature for 6 h. After centrifugation (3000 g, 15min, room temperature), the supernatant was filtered via a Whatman No. 1 filter paper before its volume was measured.

The same protocol was performed for a blank sample (without the hydrolysate powder). The oil-holding capacity (OHC) and water-holding capacity (WHC) were calculated using equations (4) and (5), respectively:

$$\text{OHC (mL oil/g protein hydrolysate powder)} = \frac{V_b - V_s}{m} \quad (4)$$

$$\text{WHC (mL water/g protein hydrolysate powder)} = \frac{V_b - V_s}{m} \quad (5)$$

where V_b (mL) and V_s (mL) are the volumes of the supernatant of the blank and sample, respectively. Casein was used as a standard, and its OHC and WHC were determined identically.

Data analysis

Triplicate experimental data were analysed using Microsoft Excel, and presented as mean±standard deviation. Statistically significant differences were determined, using the Tukey's method with Statgraphics Centurion 18 software.

Results and discussion

Effect of ultrasonic amplitude on cholesterol esterase inhibition activity of the whiteleg shrimp head hydrolysates

As shown in Figure 1, regardless of the ultrasound-assisted mode, increasing the ultrasound amplitude enhanced the cholesterol esterase inhibitory activity of whiteleg shrimp head hydrolysates, reaching a maximum at 60% amplitude before declining, which can be attributed to optimal ultrasound-induced cavitation.

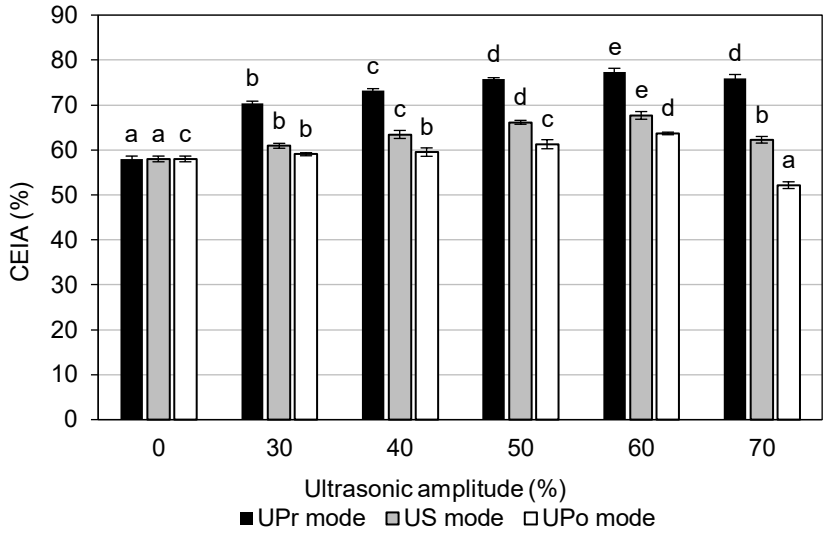

Figure 1. Effect of ultrasonic amplitude on cholesterol esterase inhibitory activity (CEIA) of hydrolysates prepared using three ultrasound-assisted modes
Bars of the same color with different letters indicate significant differences ($p<0.05$)

This cavitation created mechanical, thermal, and/or chemical effects, which might alter the shrimp head proteins by unfolding their molecular structure, reducing their size, or disrupting their non-covalent interactions (hydrogen bonding, Van der Waals forces, and dipole attractions) (Fadimu et al., 2022; Hong et al., 2022). These changes ameliorated peptide bond susceptibility to alcalase, thus improving the protein hydrolysis and heightening the bioactivity of resulting hydrolysates. Additionally, sonication at 60% amplitude possibly aided in unmasking hydrophobic amino acids at an appropriate level, which favoured cholesterol esterase inhibitory activity (Chen et al., 2024; Zhang et al., 2024).

The reduced cholesterol esterase inhibitory activity observed at low ultrasonic amplitudes (30, 40, and 50%) may be attributed to insufficient cavitation effects. Liu et al. (2022) reported that the turbulent forces and microstreaming generated by low-intensity ultrasonic cavitation could induce peptide collisions and aggregation, thereby decreasing the bioactivity of hydrolysates. Conversely, excessively high ultrasonic amplitudes may cause overexposure of non-polar groups, enhancing hydrophobic interactions among peptides and leading to the formation of large peptide aggregates. Such aggregates may obstruct the active sites of hydrolytic enzymes, limiting the release of bioactive sequences from native proteins (Yan et al., 2024). Furthermore, Justino et al. (2024), underscored peptide denaturation induced by excessive heat and mechanical force during high-amplitude ultrasound application. Thus, the declined cholesterol esterase inhibitory activity of the hydrolysates at an amplitude of 70% were observed (Figure 1). Our findings are in line with the findings of Hu and Li (2022), and Siewe et al. (2020), which indicated that there was an increase in antioxidant activity of soy protein isolate and fish (*Labeo rohita*) head hydrolysate, respectively, as ultrasound amplitude augmented up to specific thresholds. Similarly, Chen et al. (2024) observed a decline in cholesterol esterase inhibitory activity of soy protein hydrolysate when it underwent ultrasonic processing at excessively high power. Therefore, for further investigations, an amplitude of 60% was set for all ultrasound treatment modes.

Effect of ultrasonic processing time on cholesterol esterase inhibition activity of the whiteleg shrimp head hydrolysates

Ultrasonication applied for an appropriate duration can enhance protein hydrolysis by increasing the susceptibility of peptide bonds to enzymatic cleavage, thereby improving enzyme accessibility to catalytic sites. Hong et al. (2022) similarly reported that optimal ultrasound treatment refines protein particles and facilitates enzyme–substrate complex formation, leading to enhanced hydrolysis efficiency. Consequently, bioactive peptide sequences are more effectively released into the resulting hydrolysates, thereby increasing their biological activities. However, excessive ultrasonication may promote the formation of large peptide aggregates and reduce enzyme catalytic efficiency, ultimately diminishing hydrolysis performance (Pacheco et al., 2023; Rao et al., 2024). Moreover, as highlighted by Thongrattanatrai et al. (2023) and Bhetwal et al. (2024), prolonged exposure of protein hydrolysates to ultrasound can induce protein reassembly and structural rearrangements that mask peptide bonds and amino acid residues, thereby hindering enzymatic hydrolysis and negatively affecting the bioactivities of the obtained hydrolysates.

In this study, within the investigated ultrasound processing times (10–30 min), ultrasound pretreatment and ultrasound-simultaneous treatment produced the highest cholesterol esterase inhibitory activities of 77.74±1.46% and 67.21±0.60%, respectively, at 20 min (Figure 2).

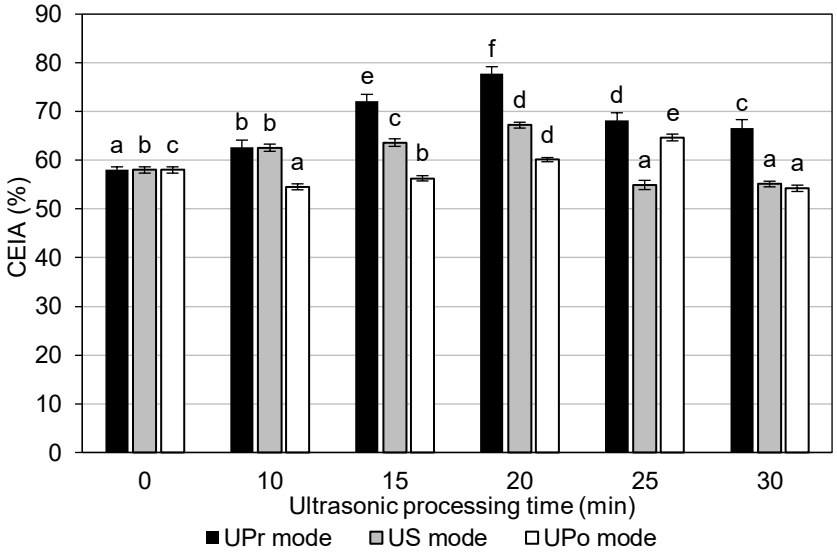

Figure 2. Effect of ultrasonic processing time on cholesterol esterase inhibitory activity (CEIA) of hydrolysates prepared using three ultrasound-assisted modes
Bars of the same color with different letters indicate significant differences ($p<0.05$)

In contrast, the ultrasound post-treatment hydrolysate exhibited its maximum inhibitory activity (60.12±0.43%) after 25 min of sonication. This is consistent with the results of Rao et al. (2024), which pointed out there was an increase in cholesterol esterase inhibitory activity of millet protein hydrolysate as the sonication time increased from 5 to 15 min, followed by a decline. Besides, Fathi et al. (2021) indicated that the ultrasound time beyond 10 min diminished both antioxidant and lipase inhibition activity of rice bran hydrolysate.

Similarly, anti-inflammatory and anti-diabetic activities of edible bird's nest protein hydrolysates increased when ultrasound processing time ranging from 15 to 30 min and decreased thereafter, as highlighted by Tang and Koh (2023). Thus, for more experiments, both ultrasound pretreatment and ultrasound-simultaneous treatment modes were performed with a duration of 20 min, while ultrasound post-treatment mode was conducted for 25 min.

Hydrolysis degree and half inhibition concentration of cholesterol esterase inhibition activity of the whiteleg shrimp head hydrolysates

A higher degree of hydrolysis corresponds to a greater release of free amino groups and short peptides in the hydrolysate (Jafar et al., 2018). Meanwhile, lower IC50 denotes stronger cholesterol esterase inhibition activity of inhibitors. As illustrated in Figure 3, all three ultrasound-assisted patterns promoted both the degree of hydrolysis and cholesterol esterase inhibitory activity of hydrolysate, with ultrasound pretreatment type expressing the highest effectiveness, followed by ultrasound-simultaneous treatment and ultrasound post-treatment models.

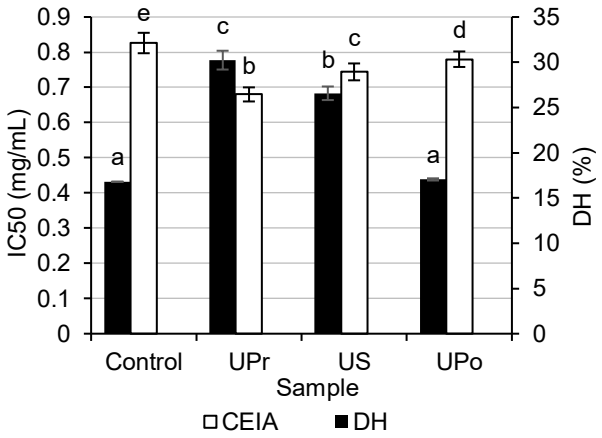

Figure 3. Degree of hydrolysis (DH) and IC50 of cholesterol esterase inhibitory activity (CEIA) of hydrolysates prepared using three ultrasound-assisted modes
Bars of the same color with different letters indicate significant differences ($p<0.05$)

Ultrasound pretreatment mode aided protein hydrolysis by preparing substrate molecules for the hydrolytic enzymes, involved in conformational changes of protein molecules and creation of micropores on the substrate particles' surfaces, promoting their susceptibility to hydrolytic enzymes (Bing et al., 2024; Hong et al., 2022). Moreover, the ultrasound-simultaneous treatment may enlarge enzymatic cleavage sites, thereby enhancing the accessibility of bulky substrate molecules. In addition, this mode improves mass transfer within the reaction mixture by generating turbulence and microstreaming in the liquid phase (Rao et al., 2024).

However, certain amino acid sequences generated during hydrolysis may be incompatible with the active sites of hydrolytic enzymes or may competitively occupy these sites, thereby hindering the binding of more suitable substrate segments (Tang and Koh, 2023). Consequently, the degree of hydrolysis of the ultrasound-simultaneous treatment

hydrolysate was 1.14-fold lower than that of the ultrasound pretreatment sample, indicating that ultrasound pretreatment was more effective than ultrasound-simultaneous treatment in enhancing alcalase-mediated protein hydrolysis. Consistent with the findings of Indriani et al. (2022) and Pacheco et al. (2023), ultrasound pretreatment was more effective than ultrasound-simultaneous treatment in enhancing the enzymatic hydrolysis of Asian bullfrog skin and pumpkin seed proteins, respectively. In contrast, Yan et al. (2024) reported that sheep hoof collagen hydrolysates produced using ultrasound-simultaneous treatment exhibited a higher degree of hydrolysis than those obtained by ultrasound pretreatment. These discrepancies may be attributed to differences in protein sources and hydrolysis conditions. With respect to cholesterol esterase inhibitory activity, the lower degree of hydrolysis observed in the ultrasound-simultaneous treatment sample may explain its reduced inhibitory activity, as evidenced by a 1.09-fold higher IC_{50} value compared with the ultrasound pretreatment sample (Figure 3). This observation is consistent with the findings of Ashraf et al. (2024), who reported that increasing the degree of hydrolysis enhanced the cholesterol esterase inhibitory activity of lentil and mung bean protein hydrolysates. Furthermore, Wang et al. (2024) demonstrated that peptides with lower molecular weights exhibit superior cholesterol esterase inhibitory activity.

On the other hand, the increase in cholesterol esterase inhibitory activity of the ultrasound post-treatment sample, compared to the non-ultrasonicated hydrolysate (Figure 3), might result from the cavitation-induced fragmentation of bioactive peptide aggregates and modifications in the peptide structure, thereby exposing additional binding sites for cholesterol esterase (Liu et al., 2022). This ultrasound-assisted mode may not affect peptide chains, since there was a statistically insignificant change in the degree of hydrolysis of the ultrasound post-treatment sample compared to the non-ultrasonicated hydrolysate (Figure 3). In addition, the ultrasound post-treatment type displayed the lowest cholesterol esterase inhibitory activity among the three ultrasound-assisted patterns, with its IC50 surpassing that of the ultrasound pretreatment and ultrasound-simultaneous treatment modes by 1.15 and 1.05 times, respectively. Similarly, Lopes et al. (2023) reported that the ultrasound pretreatment mode improved the antioxidant activity of common bean and lentil protein hydrolysates more efficiently than the ultrasound post-treatment type did.

All hydrolysates in this study demonstrated a mild cholesterol esterase inhibitory activity with their IC50 ranging from 0.68±0.02 to 0.83±0.03 mg/mL, which were significantly higher than that of simvastatin drug (IC50 of 0.083±0.001 μg/mL), a drug used for managing blood cholesterol level. However, these IC50 values were comparable to those of hydrolysates derived from date seed protein (Mostafa et al., 2022) and camel skin gelatin (Fawale et al., 2023), and significantly lower than that of *Rosa roxburghii* seeds (Yin et al., 2025). For that reason, it could be suggested that hydrolysate could be considered as a natural potential cholesterol-lowering agent.

Solubility of whiteleg shrimp head hydrolysates

Solubility is an effective indicator of assessing protein aggregation and denaturation. This characteristic not only impacts other functional properties of protein but also significantly influences sensory qualities of fortified foods (Cropotova et al., 2024). In this study, as illustrated in Figure 4, all three ultrasound-assisted modes improved the solubility of the hydrolysate across the tested pH range of 3 to 8.

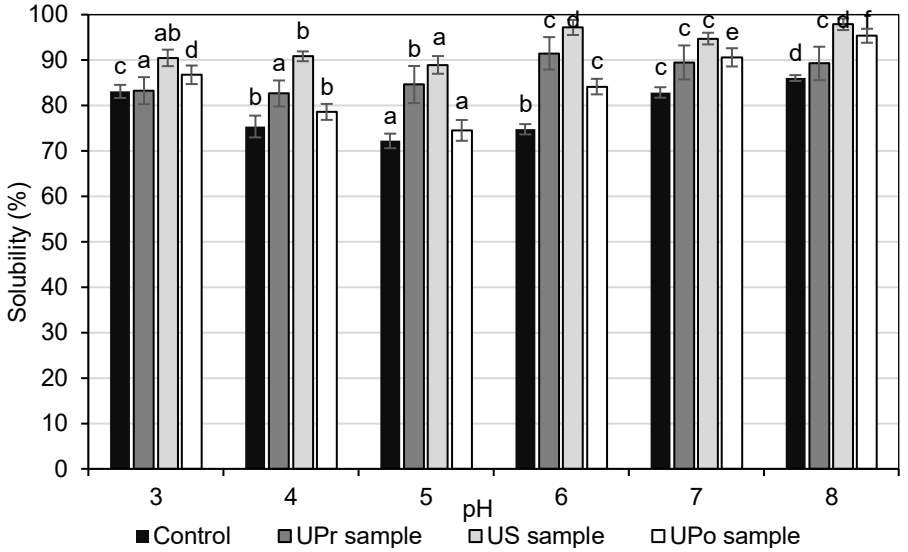

Figure 4. Solubility of hydrolysates prepared using three ultrasound-assisted modes
Bars of the same color with different letters indicate significant differences ($p<0.05$)

This increment could be ascribed to acoustic cavitation-induced unfolding of peptide molecules and disruption of their non-covalent linkages, including hydrogen bonds, electrostatic, and hydrophobic interactions. These changes enhanced the availability of hydrophilic amino acid side chains for hydrating, thereby boosting the peptide solubility (Saran et al., 2024; Yang et al., 2024). Similar observations were revealed by Gautam et al. (2025), who recorded the increase in solubility of shrimp shell and housefly larvae protein hydrolysates produced using ultrasound pretreatment and ultrasound-simultaneous treatment modes, respectively.

Within the tested pH range, among the three ultrasound-assisted modes, the ultrasound-simultaneous treatment pattern was the most effective type for elevating the shrimp head hydrolysate solubility, with an increase of 1.09 to 1.29-folds compared to the control (Figure 4). This might be attributed to the appropriate DH of the ultrasound-simultaneous treatment sample, which increased the number of hydrophilic groups, sufficiently forming hydrogen bonds with water molecules, thus facilitating its solubility (Vo et al., 2022). Conversely, lower degree of hydrolysis of the ultrasound post-treatment sample indicated the presence of a high quantity of large peptides, which may inefficiently expose their ionisable amino and carboxyl groups, mitigating their hydration and solubility (Vo et al., 2022). Meanwhile, decreased solubility of the higher DH hydrolysate (the ultrasound pretreatment sample) could be associated with the excessive exposure of hydrophobic amino acids, which favored hydrophobic interactions, leading to peptide aggregations (Barea et al., 2024).

Regarding the influence of pH on the solubility of hydrolysates, all samples demonstrated their peak solubility at pH 8, exceeding 80% of solubility (Figure 4). As reported by Xu et al. (2021), alkaline conditions imparted a strong net negative charge to peptides, fostering their interactions with the aqueous environment and thereby increasing their solubility. Besides, Vo et al. (2022) and Dhanabalan et al. (2020) found that solubility of hydrolysates from small shrimp (*Acetes japonicus*) and non-penaeid shrimp elevates at alkaline pH.

Overall, all hydrolysates in this study exhibited over 70% solubility across a wide pH range from 3 to 8, highlighting their potential to be incorporated into various food formulations.

Heat stability of whiteleg shrimp head hydrolysates

Thermal treatment influences the functional characteristics of proteins due to their intrinsic heat sensitivity. Heat-induced denaturation alters protein structure, resulting in the exposure of hydrophobic residues, which promotes protein-protein interactions and reduces protein solubility (Vo et al., 2020b). In this study, within the tested pH range from 3 to 8, all the hydrolysates remained greater than 70% solubility following heat treatment at 63 °C for 30 min or 93 °C for 30 s (Figure 5).

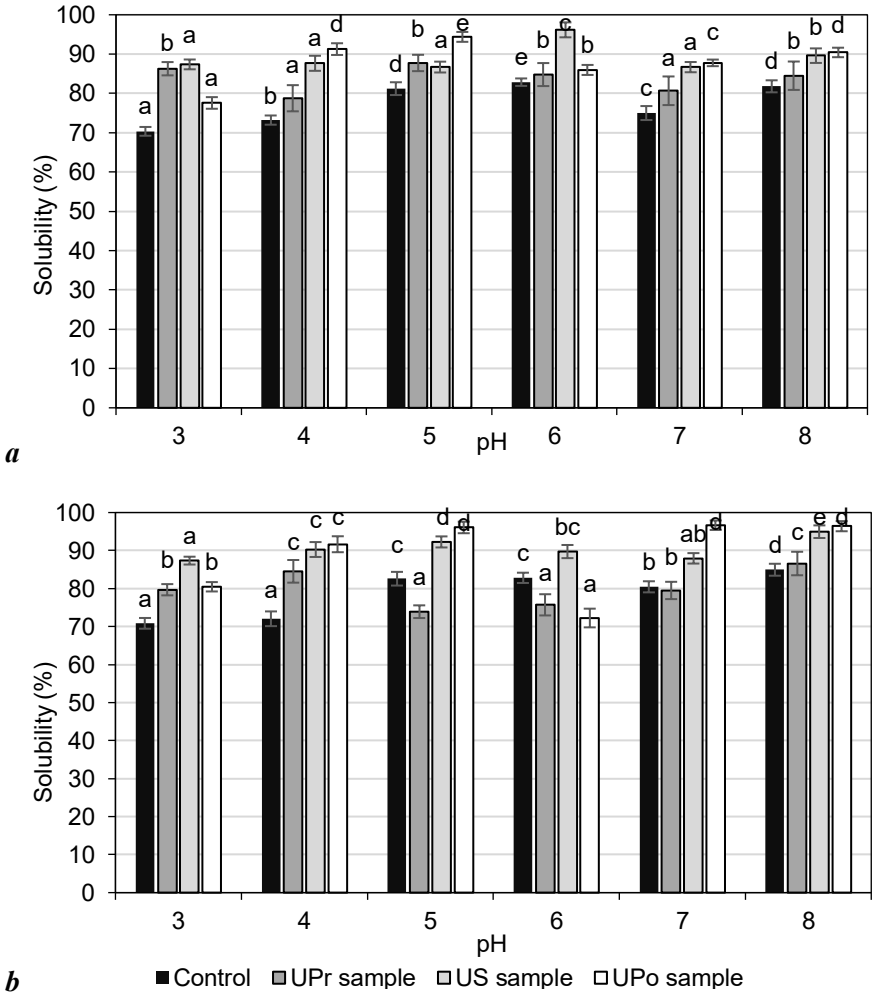

Figure 5. Heat stability ((a) 63 °C for 30 min and (b) 93 °C for 30 s) of whiteleg shrimp head hydrolysates prepared using three ultrasound-assisted modes
Bars of the same color with different letters indicate significant differences ($p<0.05$)

The results indicated that three ultrasound-assisted modes strengthened the heat stability of the hydrolysates under both thermal treatment conditions. It could be due to the fact that acoustic cavitation might disrupt noncovalent interactions between peptide molecules, making their amino acid side chains available for water molecules to form hydrogen bonds, thus impeding peptide aggregation (Luo et al., 2024).

Water-holding capacity of whiteleg shrimp head hydrolysates

As seen in Figure 6, the ultrasound pretreatment, ultrasound-simultaneous treatment, and ultrasound post-treatment modes decreased the water holding capacity of whiteleg shrimp head hydrolysates by 7.3, 2.5, and 1.3 times, respectively.

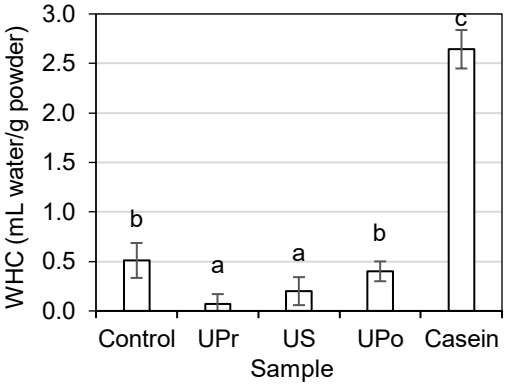

Figure 6. Water-holding capacity (WHC) of whiteleg shrimp head hydrolysates prepared using three ultrasound-assisted modes
Bars of the same color with different letters indicate significant differences ($p<0.05$)

As the degree of hydrolysis increased, the hydrolysates became more flexible, resulting in lower water-holding capacity (Qoms et al., 2023). This inverse proportion relationship between the degree of hydrolysis and WHC of protein hydrolysates was also published in our previous study (Vo et al., 2022). Compared to casein, all the ultrasonication-treated hydrolysates displayed significantly lower WHC, equivalent to 2.6–15.1% casein's WHC (Figure 6). Ghalamara et al. (2024) and Qoms et al. (2023) proposed that high solubility of protein hydrolysates led to reduction of their water-holding capacity, because their peptide molecules were highly dispersed in the aqueous medium. The findings of our present study was aligned with the study of Bhetwal et al. (2024), which indicated that the ultrasound-simultaneous treatment mode decreased the WHC of the hemp protein hydrolysate by 1.3 times compared to the control sample. In addition, Thongrattanatrai et al. (2023) found that the ultrasound pretreatment mode reduced the water-holding capacity of eri silkworm pupa hydrolysate from 0.33 to 0 ml water/g hydrolysate powder. However, Rawat and Saini (2023) published that there was an increase in water-holding capacity of sunnhemp protein hydrolysate prepared with the ultrasound pretreatment mode. This discrimination was probably due to different protein sources, hydrolysis and ultrasound conditions.

Oil-holding capacity of whiteleg shrimp head hydrolysates

Oil-holding capacity (OHC), which reflects the ability of peptides to interact with oil molecules, plays an important role in maintaining the acceptability and quality of food products. The retention of oil within the peptide matrix is primarily attributed to interactions between the hydrophobic side chains of peptides and the hydrocarbon chains of the oil (Saran et al., 2024). Ultrasound boosts the OHC of protein hydrolysates, owing to its cavitation effects that aids in exposing hydrophobic groups of the component peptides, as revealed by Saran et al. (2024). A 22% increase in OHC of eri silkworm pupa protein hydrolysate was observed by Thongrattanatrai et al. (2023), when applying ultrasound pretreatment mode. Similarly, as recorded by Bhetwal et al. (2024), and Saran et al. (2024), ultrasound pretreatment mode improved OHCs of hydrolysates derived from hemp seeds and rice bran by 1.5 and 1.2 times compared to that of non-ultrasonic treated samples, respectively. Furthermore, Bing et al. (2024), reported that ultrasound post-treatment mode significantly ameliorated the OHC of mung bean protein hydrolysate. The findings of this study are consistent with the above results. The OHC of whiteleg shrimp head hydrolysates increased from 0.98±0.12 to 1.69±0.23, 2.13±0.17, and 3.18±0.19 ml oil/g hydrolysate powder, when the ultrasound pretreatment, ultrasound-simultaneous treatment, and ultrasound post-treatment patterns were employed, respectively (Figure 7). Compared to casein, the ultrasound pretreatment, ultrasound-simultaneous treatment, and ultrasound post-treatment samples demonstrated superior OHC, surpassing by 1.2, 1.5, and 2.2 times, respectively (Figure 7). Accordingly, whiteleg shrimp head hydrolysates could be utilized to retard phase separation as well as improve palatability and taste retention of some fortified food products.

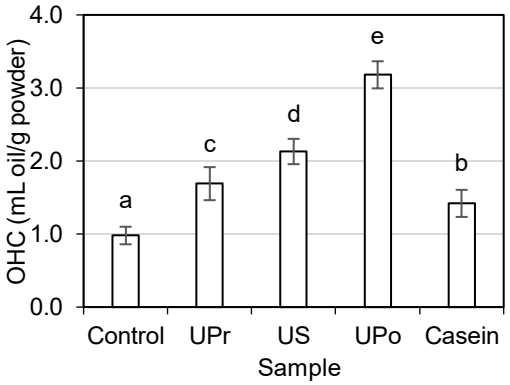

Figure 7. Oil holding capacity (OHC) of whiteleg shrimp head hydrolysates prepared using three ultrasound-assisted modes

Bars of the same color with different letters indicate significant differences ($p<0.05$)

Conclusions

This study is the first to systematically investigate the effects of three distinct ultrasound-assisted modes – ultrasound pretreatment, ultrasound-simultaneous treatment, and ultrasound post-treatment – on cholesterol esterase inhibitory activity and functional properties of protein hydrolysates derived from whiteleg shrimp (*Litopenaeus vannamei*) heads. All three ultrasound-assisted modes significantly enhanced inhibitory activity, solubility, thermal stability, and oil-holding capacity of the hydrolysates, while concomitantly reducing their

water-holding capacity. These findings provide preliminary evidence supporting further research into ultrasound-induced modifications of protein structure, process scale-up, and the potential clinical application of whiteleg shrimp head protein hydrolysates as functional ingredients for dietary management or supportive treatment of hypercholesterolemia.

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Antioxidant effects of plant ingredients on a dairy lipid base

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Abstract

Introduction. This study aimed to evaluate the antioxidant effects of plant ingredients on the stability and properties of high-fat milk-based mixtures during storage.

Materials and methods. The functional and technological properties of cocoa and sesame fibre in a lipid medium, as well as the quality indicators of the resulting mixtures during storage at (4±2) °C and (20±2) °C were assessed using standard analytical methods. These included determination of the fat-holding capacity and swelling degree of cocoa and sesame fibre, as well as measurement of peroxide and acid values of the dairy lipid base with and without added plant antioxidants over 16 days of storage.

Results and discussion. According to the obtained results, the highest fat-holding capacity and swelling capacity in cream with a 15% fat content were recorded for cocoa fibre, reaching 3.5±0.1 g/g and 2.8±0.1 g/g, respectively. In contrast, the corresponding values for sesame fibre were 1.9±0.1 g/g and 1.1±0.1 g/g. These differences may be attributed to the higher content of insoluble dietary fibre and phenolic compounds in cocoa fibre, which enhance its interaction with the lipid phase.

The effect of cocoa and sesame fibre on the spoilage rate of the lipid dairy mixture during storage at (4±2) °C was investigated. In the control sample, the peroxide value doubled by the 16th day of storage compared with the initial level. In samples containing cocoa or sesame fibre, an antioxidant effect was observed; by the end of the storage period, the peroxide value was 1.05-fold and 1.12-fold lower, respectively, than that of the control.

The influence of the antioxidant dihydroquercetin on changes in peroxide and acid values of the lipid dairy base containing cocoa or sesame fibre during 16 days of storage at (20±2) °C and (4±2) °C was also evaluated. In the control sample, the lipid dairy base supplemented with 0.03% dihydroquercetin exhibited an increase in peroxide value from 0.94 mmol ½ O/kg on day 1 to 1.30 mmol ½ O/kg on day 16, indicating a slow accumulation of primary oxidation products. In experimental samples containing plant ingredients, a slightly lower rate of peroxide value increase was observed. With increasing storage time, the acid value of the lipid–plant mixtures gradually increased, with the most pronounced increase occurring in the control sample.

Conclusions. The incorporation of cocoa or sesame fibre in combination with dihydroquercetin into dairy lipid base mixtures reduces oxidative and hydrolytic deterioration of the lipid fraction during storage.

Introduction

A wide range of natural and synthetic antioxidants is currently available (Singh et al., 2025; Yildiz et al., 2025). Antioxidants are of particular importance in the modern dairy industry, where consumers increasingly demand products with extended shelf life and minimal use of artificial additives (Gubsky et al., 2025). The application of effective natural antioxidants or plant-derived ingredients with antioxidant activity not only enhances product quality but also aligns with market expectations regarding safety and environmental sustainability (Stabnikova et al., 2024).

As reported by Alvarado-Martinez et al. (2020), concerns regarding the potential toxicity of synthetic antioxidants have intensified interest in natural alternatives. Numerous studies confirm that natural antioxidants generally lack carcinogenic and mutagenic properties, making them a safer and more promising option compared with synthetic compounds.

In dairy processing, natural antioxidants are widely used to inhibit lipid oxidation, thereby preserving sensory attributes, extending shelf life, and protecting nutritional value (Abd El-Aziz, 2023; Stobiecka et al., 2022). Enhancement of the endogenous antioxidant system of dairy products, particularly lipid-based matrices, can be achieved through the incorporation of phytochemical compounds such as vitamins C and E, polyphenols, and carotenoids (Christaki et al., 2021; Meléndez-Martínez et al., 2022; Wiley et al., 2024).

Numerous studies have shown that certain natural components, including plants and their processed products, can stabilize fats against oxidation while also exhibiting antimicrobial activity (Abd El-Aziz et al., 2023; Santos et al., 2023). The prevention of oxidative and microbiological spoilage in fat-containing products is achieved through the application of various technological approaches (Stobiecka et al., 2022).

The modern food industry requires the identification of natural components capable of extending shelf life and improving the quality of high-fat dairy products. Among promising natural sources of antioxidants with additional antimicrobial properties are plant-derived ingredients, particularly cocoa fibre and sesame fibre.

Cocoa fibre is free of preservatives and consists of cocoa shell and cocoa nibs ground during the production of cocoa butter or cocoa powder, in a non-alkalized form. It contains 6–7 times more antioxidants and magnesium than most other plant products, while being low in fat. In addition to their functional properties, dietary fibres possess specific technological characteristics, enabling the production of foods with moderately viscous textures. Cocoa fibre is particularly rich in phenolic compounds, including flavanols, procyanidins, chlorogenic acids, and alkaloids (Sánchez et al., 2023). Therefore, cocoa fibre can be regarded as a carbohydrate–protein complex that not only provides valuable nutrients but also exhibits antioxidant activity (Anoraga et al., 2024).

Sesame fiber is a natural product and a source of dietary fiber, micro- and macronutrients, mono- and polyunsaturated fatty acids (Chen et al., 2023). Sesame fiber is a valuable source of natural antioxidants that effectively protect biomolecules from the action of reactive oxygen species and free radicals. The main antioxidant components are phenolic compounds, namely sesamol, sesamin, and sesamolin, which belong to the class of lignans inherent to the seeds of *Sesamum indicum* L. These substances are capable of binding free radicals, inhibiting chain reactions of lipid peroxidation, and stabilizing cell membranes (Lee et al., 2021). Due to the presence of hydroxyl and methoxyl groups, these compounds exhibit high electron-donor capacity, forming stable radical structures that enhance the antioxidant effect. The antioxidant action of sesame fiber is realized through a dual mechanism – physical adsorption of oxidation products and chemical neutralization of free radicals (Park et al.,

2024). Thus, sesame fiber is a promising functional ingredient for improving the oxidative stability of fat-containing food products.

In the food industry, antioxidants with proven efficacy and well-studied effects on various fat bases, including dairy, are widely used. Dihydroquercetin (DHQ), also known as taxifolin (2,3-dihydro-3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-1-benzopyran-4), belongs to the class of bioflavonoids and exhibits a broad range of biological, antioxidant, and vitamin-like activities (Patil et al., 2025). DHQ contains five active hydroxyl groups in its structure and is considered a P-vitamin compound, acting as a catalyst in numerous biochemical processes. Its antioxidant and capillary-protective effects are reported to be 3–5 times greater than those of commonly used preparations (Unver et al., 2024). Dihydroquercetin is a plant extract obtained from the roots of larch and is found in Chinese yew. The substance is a flavonoid and a representative of the P-vitamin group, typically available as a fine white to light-yellow powder (El-Hadad et al., 2020; Muramatsu et al., 2020).

A technology for producing functional soybean yogurt containing blueberries, stevioside, and dihydroquercetin is known. It has been established that dihydroquercetin exhibits the highest antioxidant activity compared to other antioxidants (Bal-Prylypko et al., 2025).

In addition to the effectiveness of the above-mentioned antioxidants, there are technological challenges associated with the use of plant polyphenols in food systems, related to their low solubility in the medium, chemical lability, tendency to polymerization, and loss of biological activity. Furthermore, the ability of plant polyphenols to retain their properties in complex food systems with increased fat content, as well as their impact on technological processes in dairy production, is insufficiently studied. In particular, literature sources contain studies indicating that some polyphenols are capable of inhibiting the development of starter culture microorganisms (Yang et al., 2023). Considering the aforementioned, it is relevant to study the antioxidant effect of plant ingredients on the dynamics of spoilage of milk-based mixtures with increased fat content during storage. A promising solution to this task may be the use of cocoa and sesame fiber in combination with the antioxidant dihydroquercetin as functional food components of the mixtures.

Materials and methods

Materials

The basis for the preparation of experimental samples of lipid milk base mixtures was cream pasteurized at a temperature of (85±2) °C with a holding time of 15-20 s, homogenized at a pressure of 10-15 MPa, which had a liquid, homogeneous consistency without fat lumps or protein flakes; a slightly sweet, clean taste, free from foreign flavors and odors not characteristic of fresh cream, and the following physicochemical indicators: mass fraction of fat 15%, protein – 2.9±1%, carbohydrates – 4.2±0.3%, mineral substances (mg): sodium – 40, potassium – 124, calcium – 90, magnesium – 10, phosphorus – 83, iron – 0.1, carotene – 0.03, vitamins (mg): A – 0.06, B2 – 0.1, PP – 0.2, C – 0.5, titratable acidity 17±1 °T, density 1010 kg/m^3.

Cocoa fiber or sesame fiber was added to the lipid milk base for 3.0% – a rational dosage established by previous physicochemical and organoleptic studies. Mixing was carried out at a temperature of 45 °C followed by pasteurization of the mixture at 85-87 °C (Grek et al., 2025).

To determine the shelf life, samples of the lipid milk base with cocoa or sesame fiber and the natural antioxidant dihydroquercetin were produced. Samples without the addition of plant ingredients, as well as samples with and without the antioxidant, were used as reference (control). According to the manufacturer's recommendations, the amount of dihydroquercetin added to the lipid milk base was 0.03% of the fat mass. Dihydroquercetin was dissolved in cream, after which the resulting solution was added to the container before pasteurization.

Cocoa fiber has the following chemical composition, %: fat – 2.5, protein – 17.0, carbohydrates – 0.7, and salt – 0.1. The main mass consists of 72.0% fiber, including 61.0% insoluble and 11.0% soluble dietary fiber. According to consumer characteristics, the product is a brown powder with a homogeneous structure and an intense chocolate taste and aroma, without foreign flavors (Rojo–Poveda et al., 2020).

Sesame fiber ("Richoil", Ukraine) has the following chemical composition, %: protein – 48.9, carbohydrates – 29.1, fat – 11.2, dietary fiber – 7.8, moisture – not more than 8.

Dihydroquercetin (Bioflavid LLC) was dissolved in distilled water, after which the resulting solution was added to the container before pasteurization.

Methods

Standardized and validated methods for analyzing technological properties and quality indicators were applied during the research process, ensuring the reliability of the obtained results and achievement of the stated objective.

Technological properties of cocoa and sesame fiber

The fat-binding capacity of cocoa or sesame fiber mixed with cream with 15% fat content was determined using the centrifugation method at a temperature of 20±2 °C (Wagner et al., 2024).

The swelling capacity of cocoa or sesame fiber was determined by the gravimetric method, which involves determining the change in mass after immersion in cream with 15% fat content for the corresponding time (Grek et al., 2022). Quantitatively, this indicator is characterized by the swelling degree (K), which reflects the relative increase in the mass of the system. The study was carried out at a temperature of 20±2 °C.

Indicators characterizing oxidation of lipid milk base mixtures with cocoa or sesame fiber and antioxidant

The peroxide value, which characterizes the amount of peroxides and hydroperoxides, was determined by the iodometric method. The principle of the method consists in the release of iodine from potassium iodide under the influence of peroxide compounds of fat. The peroxide value is expressed in millimoles of ½ oxygen per kilogram (mmol ½ O/kg) (Pashaei, 2025).

The acid value was determined by titration. This indicator reflects the concentration of free fatty acids in 1 g of fat and is measured as the volume of 0.1 N KOH solution required for complete neutralization. To calculate the final value of the acid value, the volume of 0.1 N alkali solution used for titration of milk fat was multiplied by a factor of 2 (Pashaei, 2025).

Statistical Analysis

Data were expressed as means±standard deviations for triplicate determination. Statistical analysis was performed using Microsoft Excel 2007.

Results and discussion

Technological properties of cocoa and sesame fiber

At the first stage, the fat-binding capacity and the swelling degree of sesame and cocoa fiber in cream with 15% fat content at a temperature of 20±2 °C were determined. The results of the research are presented in Table 1.

Table 1

Characteristics of cocoa and sesame fibers

Parameters	Type of fiber	
	Cacao	Sesame
Fat-binding capacity of fiber, g/g	3.5±0.1	2.8±0.1
Swelling degree, g/g	1.9±0.1	1.1±0.1

According to the values presented in Table 1, the highest fat-binding capacity in cream with a mass fraction of fat of 15% was recorded for cocoa fibre 3.5±0.1 g/g. At the same time, this sample is characterized by an increased swelling degree 1.9±0.1 g/g. The obtained effect is due to the specific chemical composition of cocoa fibre and is consistent with the data provided by other researchers for similar systems (Delgado-Ospina et al., 2021). For comparison, the fat-binding capacity of sesame fibre is lower by 0.7±0.1 g/g, and the swelling degree is lower by 0.8±0.1 g/g, respectively. In cream with 15% fat content, cocoa fibre better retains fat and stabilizes the emulsion. This is explained by the presence of surface-active compounds (polyphenols and residues of cocoa butter), which promote the formation of a stable "fat-water-solid phase" system (Merachli et al., 2021).

The swelling kinetics of sesame and cocoa fibre were studied at a temperature of 20±2 °C in cream with 15% fat content for 60 minutes (Figure 1).

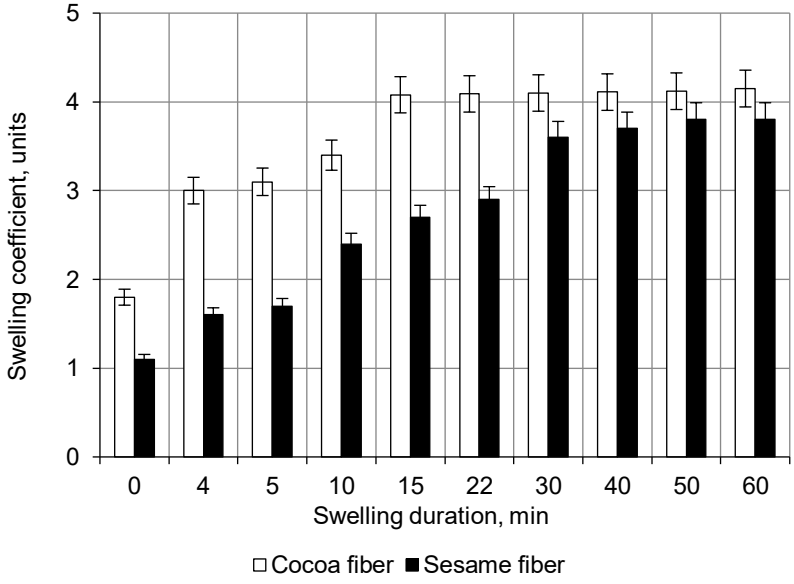

Figure. 1. Swelling kinetics of sesame and cocoa fibre at 20±2 °C in cream with 15% fat content

According to the research results (Figure 1), the most intensive swelling of all fibre types occurs within the first 15 minutes (for cocoa fibre, and 30 minutes for sesame fibre). The maximum swelling coefficient in cream reaches 4.10±0.02 units for cocoa fibre and 3.8±0.01 units for sesame fibre, respectively. The rational swelling duration for all types of fibre ranges from 15 to 30 minutes. An increase in the contact time with cream to 60 minutes leads to a slowdown in the swelling dynamics. Because of the volume increase of fibre during swelling in the spatial network, tension likely arises, which leads to the cessation of swelling (Van de Velde et al., 2024).

Temperature significantly affects the intensity and degree of swelling of plant ingredients in various technological media. The influence of temperature on the swelling degree of cocoa and sesame fibres in cream with a fat mass fraction ranging from 10% to 20% was specified. The highest swelling coefficients were observed at (45±5) °C, reaching 5.41±0.1 units for cocoa fiber and 4.10±0.1 units for sesame fiber, respectively. This is consistent with the findings of Grek et al. (2025).

Changes in milk fat quality during storage were monitored by measuring the amount of hydrolysis and oxidation products. These compounds do not immediately deteriorate organoleptic properties; however, they indicate significant degradation of the studied samples. Thus, measuring peroxide accumulation in the lipid milk base with fibre makes it possible to predict oxidation stability before consumer characteristics deteriorate.

Effect of cocoa and sesame fiber on changes in the peroxide value of milk lipid-based mixtures

An assessment of the influence of cocoa and sesame fibre on the spoilage rate of lipid milk base mixtures was conducted. The experimental samples were stored at a temperature of (4±2) °C. The effect of plant ingredients on the peroxide value of lipid–milk base mixtures during storage at (4±2) °C is shown in Figure 2.

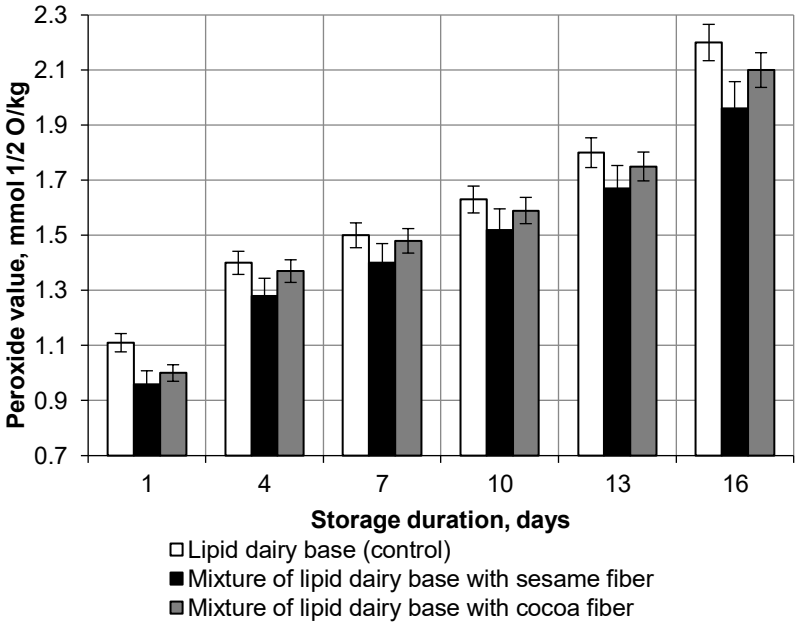

Figure 2. Influence of cocoa and sesame fibre on peroxide value of lipid–milk base mixtures during storage at (4±2) °C

According to the research results, the peroxide value in the control sample increased continuously throughout the storage period, doubling by the 16th day compared to its initial level. The sample with cocoa fibre demonstrates a noticeable antioxidant effect. By the end of the storage period, its peroxide value was 1.05 times lower than the control, confirming the antioxidant effect. This result indicates moderate effectiveness in inhibiting primary oxidation. These observations are consistent with the findings of Soares and Oliveira (2022) and Benítez et al. (2023), which showed that cocoa by-products, due to their high fibre and polyphenol content, slow down oxidative processes in lipid systems.

The lipid milk base sample with sesame fibre had a peroxide value 1.12 times lower than the control sample and 1.07 times lower than the sample with cocoa fibre by the end of the storage period. The higher antioxidant activity of sesame fibre (the peroxide value on day 16 is) is explained by several factors. It contains a significant amount of phenolic compounds such as sesamol, sesamin, and sesamolin, which have high antiradical activity and can stabilize free radicals formed during lipid oxidation (Abbas et al., 2022; Morsy et al., 2022). The structural features of sesame fibre, particularly the presence of hydrophilic and hydrophobic fractions, allow it to interact effectively with the lipid phase, providing local inhibition of oxidation processes. Similar results were reported by Morsy et al. (2022), where sesame extracts and by-products effectively reduced primary oxidation indicators in lipid systems. Thus, comparison with literature data confirms that both fibres (cocoa and sesame) exhibit antioxidant activity; however, sesame fibre demonstrates a higher ability to inhibit lipid oxidation due to a combination of high phenolic content, the ability to chelate catalytic metal ions, and effective interaction with the lipid matrix.

The obtained results indicate that the antioxidant potential of plant ingredients is limited, which necessitates the introduction of more effective antioxidants into lipid milk base mixtures with fibre to prevent oxidative processes during storage.

Effect of dihydroquercetin on changes in the peroxide value of milk lipid-based mixtures with cocoa or sesame fiber during storage

At the next stage, the effect of the antioxidant dihydroquercetin, at the manufacturer-recommended concentration, on the spoilage rate of the lipid milk base enriched with cocoa or sesame fibre was studied. The experimental samples were stored at (4±2) °C for 16 days. The results of the study are presented in Figure 3.

By the end of storage, the peroxide values of the lipid milk base mixtures containing plant ingredients and dihydroquercetin (DHQ) slightly decreased compared to the values on the 13th day, likely due to the transformation of primary oxidation products into secondary ones (Li et al., 2025). During this period, the difference between the peroxide values of the control and experimental samples ranged from 0.4% to 1.8%, indicating a slowdown in oxidation processes in the presence of plant components (Oboulbiga et al., 2023; Yin et al., 2024). The more pronounced antioxidant effect of the sample with sesame fibre is explained by the presence of specific lignans such as sesamol, sesamin, and sesamolin, – which have high thermal stability and effectively inhibit both the initiation and propagation of lipid oxidation chain reactions (Oboulbiga et al., 2023; Yin et al., 2024).

Temperature is a key factor influencing the shelf life of lipid milk bases (Khan et al., 2020; Yan et al., 2025). Higher temperatures accelerate oxidative and hydrolytic reactions in milk fat products, while lower temperatures slow these processes. Therefore, it is important to study the oxidation kinetics of lipid milk base mixtures under auto-oxidation conditions, particularly at elevated storage temperatures. To evaluate the effect of dihydroquercetin on the quality of lipid milk base mixtures with different types of fibre, samples were stored in a thermostat at 20±2 °C for 16 days.

The effect of the antioxidant dihydroquercetin on the change in peroxide value of lipid milk base mixtures with plant ingredients during storage at (20±2) °C is presented in Figure 4.

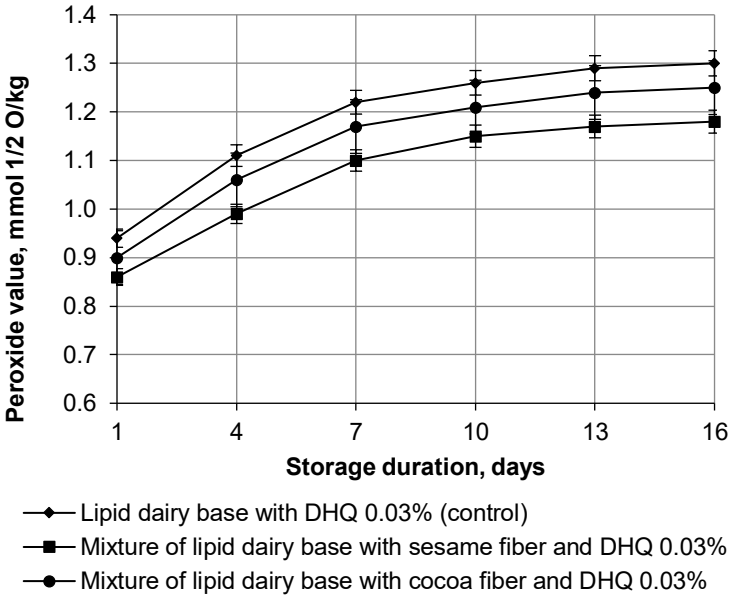

Figure 3. Influence of the antioxidant dihydroquercetin on peroxide value of lipid milk base mixtures with cocoa or sesame fibre during storage at (4±2) °C

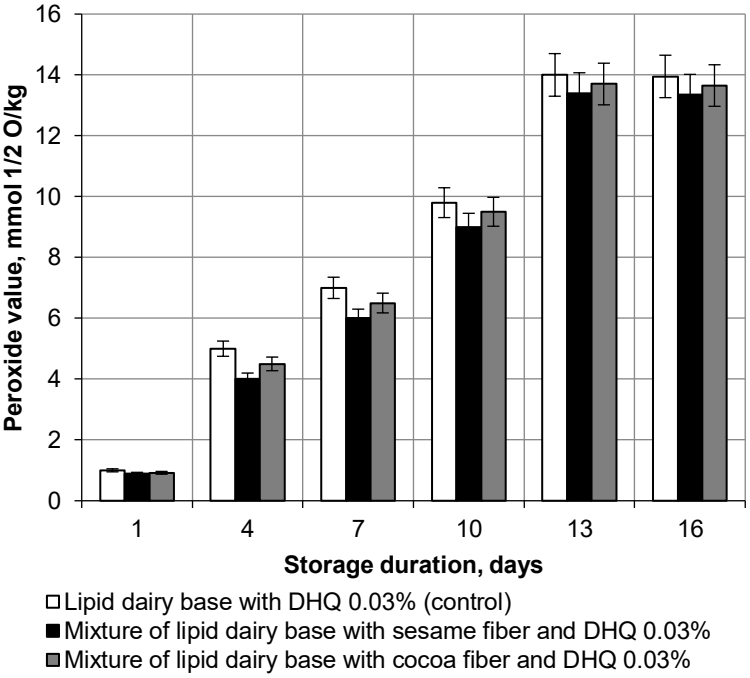

Figure 4. Influence of the antioxidant dihydroquercetin on the peroxide value of lipid milk base mixtures with plant ingredients during storage

The obtained data indicate that a significant increase in the peroxide value of mixtures of the lipid dairy base with plant ingredients and DHA, which occurs at a temperature of (20±2) °C, is consistent with the findings of other researchers. Lu et al. (2019) demonstrated that elevated temperatures significantly accelerate milk fat oxidation, while Yan et al. (2025) showed that substantial oxidation could occur even at moderate temperatures, as early as the 6th day of storage. Data on dry dairy products and pasteurized milk indicate a clear correlation between the accumulation of lipid oxidation products and the deterioration of sensory properties (Ajmal et al., 2018; Clarke et al., 2020). For example, after seven days of storage, the peroxide value of the control lipid dairy base with DHA increased sevenfold, whereas mixtures containing an antioxidant and plant ingredients increased slightly less, by 6.8 times for cocoa fibre and 6.7 times for sesame fibre. This effect is likely due to the phenolic compounds in cocoa and sesame fibres, which can neutralize free radicals and slow primary lipid oxidation (Tušek et al., 2024). Unlike cocoa polyphenols, whose antioxidant activity partially decreases during prolonged contact with oxygen and at elevated temperatures, sesame lignans are characterized by thermal stability and lipophilicity, which ensures their better integration into the fat phase and enhances the efficiency of peroxide formation inhibition (Oboulbiga et al., 2023). Modern studies confirm these mechanisms. In particular, experimental work has shown that sesamol and other lignans improve the oxidative stability of fatty matrices and reduce the formation of volatile oxidation products during heat treatment and storage (Cheng et al., 2024). These observations correspond to the results of studies by Morsy et al. (2022) and Oboulbiga et al. (2023), which additionally report high overall antioxidant activity of sesame by-products and the stability of these compounds in fat systems.

According to the findings by Tušek et al. (2024), the polyphenols contained in cocoa fibre demonstrate high antioxidant activity in aqueous and emulsion systems; however, their effectiveness in purely lipophilic environments may decrease under the influence of oxygen and elevated temperatures due to oxidative degradation and the loss of conjugated structures. At the same time, studies of cocoa fibre indicate their potential as a source of bioactives, but the effectiveness of these components in fatty matrices depends on the extraction method, the polarity of fractions, and application conditions (Braojos et al., 2024). Thus, the highest antioxidant activity during storage of mixtures of the lipid dairy base at a temperature of (20±2) °C is exhibited by the sample with the addition of sesame fibre and DHA. For all samples, on the 10th day of storage, the peroxide value was less than 10 mmol/kg ½ O, which is the maximum permissible level for ensuring storage duration according to the literature (Grek et al., 2021; Ustymenko et al., 2023). The studies showed that all antioxidants of various origins slowed down the oxidation processes in the lipid dairy base.

Effect of dihydroquercetin on the acid value of milk lipid-based mixtures with fiber during storage

During storage, in addition to oxidation products, the content of free fatty acids in the lipid dairy base also increased. The influence of the antioxidant dihydroquercetin on the acid value of mixtures of the lipid dairy base with different types of fibre during storage at (20±2) °C is shown in Figure 5, and at (4±2) °C in Figure 6.

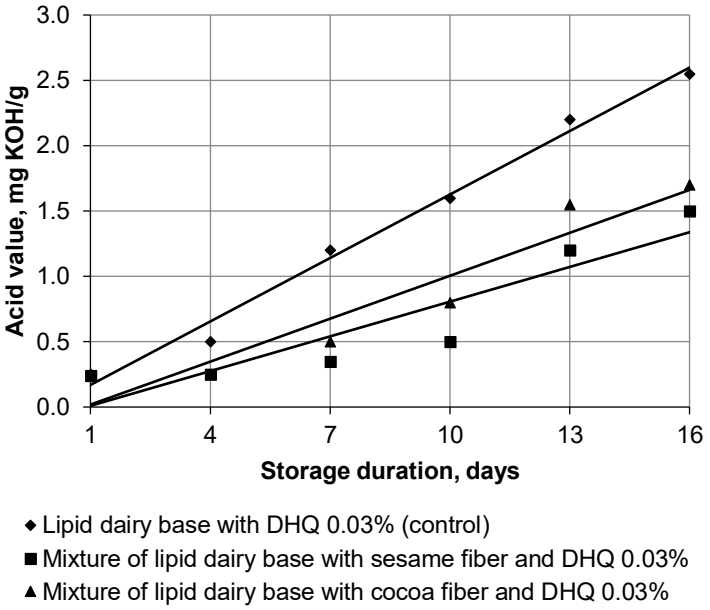

Figure 5. Influence of the antioxidant dihydroquercetin on the acid value of mixtures of the lipid dairy base with cocoa or sesame fibre during storage at (20±2) °C

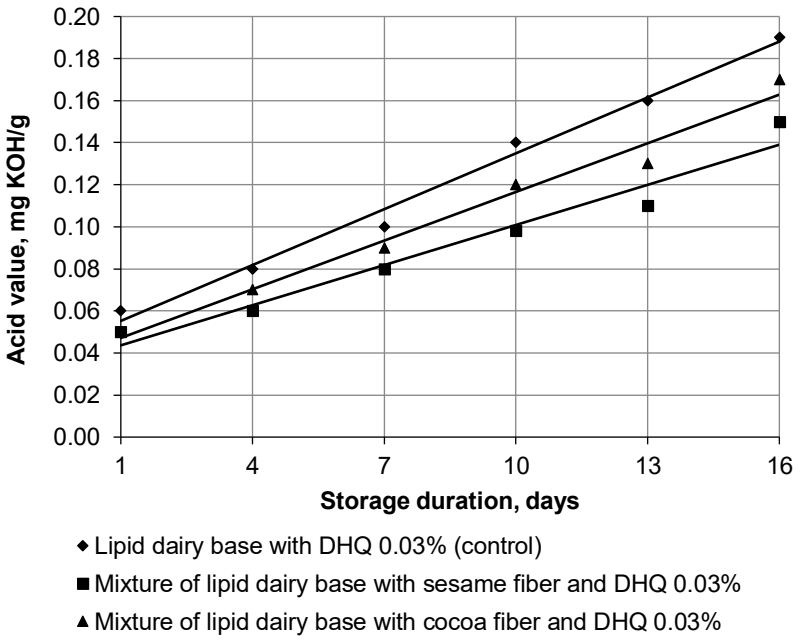

Figure 6. Influence of the antioxidant dihydroquercetin on the acid value of mixtures of the lipid dairy base with cocoa or sesame fibre during storage at (4±2) °C

According to the results presented in Figure 5, the change in the acid value of the lipid dairy base after 4 days of storage at (20±2) °C was insignificant. With an increase in the storage duration of the mixtures of the lipid dairy base, the acid value gradually increased, and the increase was most intensive in the control.

The increase in acid value on the 10th day of storage at (20±2) °C was noticeable only for the lipid dairy base with the antioxidant dihydroquercetin (6.4-fold), whereas for the mixture of the lipid dairy base with cocoa fibre and DHA the amount of free fatty acids increased 3.6-fold, and for the mixture with sesame fibre and DHA – 2.4-fold. After 13 days of storage, a significant increase in the acid value was recorded in the samples of the lipid dairy base with DHA – 8.8-fold, and in samples with added cocoa fibre and sesame fibre – 6.2-fold and 4.8-fold, respectively. As for the samples of mixtures of the lipid dairy base stored at (4±2) °C (Fig. 6), a similar trend toward increasing acid value is observed, comparable to mixtures stored at (20±2) °C.

Based on the results of the conducted studies, the developed mixtures of the lipid dairy base with cocoa or sesame fibre and the antioxidant dihydroquercetin maintain the stability of the fat phase (without significant oxidation) for no more than 13 days at a temperature of (4±2) °C. Thus, the antioxidant effectiveness of plant ingredients is determined by their ability to interact with peroxide radicals in the dairy lipid base. This interaction makes it possible to slow down or completely prevent further formation and accumulation of secondary oxidation products when dihydroquercetin is added.

Conclusions

The study demonstrates that cocoa and sesame fibre, alone or in combination with the antioxidant dihydroquercetin, effectively enhance the stability of lipid dairy base mixtures during storage. These plant fibres exhibit antioxidant and functional properties that reduce lipid oxidation and hydrolytic changes, while also contributing to the technological characteristics of the product. The combined use of fibre and dihydroquercetin produces a synergistic effect, improving oxidative stability and extending the shelf life of fat-containing dairy systems.

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Influence of YoFlex® Premium 5 starter culture on fermentation process and yogurt quality

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Abstract

Introduction. The aim of this study was to investigate the multifunctional properties of the novel yogurt starter culture YoFlex® Premium 5 for its potential application in the production of yogurts with extended shelf life.

Materials and methods. Milk bases were fermented using the yogurt starter cultures YC-X16 and YoFlex® Premium 5. Sample viscosity was measured using a Kinexus Pro+ rotational rheometer; the degree of structural recovery was determined by a calculation method; active acidity was assessed by the potentiometric method; and sensoryeptic properties were evaluated by sensory profiling.

Results and discussion. The YoFlex® Premium 5 yogurt starter culture, compared to YC-X16, halves fermentation time without a pronounced lag phase and fully stabilizes yogurt acidity during the first 7 days of storage. Beyond this period, pH changes become more pronounced, indicating the need for targeted technological measures to limit acidification of the milk-based medium. Yogurt produced with YoFlex® Premium 5 approaches the effective viscosity of the most structured yogurt obtained using YC-X16 on a milk base fortified with 1% skimmed milk powder. Exopolysaccharides produced by YoFlex® Premium 5 not only increase yogurt viscosity but also markedly enhance its thixotropic properties and moisture retention, contributing to a smoother texture and improved mouthfeel. Specifically, the degree of structural recovery of disrupted clots rises from 18.6% in the control and 30% in the YC-X16 + 1% skimmed milk powder sample to 42% in the YoFlex® Premium 5 sample, demonstrating its superior gel-forming ability. Therefore, combining the structuring effect of skimmed milk powder with the thixotropic benefits of exopolysaccharides from YoFlex® Premium 5 is recommended, particularly for stirred yogurt production, where consistency and stability are critical. Furthermore, achieving excellent quality in fresh yogurts fermented with YoFlex® Premium 5 requires additional stabilization during storage for up to 14 days or longer. The use of protective cultures may help maintain yogurt quality over extended storage, representing a promising direction for future research. These findings highlight the potential of YoFlex® Premium 5 to improve both the technological efficiency and sensory attributes of yogurt, making it a valuable tool for industrial applications.

Conclusions. The application of the YoFlex® Premium 5 starter culture in yogurt production shortens fermentation time, improves viscosity and thixotropic properties, and yields an excellent sensory profile, but requires additional measures to stabilize yogurt quality during storage.

Introduction

Yogurt is a widely consumed fermented milk product with proven functional effects on human health (Munteanu-Ichim et al., 2024). Preventing the growth of undesirable microorganisms is important for ensuring yogurt quality and safety and for extending its shelf life (Wang et al., 2025). Although yogurt is generally considered microbiologically stable (Garnier et al., 2017), there has recently been growing interest in its biological preservation using selected microorganisms and their metabolites (Chaves et al., 2021; Siedler et al., 2020). These approaches act mainly through pH reduction, competition for nutrients, and the production of bacteriocins. The use of probiotic lactic acid bacteria strains helps maintain fermentation stability and improves the functional properties of the final product (Nielsen et al., 2021; Prócel et al., 2025; Souza et al., 2023; Stabnikov et al., 2025).

Lactic acid bacteria can perform several additional technological functions in yogurt production. In particular, a significant increase in yogurt viscosity was observed when using Chr. Hansen YoFlex® Premium 1 cultures compared with YF-L901 and YF-L701 cultures, including under conditions with the addition of 1–4% nonfat dry milk (Wang et al., 2023). The possibility of reducing the required amount of nonfat dry milk by 1–2% has been demonstrated due to the structuring effect of exopolysaccharides (EPS) produced as metabolites of bacterial starter cultures (Chaharovskyi and Lukashchuk, 2024). EPS have been shown to improve the sensory properties and stability of yogurt, even at low concentrations (Buldo et al., 2016). Their ability to interact with water molecules and milk proteins enhances the viscosity and mechanical strength of the acid gel while reducing syneresis. In particular, co-fermentation of commercial starter cultures with the probiotic EPS-producing strain *Lactiplantibacillus plantarum* MC5 in compound starter systems positively affected the texture, rheological behavior, and storage stability of yogurt (Zhao et al., 2022). The specific technological functionality of EPS is largely determined by the structure and molecular weight of the biopolymer macromolecules. These molecular differences govern the specific interactions between exopolysaccharides and the protein matrix, resulting in increased viscosity and/or elasticity (thixotropic behavior) of yogurt (Brüls et al., 2023). The use of EPS-producing strains in low-fat and non-fat yogurt has been shown to reduce the need for stabilizers and to minimize post-fermentation mechanical stress on the curd when skim milk powder is incorporated (Zhang et al., 2016). The unique technological role of EPS depends on the structure and molecular weight of the biopolymer macromolecules. These molecular-level differences determine the specificity of EPS interactions with the protein matrix, which can result in increased viscosity and/or elasticity (thixotropy) of yogurt (Brüls et al., 2023). It has been proven that the use of EPS-producing strains in non-fat and low-fat yogurts reduces the need for stabilizers and minimizes the post-fermentation mechanical impact on the curd due to the addition of skim milk powder (Zhang et al., 2016). Therefore, the selection of EPS strains of lactic acid bacteria for use in yogurt production should be based on a preliminary study of the specifics of the influence of each commercial starter culture on the characteristics of the finished product. EPS production, dynamics of acidity changes, consistency and stability of yogurt during storage significantly depend on the bacterial composition of the starter, in particular, on the ratio between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Ivashchenko et al., 2023). A high proportion of *Streptococcus thermophilus* increases the abundance of EPS-producing strains, improves curd structure, but may alter flavor and increase acidity (Lange et al., 2020). Therefore, it is recommended for yogurt culture developers to test the specified ratio in the range of 1:1 to 1:4 by evaluating the fermentation profile and sensory parameters of the yogurt (Dan et al., 2023). EPS also have bioactive properties, including prebiotic,

immunomodulatory, and antioxidant properties (Wang et al., 2025). Thus, research and innovation in the biosynthesis and application of EPS contribute to the further development of functional dairy product technologies (Ouarabi et al., 2025).

A new generation of yogurt starter cultures has recently emerged, including YoFlex® Premium 5 (Chr. Hansen). This culture can increase yogurt viscosity even at lower protein levels and reduce the need for stabilizers and skimmed milk powder. It also exhibits reduced post-acidification and positively affects product quality during storage. However, the specific functional and technological properties of YoFlex® Premium 5 require further detailed and comprehensive investigation, which underlines the relevance of the present study.

Materials and methods

Materials

Raw milk that meets regulatory requirements was used to produce the yogurt test samples. As a source of dry nonfat milk solids, skimmed milk powder was used with a fat content of 1.5% and a protein content of 32% (Rybak et al., 2014). Standardized milk samples were fermented by technologically efficient direct vat set cultures from Chr. Hansen: (a) YC-X16 – classic starter culture (*Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus*); (b) YoFlex® Premium 5 – new culture that produces EPS during fermentation (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*).

Preparation of test samples

Milk mixtures were standardized for fat content (2.5%) and nonfat dry milk solids (NFMS) content (9.5%), were pasteurized and cooled to a temperature of 40-43 °C and were inoculated by direct vat set cultures. Three samples with YC-X16 culture were additionally fortified with skim milk powder (SMP) in an amount of 0.5 to 1.0%. Fermentation of milk mixtures was carried out at the specified temperature until the acidity values reached not higher than pH 4.8.

The designations of samples are given below:

- control (YC-X16);
- sample 1 (YC-X16 with 0.5% SMP);
- sample 2 (YC-X16 with 0.75% SMP);
- sample 3 (YC-X16 with 1.0% SMP);
- sample 4 (YoFlex® Premium 5).

Research procedure

The samples were prepared according to the technological scheme of stirred yogurt production. The pH of samples after inoculation of yogurt cultures was determined every 2 hours of the fermentation process for 8 hours. After fermentation, the samples were stirred, cooled to a temperature of 4±2 ºC, and kept under these conditions for at least 6 hours. After that, the pH of the samples and syneresis were determined and these indicators continued to be recorded during yogurt storage at the specified temperature conditions after 7, 14, and 28 days. The effective viscosity and thixotropic ability of the samples were determined in samples on the 14th day of storage, in accordance with the usual yogurt shelf life. The quality

level of the samples according to the complex of sensory indicators was determined in fresh yogurt samples and samples after 14-day storage.

Methods

Active acidity was assessed by the potentiometric method by lab pH-meter pH/mV/Temp Knick Portamess® 911.

The viscosity of yogurt samples was measured on the 14th day of storage at 4±2 °C. Measurements were carried out using a Kinexus Pro+ rotational rheometer (Malvern Instruments Ltd, United Kingdom) with top geometry C25 DIN L0142 SS (cylinder) and bottom geometry PC25 DIN C0350 AL. Before measurement, samples were gently stirred for 30 seconds and brought to a temperature of 10 °C. The effective viscosity of the yogurt samples was determined during both forward and reverse shear sweeps, with the shear rate increasing from 0.1 to 400 s^{-1} and then decreasing from 400 to 0.1 s^{-1}.

The degree of restoration of the structure of yogurt samples was calculated from the value of the effective viscosity at the end of the measurement at a shear rate gradient of γ = 0.1 s^{-1} (reverse stroke), taking the effective viscosity of the practically undestroyed structure at the beginning of the measurement as 100% (γ = 0.1 s^{-1}) (Liang et al., 2022).

Syneresis of yogurt samples was calculated using a modified method described by Arab et al. (2023). For this purpose, yogurt samples cooled to 4 °C were placed in graduated centrifuge tubes and centrifuged at 350×g (model K-24; Sigma Laborzentrifugen GmbH, Germany) for 10 minutes. The clear supernatant was collected and weighed. The syneresis of the yogurt samples was calculated using the equation:

$$\text{Syneresis}(\%) = \frac{\text{weight of supernatant (g)}}{\text{weight of yogurt sample (g)}} \times 100\%$$

Sensory properties of yogurt samples were assessed by sensory profiling on a twenty-five-point descriptive scale according to method (ISO 13299:2016) for sensory attributes: appearance, texture, color, taste, and flavor. The scores obtained for each of the 5 characteristics were recalculated taking into account the weighting factors: taste – 0.3, texture – 0.3, flavor – 0.2, appearance – 0.1, color – 0.1. The quality level of each yogurt sample was assessed according to the following differentiation: 21-25 points excellent; 16-20.9 points – good; 11-15.9 points – satisfactory; 6-10.9 points – poor (practically unacceptable); less than 6 points – very poor (unacceptable).

Statistical analysis

The studies were performed in 3 or more replicates, and the results were expressed as mean±standard deviation. Data were analyzed using one-way analysis of variance (ANOVA) and Tukey HSD tests using the SPSS statistical package. Statistical difference was defined at $p \leq 0.05$.

Results and discussion

Direct vat set cultures activity during milk samples fermentation

The changes in the active acidity of samples during fermentation lasting up to 8 hours is shown in Fig. 1. The end of the fermentation process was recorded by the time of reaching the active acidity pH = 4.8.

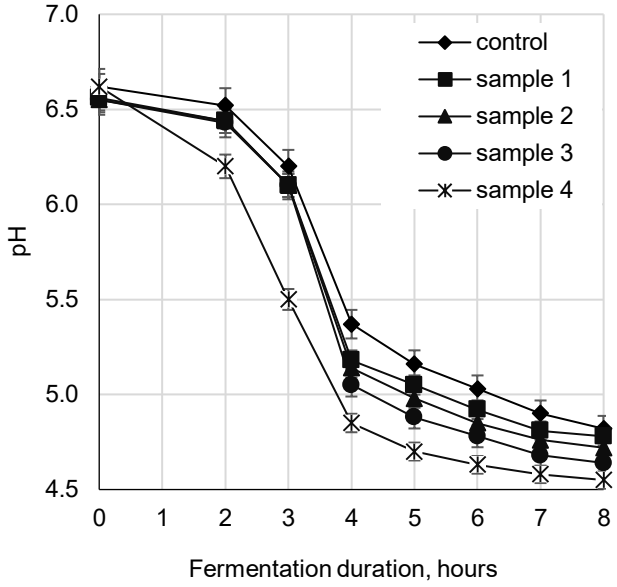

Figure 1. Changes in active acidity during fermentation of yogurt samples

Although all samples had nearly identical initial active acidity (Fig. 1), the pattern of pH changes during fermentation differed. In particular, the control sample with the YC-X16 culture exhibited the longest lag phase and a relatively moderate decrease in pH, reaching a value of 4.8 only at the 8th hour of fermentation. In contrast, sample 4 with the YoFlex® Premium 5 starter culture showed almost no lag phase, reaching a pH of approximately 4.8 by the 4th hour, indicating the high activity and technological advantage of this new culture.

Samples 1-3, containing skimmed milk powder, are close to the control sample in terms of the change in active acidity at the beginning of fermentation, but over time, they show an intermediate value between the control sample and sample 4, and their fermentation process takes place within 6-7 hours. The dynamics of fermentation of samples 1-3 becomes more noticeable with an increase in the content of skimmed milk powder in them from 0.5 to 1.0%, which changes not only the texture, but also affects the kinetics of fermentation. This effect can be explained by a change in the composition, buffer capacity and nutritional content of the medium (Honesová et al., 2024, Karam et al., 2013).

Changes in the active acidity of yogurt samples were also monitored during storage for up to 28 days (Fig. 2).

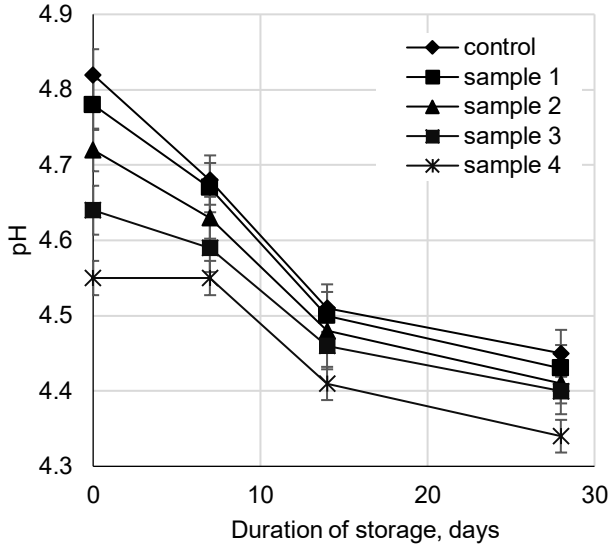

Figure 2. Changes in active acidity during storage of yogurt samples

After fermentation, post-acidification during storage was more pronounced in the control sample and less in samples 1 and 2, due to the slower accumulation of lactic acid. Active acidity decreased by 0.3 (control), 0.28 (sample 1), and 0.24 (sample 2) pH units during the first 14 days of storage. Continued storage from day 14 to 28 showed a slowdown in the decline of lactic acid bacteria activity, likely due to lactic acid accumulation, which increases intracellular acidity (Béal et al., 1999).

Sample 3 exhibited three stages of pH change: during the first 7 days, acid formation was somewhat inhibited; from day 7 to 14, lactic acid accumulated more actively; and from day 14 to 28, this process slowed again. In contrast, sample 4, with the highly active YoFlex® Premium 5 starter, showed complete stabilization of yogurt acidity during the first 7 days, after which the pH trend resembled that of sample 3. This indicates that this starter requires a modified technological approach to limit acidification during storage beyond 7 days, for example, by reducing the content of available substrates (Deshwal et al., 2021). Nevertheless, the active acidity of all samples remained within the acceptable range for yogurt ($4.0 \leq pH \leq 4.5$) throughout storage (Kang et al., 2019).

Viscosity and syneresis of yogurt samples

Changes in the effective viscosity of yogurt samples, measured by rotational viscometry with logarithmic scales on both axes, are shown in Fig. 3. Measurements were performed in both forward ($0.1\ s^{-1}$ to $400\ s^{-1}$) and reverse ($400\ s^{-1}$ to $0.1\ s^{-1}$) shear rates to provide a comprehensive assessment of the samples' rheological properties.

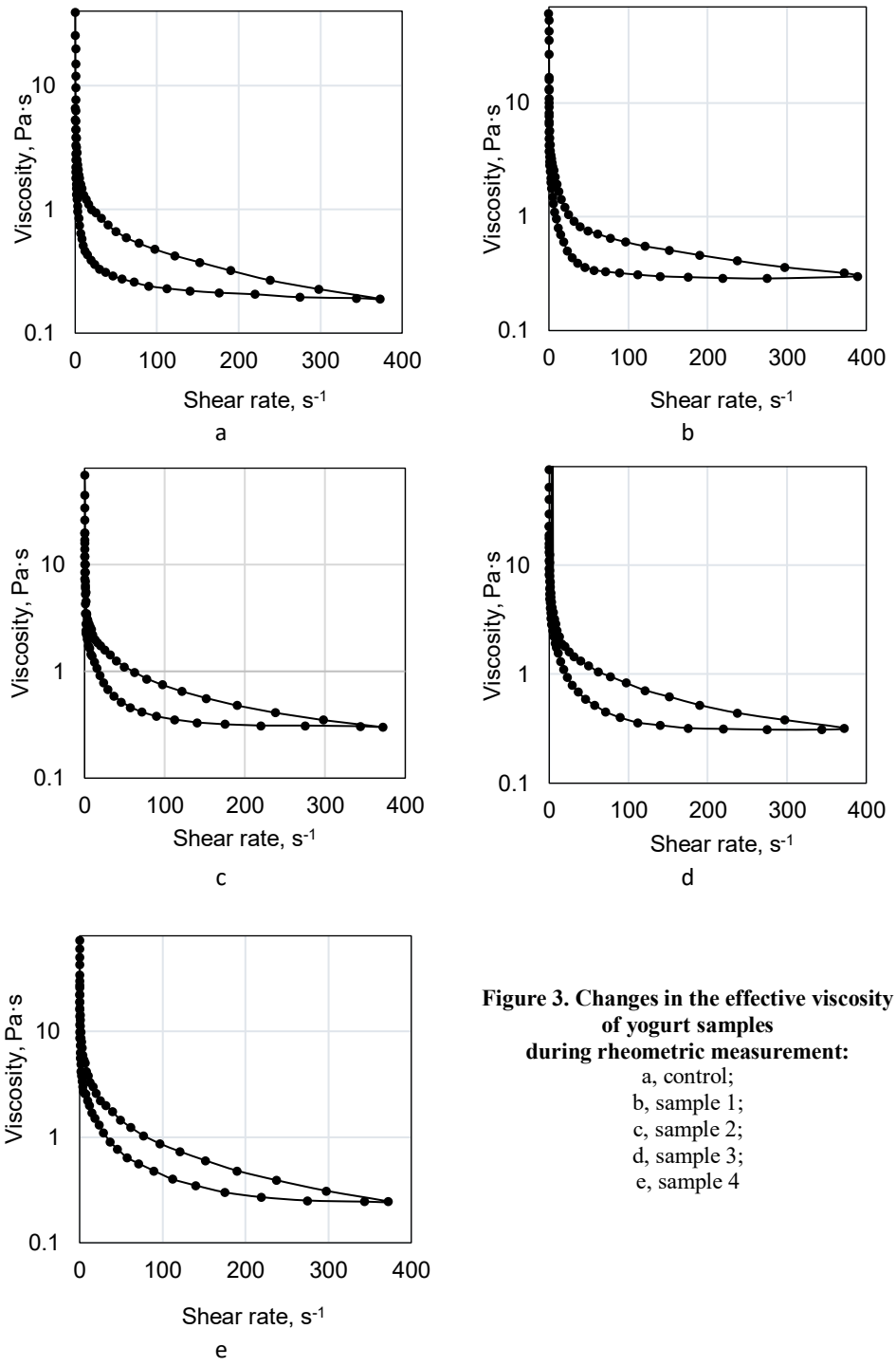

Figure 3. Changes in the effective viscosity of yogurt samples during rheometric measurement:
a, control;
b, sample 1;
c, sample 2;
d, sample 3;
e, sample 4

From Figure 3, it is obvious that the significant effect of skimmed milk powder on the effective viscosity of yogurt samples with a practically undestroyed texture at a shear rate of γ=0.1 s^{-1} is observed. Compared with the control (η=35.1132 Pa·s), the effective viscosity of samples 1-3 increased by 1.7-2.1 times with an increase in the content of skimmed milk powder from 0.5 to 1.0%**.** This effect can be explained by the increase in protein content in the milk base, which strengthens the protein matrix of the curd (Isleten et al., 2006) and, consequently, increases the viscosity of the yogurt. Sample 4 shows slightly lower, but similar, viscosity characteristics to sample 3. It can be assumed that this is a consequence of the lower bond energy formed by EPS compared to milk proteins.

While casein gels at pH ≈ 4.6 form energetically stable and practically irreversible clots, with hydrophobic interactions between proteins estimated in the range of 30–80 kJ/mol (De Kruif, 2012), polysaccharide gels, EPS systems, and protein–polysaccharide complexes are dominated by lower-energy hydrogen and hydration bonds (Everett et al., 2005). As a result, the most structured texture was observed in sample 3, which contained 1% skimmed milk powder. Sample 4, produced by fermenting milk with the YoFlex® Premium 5 culture, exhibited a similar textural characteristic.

To study the thixotropic behavior of the yogurt samples, the degree of recovery of the nearly destroyed structure at γ = 400 s^{-1} was calculated during the reverse measurement at a minimum shear rate of γ = 0.1 s^{-1}, and compared with the initial values of effective viscosity (Fig. 4).

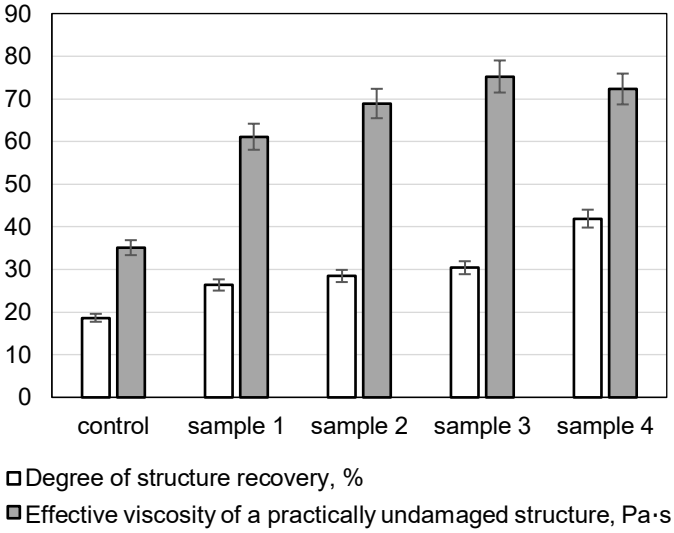

Figure 4. Comparative assessment of the initial values of effective viscosity and thixotropic ability of yogurt samples

As shown in Fig. 4, samples 1–3 containing skimmed milk powder exhibited higher textural integrity and a greater ability to recover the structure at reduced shear rates compared with the control. Data on the effect of skimmed milk powder on yogurt thixotropy are limited, but it is known that increasing the content of nonfat milk solids (NFMS) enhances the viscosity modulus and strength of the protein gel (Honesová et al., 2024; Karam et al., 2013), thereby improving its recovery ability.

As for sample 4, obtained by fermentation of the YoFlex® Premium5 culture, it should be noted its slightly lower effective viscosity at the beginning of the rheometric measurement, compared to sample 3 (72.3105 versus 75.2701 Pa·s). However, the thixotropic ability of sample 4 significantly exceeds the degree of recovery of the effective viscosity of the destroyed texture of all other samples and reaches a rather high value – 41.92%. It can be assumed that EPS form additional low-energy bonds in the structure of protein gel, which have the ability to restore the formed matrix more quickly after destruction (Akin et al., 2009). A similar effect was observed by other scientists (Jurášková et al., 2022; Zheng et al., 2013), who found the formation of a more elastic and rapidly recovering protein gel in the presence of EPS, compared to the protein clot as a control.

In the information environment, there is no clear and unambiguous classification of yogurts according to the levels of "low / medium / high thixotropy" (i.e., by threshold values). Some studies indicate that after a strong shear, the restoration of the structure of yogurts is possible in the range of 20-30% to 40-50% (Chen et al., 2024; Lee et al., 2010). Therefore, it was decided to apply the following criteria to assess the level of thixotropic ability of yogurt by the degree of structure restoration:

- more than 70% - high thixotropy (the texture is almost completely restored after destruction);
- 30-70% - medium thixotropy (partial restoration of the texture);
- less than 30% - low thixotropy (the system is almost not restored).

According to the proposed classification, only samples 3 and 4 show average thixotropy due to the increased content (1%) of skimmed milk powder and in the presence of EPS produced by the YoFlex® Premium 5 culture.

Thus, milk proteins in the composition of skimmed milk powder show high texturing ability, and EPS more effectively improve the thixotropic properties of the gel. Therefore, it is possible to predict a possible synergistic interaction between skimmed milk powder and EPS to combine their positive effects on the textural and mechanical characteristics of the yogurt, in particular to increase its thixotropic ability. This assumption will be tested in further studies.

The synergetic capacity of yogurt samples during storage was also determined (table), taking into account the possibility of modification of the yogurt microstructure by exopolysaccharides produced by lactic acid bacteria (Arab et al., 2023).

Table Syneresis of yogurt samples during storage, %

Sample	Storage duration, days			
	0	7	14	28
Control	$15.90^{a}\pm0.71$	$14.36^{a}\pm0.68$	$14.21^{a}\pm0.60$	$14.32^{a}\pm0.68$
Sample 1	$14.96^{ab}\pm0.52$	$13.73^{ab}\pm0.57$	$13.11^{ab}\pm0.55$	$12.99^{ab}\pm0.65$
Sample 2	$12.83^{b}\pm0.61$	$11.29^{b}\pm0.48$	$10.69^{b}\pm0.49$	$11.26^{b}\pm0.43$
Sample 3	$9.82^{c}\pm0.41$	$9.19^{c}\pm0.44$	$9.22^{c}\pm0.42$	$9.31^{c}\pm0.40$
Sample 4	$9.01^{cd}\pm0.46$	$8.36^{cd}\pm0.39$	$8.27^{d}\pm0.38$	$8.11^{d}\pm0.32$

Note: Values are the means±standard deviation. Means within the same column with different superscripts are significantly different at $p \leq 0.05$

Syneresis was highest in all fresh yogurt samples, which can be attributed to the large number of temporarily formed low-energy bonds capable of causing substantial reconfigurations in the protein gel structure (Brüls et al., 2024). Syneresis decreased during

storage, but the variability was minimal, and no clear trends were observed. Therefore, further studies of this parameter will be conducted on a larger set of fermented samples, including those combined with skimmed milk powder (SMP).

It should also be noted that no statistical differences ($p > 0.05$) were observed for the control sample and sample 1 throughout the storage period, indicating that 0.5% SMP had little effect on yogurt syneresis. In contrast, sample 4, fermented with the YoFlex® Premium 5 culture, showed the lowest whey separation, reflecting the high moisture-holding capacity of EPS and associated modifications in the yogurt microstructure (Han et al., 2026). These results confirm the technological effectiveness of the YoFlex® Premium 5 starter culture.

Sensory evaluation of yogurt samples

The results of the sensory evaluation of yogurt samples, carried out by sensory profiling according to the main sensory attributes (appearance, texture, color, taste, and flavor) with subsequent calculation of the total weighted score taking into account the weighting coefficients, are shown in Fig. 5.

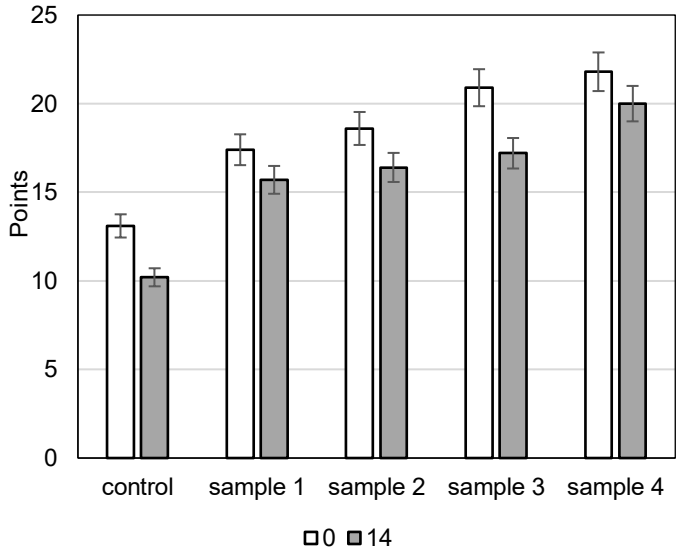

Figure 5. Sensory evaluation of yogurt samples according to a set of quality indicators immediately after production and after 14 days of storage

Based on the data obtained, fresh samples 3 and 4 exhibited excellent quality. During storage up to 14 days, some sensory characteristics (taste, flavor) require improvement, which remains an important task for further research.

Overall, considering the evaluated quality indicators, samples 3 and 4 showed the highest performance, highlighting the technological impact of 1% skimmed milk powder (sample 3) and EPS produced by the YoFlex® Premium 5 culture (sample 4). The use of this new starter culture allows a significant reduction in fermentation time and prevents post-acidification during the first 7 days of storage. Skimmed milk powder effectively structures the yogurt gel but increases production costs compared with yogurt made using YoFlex®

Premium 5. The thixotropic ability of yogurt containing 1% skimmed milk powder (lower dosages are ineffective) was rated as moderate to low, whereas the use of YoFlex® Premium 5 increased the degree of gel recovery to 42%. Therefore, a rational approach for future research may be to combine the functional and technological benefits of the YoFlex® Premium 5 starter culture with varying amounts of skimmed milk powder.

Conclusions

The YoFlex® Premium 5 yogurt starter culture exhibits higher activity than YC-X16, significantly shortening fermentation without a pronounced lag phase and stabilizing yogurt acidity during the first 7 days of storage. Skimmed milk powder at 1% increases yogurt viscosity through protein matrix formation, while exopolysaccharides improve thixotropy, increasing gel recovery from 30% to 42% and reducing syneresis. Combining YoFlex® Premium 5 with skimmed milk powder may further enhance functional and technological properties. Fresh samples with skimmed milk powder or YoFlex® Premium 5 showed excellent sensory quality, but additional stabilization is needed during storage beyond 14 days. Future research should explore synergies between skimmed milk powder and YoFlex® cultures to achieve high viscosity, thixotropy, and stable quality over extended storage using bioprotective cultures.

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Structural and rheological behaviour of mayonnaise-type emulsions containing aquafaba and functional oil blends

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Abstract

Introduction. This study examined the potential of aquafaba as a plant-based emulsifier in low-fat mayonnaise sauces prepared with blended oils.

Materials and methods. The oil phase was prepared from blends of sunflower and hemp oils, with the addition of black cumin oil. Aquafaba obtained from industrially boiled beans was used as a natural emulsifier. The sauces were characterized through sensory evaluation, structural analysis, and rheological measurements using laser diffraction and viscometry.

Results and discussion. The effect of blended vegetable oils (hemp and black cumin) and aquafaba on the sensory, rheological, and structural properties of mayonnaise-type sauces was examined. Incorporation of blended oils at specific ratios enhanced the flavor profile by lowering acidity and imparting characteristic spicy notes. At hemp oil contents up to 25%, a high degree of homogenization and uniform particle size distribution of the dispersed phase were observed. Higher levels of α-linolenic acid in the oil phase increased emulsion polydispersity and promoted floc formation, as indicated by a higher flocculation index, irregular particle distribution, and greater surface area of the fat phase.

Laser diffraction and microscopy revealed that ultrasonic treatment reduced average droplet size and narrowed size distribution, resulting in greater system homogeneity, as evidenced by lower flocculation index and polydispersity values across all sauces. Rheological analysis using the Ostwald–de Waele and Casson models confirmed pseudoplastic behavior in all systems and demonstrated that rheological properties were dependent on the composition of the oil phase.

Sauces containing more than 75% sunflower oil exhibited similar rheological characteristics. Viscosity and ultimate shear stress increased with higher hemp oil content, while greater flocculation reduced structural compactness and stability. The sauce with the highest hemp oil content demonstrated pronounced thixotropy and a high structural recovery coefficient.

Conclusions. Incorporating aquafaba with blended oils makes it possible to create stable emulsions that not only remain consistent over time but also demonstrate enhanced functional and technological qualities.

Introduction

Food emulsions play a significant role in the food industry. One of the most popular emulsions is mayonnaise, a widely used sauce in various culinary applications. In traditional formulations, emulsifiers are primarily animal-derived proteins (Urbánková et al., 2019; Vathsala et al., 2023), along with plant-based stabilizers and thickeners, including gums, starch, and pectin. The use of mixed ionic and non-ionic surfactants as emulsifiers has also been reported (Mikulcová et al., 2017). However, the increasing demand for plant-based mayonnaise alternatives has encouraged the substitution of animal ingredients with plant-derived components. Such reformulations not only lower the caloric content (Huang et al., 2022; Stabnikova & Paredes-López, 2024) and reduce product allergenicity (Gómez-Candela et al., 2011; Sarkar et al., 2020), but also enhance microbiological safety. Plant-based emulsifiers that may be employed include sucrose palmitate (Jufret et al., 2022), quinoa and amaranth proteins (Gürbüz et al., 2018), tea saponins (Usman et al., 2025), sesame protein isolate (Niazi et al., 2025), a range of unconventional plant proteins isolated from green biomass, leaves, and grasses (Gubsky et al., 2025), as well as aquafaba proteins (Sachko et al., 2023).

Utilizing by-products from plant processing to improve food quality is a sustainable strategy for converting agricultural residues into value-added products (Mironeasa et al., 2024; Stabnikova et al., 2023a, b). For example, aquafaba, the liquid obtained from cooked chickpeas, can be repurposed from waste into a functional ingredient owing to its valuable protein and polysaccharide content. The quantitative characteristics of aquafaba are strongly influenced by the legume variety and processing conditions, including cooking time, legume-to-water ratio, temperature, pressure, pH, and salt content (Schmidt et al., 2023).Aquafaba proteins are mainly water-soluble, low-molecular-weight albumins and globulins, accounting for 18–29% on a dry matter basis (DMW), and are characterized by a non-compact structure. Its mineral profile shows relatively high concentrations of potassium (25–37 mg/100 g), copper (0.06–0.17 mg/100 g), and manganese (0.04–0.11 mg/100 g). The carbohydrate fraction consists primarily of monosaccharides such as D-galactose, D-glucose, and L-arabinose, with smaller amounts of D-xylose and D-fructose. The elevated saponin content (4–40 mg/g DMW), plant-derived glycosides with surface-active and foaming abilities, contributes to aquafaba functional properties (Sahin et al., 2024). Owing to its compositional characteristics, aquafaba serves as an effective plant-based emulsifier capable of forming stable emulsions with prolonged storage stability (Raikos et al., 2020; Włodarczyk et al., 2022).

Another aspect of improving the sensory and physicochemical properties of mayonnaises and mayonnaise-based sauces is the use of blends instead of single oils. Firstly, such an approach makes it possible to optimise the fatty acid composition of mayonnaise. To create blends, oils from both traditional and niche crops are used, such as dill, flax, black cumin, arugula, mustard, milk thistle, pumpkin, and sesame, among others (Sharma et al., 2019; Starikov et al., 2023). The incorporation of niche crop oils into blends extends the shelf life of the final product due to the presence of essential compounds such as polyphenols, quinones, flavonoids, alkaloids, and tocopherols, which exhibit antioxidant and antimicrobial activities. The antioxidant activity of many of these compounds exceeds that of synthetic antioxidants. For instance, the antioxidant effect of thymoquinone, which is contained in black cumin oil, ranges from 2.1% to 3.5%, is higher than that of butylated hydroxyanisole (E320) and butylated hydroxytoluene (E321) (Asfaw, 2023; Kmiecik et al., 2025). Secondly, oil blending allows fine-tuning of the physicochemical and sensory characteristics of mayonnaise. Different oils possess unique flavor profiles and aromas. Thus, the use of blends

not only improves nutritional value but also enables the development of innovative products with unique textures and flavor qualities that meet the demands of the modern market.

Encapsulation of oils and their blends into nanoemulsions is a promising strategy both for enhancing oxidative stability (Gürbüz et al., 2018), and for improving the bioavailability of individual components (Bajerski et al., 2016).

The aim of this study was to develop low-fat mayonnaise emulsions based on blends of sunflower, hempseed, and black cumin oils, using aquafaba from canned white beans as the primary emulsifier. The focus was placed on investigating the structural, rheological, and sensory characteristics of the obtained emulsions, which are crucial for understanding their stability and consumer properties.

Materials and methods

Materials

The ingredients used in the formulation of the mayonnaise samples were obtained from the Ukrainian retail network and met the established food quality criteria, confirming their compliance with safety standards for food production.

Mayonnaise samples preparation

The mayonnaise samples were prepared according to the method (Sachko et al., 2023). The formulation of all sauces (per 100 g of product) included the following ingredients: mustard powder – 1.0 g, cream powder (46%) – 1.0 g, sodium chloride – 1.0 g, sucrose – 1.5 g, sodium bicarbonate – 0.05 g, pectin – 0.04 g, xanthan gum – 0.03 g, carboxymethylcellulose – 0.03 g, food-grade vinegar – 8.0 g, concentrated lactic acid – 0.3 g, water – 41.15 g, aquafaba obtained from canned white beans (Veres trademark) – 15.0 g, and vegetable oil – 30.0 g. In the control mayonnaise sample (M0), the oil phase was composed exclusively of refined sunflower oil. Samples M1 and M2 were formulated using blends of sunflower, hempseed, and black cumin oils at ratios of 70:25:5 and 45:50:5, respectively. These formulations provided ω-6:ω-3 fatty acid ratios of 10:1 and 5:1. The composition of the oil blends was calculated according to the method described by Matveeva et al. (2013). The addition of 5% black cumin oil was intended to enhance lipid oxidative stability while maintaining acceptable organoleptic properties of the final product (Khormizi et al., 2019).

Methods

Sensory analysis

The sensory properties of the mayonnaise samples were assessed using a descriptive profiling method on a 0–10 scale, where 0 represented the absence or minimal expression of a characteristic and 10 indicated its maximum intensity. The evaluated attributes included aroma, appearance, texture (consistency), color, taste, aftertaste, pungency (spiciness), and acidity. Each sause was evaluated individually to enable a quantitative description of consumer-relevant characteristics. The evaluations were carried out in a specialized sensory laboratory in accordance with ISO 8589:2007. Mayonnaise samples were presented in numbered disposable containers in random order. The final score for each attribute was calculated as the arithmetic mean of the values assigned by all panel members.

Emulsion sedimentation stability

The sedimentation stability of the emulsion was evaluated using an accelerated centrifugation method. For this purpose, 10 mL sauce of the mayonnaise sample was transfered into centrifuge tubes and subjected to a centrifugal force at 5000 rpm for 5 minutes. After centrifugation, the height of the separated liquid laysers (aqueous and oil fractions) was measured. The sedimentation stability coefficient (S, %) was then calculated using the following equation:

$$S = [(V_e - V_f)/V_e] \cdot 100\%,$$

where V_e is the volume of the undisturbed (or undestroyed) emulsion, and V_f is the volume of the separated aqueous or oil phase.

Determination of particle size distribution

The particle size distribution of the emulsion was measured using a laser diffraction instrument (PCA 1190, Anton Paar, Austria). The emulsion was dilutted with distilled water at a 1:100 ratio at room temperature (~20 °C). Measurements were performed under two conditions: without ultrasonic treatment and with ultrasonic treatment, which was applied for 5 minutes while continuously stirring the sample. Based on the measurements, the following parameters were calculated:

Surface-weighted (Sauter) mean diameter D[3,2]:

$$D[3,2] = \sum_i^N n_i d_i^3 / \sum i^N n_i d_i^2$$

Volume-weighted (De Brouckere) mean diameter D[4,3]:

$$D[4,3] = \sum_i^N n_i d_i^4 / \sum i^N n_i d_i^3$$

Polydispersity of the emulsions (Span):

$$Span = (D_{90} - D_{10})/D_{50},$$

where n_i is the number of droplets with diameter d_i, and D_{10}, D_{50}, and D_{90} are the percentile values indicating the size below which 10%, 50%, or 90% of all particles are found.

The flocculation index was calculated according to the formula:

$$FI= D[4,3])/(D[3,2].$$

Microphotographic analysis

For microphotographic analysis, the mayonnaise samples were diluted with distilled water at a 1:10 ratio. Microphotographs were captured using a Micromed microscope equipped with an integrated Pixelink 1K camera and a 60× objective lens.

Evaluation of rheological properties of emulsions

The rheological properties of the mayonnaise samples were investigated using a rotational viscometer Visco QC 300R (Anton Paar, Austria) at 20 °C. The dependence of shear stress on shear rate in the range of 0.1–100 s was measured using a coaxial cylinder

system (CC12) in accordance with ISO 3219-2:2021 and DIN 53019-1:2008. To determine the yield stress (τ_y), measurements were conducted at a shear rate of 5 s^{-1} using a vane-type spindle, V75.

To describe the rheological behavior of the sauces, the experimental data were approximated using the Ostwald–de Waele (power-law) model, which is commonly applied for food products:

$$\tau = K \cdot \gamma^{n-1},$$

where K is the consistency coefficient, which indicates the system viscosity; *n* is the flow behaviour index,

and the Casson equation

$$\tau^{1/2} = (\tau_c^{1/2} \cdot \gamma^{1/2})/(\chi+\gamma^{1/2}) + \eta_c^{1/2} \cdot \gamma^{1/2},$$

where χ is the compactness coefficient of the structure, $\tau_c^{1/2}$ is the yield stress according to the Casson model, and η_c½ is the plastic viscosity.

The thixotropic behavior of the mayonnaise samples was evaluated using a three-interval thixotropy test (3ITT). The first and third intervals were conducted at a shear rate of 20 s^{-1}, while the second interval was conducted at 200 s^{-1}, with each stage lasting 5 minutes. The Thixotropic Breakdown Coefficient was calculated according to the following equation:

$$T_b = (\eta_1 - \eta_2)/\eta_1,$$

where η_1 is the initial viscosity, and η_2 is the viscosity of the system at the end of the second measurement interval.

The Structural Recovery Coefficient (SRC, %) was calculated using the following equation:

$$SRC = [(\eta_3 - \eta_2)/(\eta_1 - \eta_2)] \cdot 100\%,$$

where η_1 is the maximum viscosity value reached at the end of the first stage; η_2 is the lowest viscosity value recorded at the end of the second stage; and η_3 is the viscosity value measured at the end of the third stage.

The total surface area of the fat phase in the emulsion system (S_{fat}) was be calculated by the following formula:

$$S_{fat}=6V_{fat}/(D[3,2]),$$

where V_{fat} is a fat phase content.

Statistical analysis

A minimum of three parallel measurements was performed for each parameter. The quality of data fitting was assessed using the coefficient of determination (R^2) and the standard error of estimate (σ_{est}). The final results are presented with their corresponding standard deviation (SD). Statistical analysis was conducted using one-way Analysis of Variance (ANOVA), and differences in mean values relative to the control sauce were evaluated using Dunnett's post hoc test (significant differences are indicated by a–b indices next to the reported values).

Results and discussion

Sensory evaluation of mayonnaise samples

The sensory characteristics of mayonnaise samples are a critical determinant of consumer appeal and market success. Therefore, a detailed evaluation of these parameters is a key step in developing and ensuring the quality of novel mayonnaise formulations. The components of the oil blends used, particularly hempseed and black cumin oils, have distinctive aftertastes, requiring careful adjustment of their proportions to achieve a balanced flavour profile without excessive intensity that could be perceived negatively by consumers. Figure 1 presents comprehensive data, including the surface appearance of the mayonnaise samples in consumer packaging, their texture upon extrusion (dispensing from the tube), and the sensory attribute profilogram.

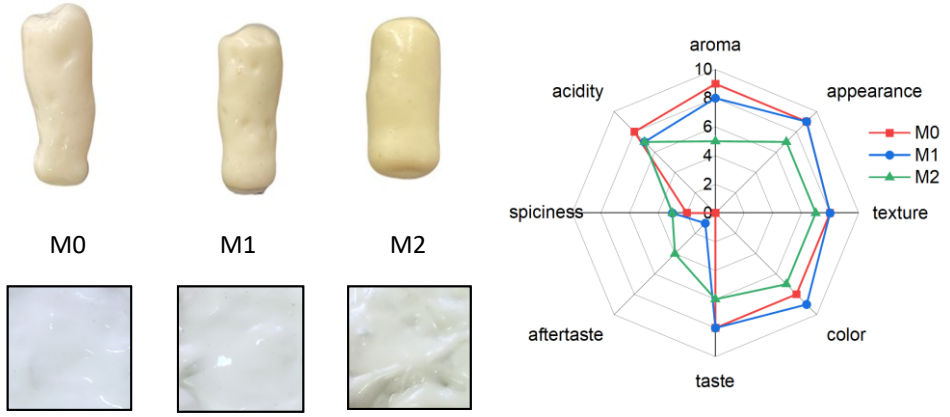

Figure 1. Appearance and sensory profile (profilogram) of the investigated mayonnaise samples:
M0, control with sunflower oil; M1 and M2 with blends of sunflower, hempseed, and black cumin oils at ratios of 70:25:5 and 45:50:5, respectively

Mayonnaise samples M0 and M1 were characterised by a larger curve area compared with M2, indicating more favourable sensory properties. At the same time, the perceived "acidity level" in samples M1 and M2, prepared with blended oils, was lower than in the control sample M0. The pH values of all mayonnaise samples were similar, ranging from 3.58 to 3.64. This effect can be attributed to the distinctive spicy notes of hempseed and black cumin oils, which may partially mask the acidic taste.

These findings are consistent with the literature (Helikh et al., 2021; Kryskova et al., 2023), which reports that the incorporation of hempseed oil up to 50% does not negatively affect sensory characteristics, whereas higher levels (above 75%) significantly reduce product quality. Although aquafaba from canned white beans contains a relatively low protein content (0.36 g/100 g), it was sufficient to form a stable emulsion. Legume-derived aquafaba is known for its high emulsifying activity index (EAI); for example, the EAI of chickpea aquafaba is nearly eight times higher than that of egg yolk (13.75 m^2/g versus 1.78 m^2/g, respectively) (Włodarczyk et al., 2022). The sedimentation stability coefficient

measured on the second day after preparation was 98%. This high stability can be attributed to the presence of heat-soluble hydrophilic proteins in legume aquafaba, which exhibit high thermal stability and strong emulsifying capacity, as well as to the sufficient content of saponins that help prevent emulsion destabilisation (Schmidt et al., 2023). All studied mayonnaises exhibited 99% sedimentation stability after 24 hours. Furthermore, no visual changes or signs of separation were detected in the emulsions after one month of storage.

The stability of food emulsions largely depends on droplet size: the smaller the droplets, the greater the kinetic stability of the emulsion against physical destabilisation phenomena such as creaming, coalescence, and Ostwald ripening. According to Kim et al. (2020), relative stability of food emulsions is typically achieved when the droplet size is below 500 nm. The size of the dispersed phase is strongly influenced by the type and content of oil (Urbánková et al., 2019), the nature and concentration of the emulsifier (Gürbüz et al., 2018), and the technological conditions used for emulsion preparation (Mikulcová et al., 2017).

Gürbüz et al. (2018) reported the formation of coarse rapeseed oil emulsions (5%) stabilised with quinoa and amaranth proteins, exhibiting droplet sizes below 1 μm in all samples. Comparable values (~0.3 μm) were observed for black cumin oil emulsions (5–7.5%) stabilised with sucrose palmitate (Jufri et al., 2022) and for hempseed oil emulsions stabilised with the non-ionic surfactants Span 85/Tween 85 (Mikulcová et al., 2017). The simplest method for confirming flocculation phenomena in such systems is optical microscopy (Fig. 2).

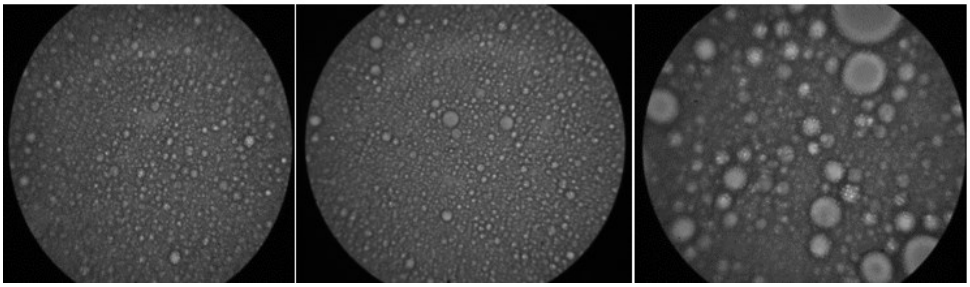

Figure 2. Microphotographs of emulsion samples M0, M1, and M2: M0, control with sunflower oil; M1 and M2 with blends of sunflower, hempseed, and black cumin oils at ratios of 70:25:5 and 45:50:5, respectively

Mayonnaise samples M0 and M1 predominantly consisted of particles of comparable size, although some larger droplets were also visible. In contrast, analysis of the magnified image of sample M2 revealed a noticeably broader particle size distribution, ranging from very fine to considerably larger droplets. A distinct localisation of smaller droplets on the surfaces of larger ones was observed, indicating particle flocculation, which is reflected in the increased D[4,3] value.

To assess the effect of flocculation, the droplet size of the emulsion phase was measured both before and after ultrasonic treatment of the mayonnaise samples (Table 1), while the distribution curves themselves are shown in Fig. 3.

Table 1

Characteristics of particle size distribution in mayonnaise samples

Sauce	Measurement regime	Volume weighted distribution, μm						D[3,2], μm
		D10	D50	D90	D[4,3]	Span	FI	
M0	wUS	1.88	5.33	10.61	6.20	1.64	1.63	3.79
	US	1.74	4.49	8.11	5.04	1.46	1.47	3.42
M1	wUS	1.90	5.56	11.53	6.57	1.73	1.72	3.81
	US	1.72	4.38	8.09	5.01	1.45	1.50	3.35
M2	wUS	1.88	5.11	17.81	8.58	3.11	3.47	2.47
	US	1.53	3.41	7.62	4.36	1.78	1.88	2.31

Note: wUS – without ultrasonic treatment; US – with ultrasonic treatment.

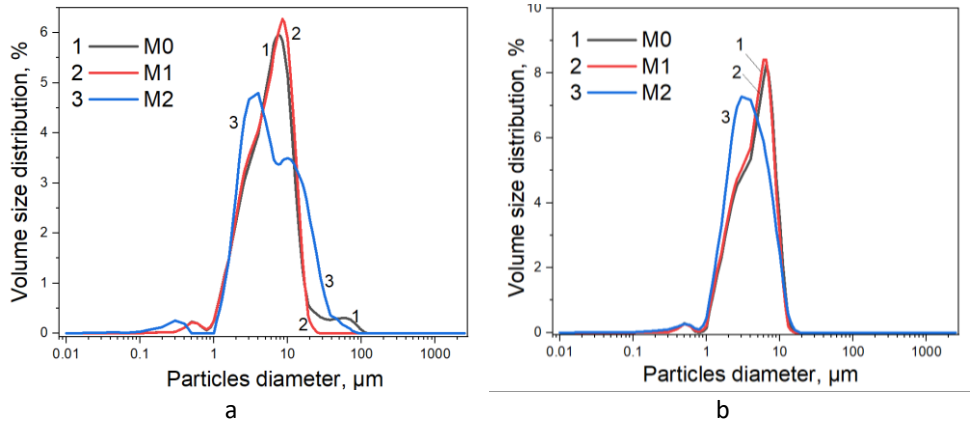

Figure 3. Volume-based particle size distribution of mayonnaise emulsions on the first day after preparation:
(a) without sonication, (b) with sonication

Analysis of the resulting curves showed that sonication caused a shift in the droplet size distribution toward smaller diameters in all samples, while in sample M2 the distribution changed from bimodal to monomodal. These observations confirm the occurrence of flocculation phenomena in the studied systems. To evaluate the effect of flocculation, droplet size measurements of the emulsion phase were conducted both with and without ultrasonic treatment (Fig. 3). Analysis of the resulting curves showed that sonication shifted the droplet size distribution toward smaller diameters in all samples, while in sample M2 the distribution changed from bimodal to monomodal. These results confirm the presence of flocculation processes in the studied systems. For samples M0 and M1, the D[4,3] values were similar (~5.01–5.04 μm). In contrast, sample M2 exhibited a lower D[4,3] value (4.36 μm) after ultrasonication, reflecting the disruption of floccules. The flocculation index (Table 1) increased in the order M0 < M1 < M2, irrespective of treatment. Sample M2, containing the oil blend at a 45:50:5 ratio, exhibited the highest degree of flocculation.

Under wUS conditions, Span values were similar for samples M0 and M1 (1.61 and 1.63, respectively) but nearly twice as high for sample M2. This indicates a higher degree of homogenization and a more uniform droplet size distribution in M0 and M1. The substantial increase in Span and flocculation index (FI) for M2 may be attributed both to the higher α-linolenic acid content in the oil phase and the presence of floccules. Włodarczyk et al. (2022) reported that replacing camelina oil (α-linolenic acid ~37%) with flaxseed oil (α-linolenic acid ~52%) increased mayonnaise droplet size from 45.28 μm to 56.75 μm. Similarly, Urbánková et al. (2019) observed flocculation when emulsifier concentrations were insufficient.

The total surface area of the fat phase in the emulsion system (S_{fat}) is a key factor in emulsion stability, as a larger surface area requires a greater amount of emulsifier to achieve stabilization. In the studied systems, M0 and M1 exhibited the same fat phase surface area, 4.7×10^5 m²/m³, whereas sample M2 showed a significantly higher value of 7.3×10^5 m²/m³. This confirms the importance of considering the type of oil when determining the emulsifier quantity needed to form a stable dispersed system. A similar effect was reported by Urbánková et al. (2019), where the particle size of an emulsion containing black cumin and tamanu oils, emulsified with sodium caseinate, increased from 2.2 to 7.8 μm as the tamanu oil content rose from 5% to 30%.

Viscosity is an important characteristic of emulsions, directly influenced by the size of the dispersed phase. The experimentally obtained flow curves indicate that the studied systems exhibit typical pseudoplastic (shear-thinning) behavior (Fig. 4a).

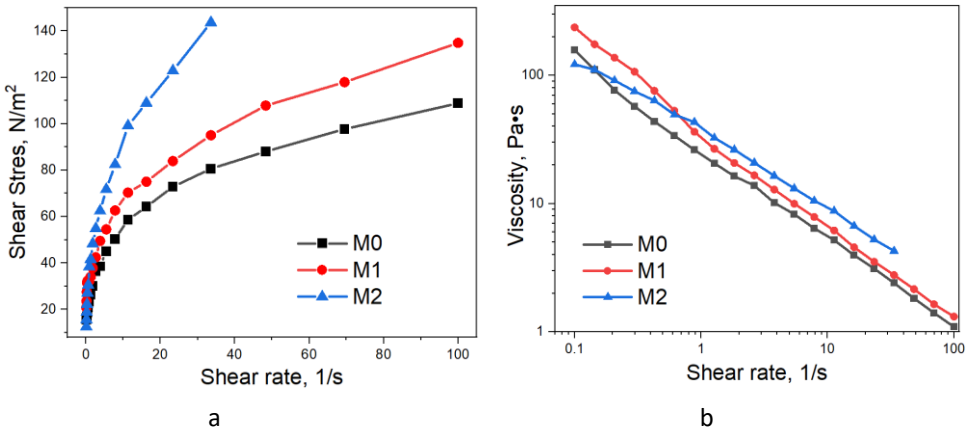

Figure 4. Rheological behavior of the emulsion samples: flow curves for the emulsion samples (a) and changes in emulsion viscosity under the shear rate (b)

Shear thinning behavior was observed for all emulsions, with viscosity decreasing as the shear rate increased from 0.1 to 100.0 s^{-1}. The flow curves of sauces M0 and M1 were nearly identical, reflecting the similar properties of their dispersed phases due to the high sunflower oil content in the blends. In contrast, the flow curve of sample M2 indicated that higher shear stress was required to disrupt its internal structure. Across the entire measurement range, sauce M0 exhibited the lowest viscosity (Fig. 4b), while sauce M2, containing the highest proportion of hempseed oil, showed the highest initial viscosity at a shear rate of 0.1 s^{-1}. At shear rates above 1.1 s^{-1}, the curves for M1 and M2 crossed, indicating that sauce M2 displayed greater resistance to deformation at higher shear rates.

The obtained flow curves were analysed using the Ostwald–de Waele and Casson models. The results indicate that both models provided a satisfactory description of the shear stress–shear rate relationship for all samples (Table 2).

Table 2

Yield stress values and coefficients of the rheological model equations

Sauce	Ostwald-de Waele model			Experimental data
	K	N	R^2	τ_y
M0	26.35±0.48	0.312±0.005	0.996	76.5[a]
M1	38.41±0.94	0.367±0.008	0.995	106.3[b]
M2	35.48±0.91	0.276±0.007	0.990	81.7[a]
Casson's model				
Sauce	χ	η_c	τ_c	R^2
M0	0.372±0.046[a]	0.08±0.06[b]	72.2±9.8[a]	0.996
M1	0.311±0.022[a]	0.15±0.08[b]	81.9±5.9[a]	0.999
M2	0.168±0.023[b]	1.93±0.38[a]	40.4±4.1[b]	0.998

Note: Different letters in the column indicate a statistical difference of the mean relative to the control sauce, according to Dunnett's post hoc test at a significance level of $p<0.05$.

This is supported by the high correlation coefficients and low root mean square deviations between the experimental data and values calculated from the non-linear regression equations. For the Ostwald–de Waele model, the obtained n values <1 confirm the pseudoplastic behavior of the emulsions. Lower n values correspond to a greater decrease in viscosity with increasing shear rate, as observed for the highly flocculated sauce M2. The experimentally determined yield stress (τ_y), representing the point at which the elastic structure of the emulsion begins to break down, is presented in Table 2. The lower yield stress of sample M2 compared with M1 is consistent with Casson model calculations, indicating reduced structural strength. These results are in agreement with the microstructural observations (Fig. 2), which show that sample M2 is more flocculated, leading to a looser internal structure.

According to the Casson equation, the dynamic yield stress (τ_c) for samples M0 and M1 exhibits statistically identical values, which are higher than that of sample M2. This indicates the greater structuredness of emulsions M0 and M1, which contributes to increased viscoelasticity. This fact is corroborated by the correlation between the structure compactness coefficient (χ) and the emulsion droplet size distribution parameters (Fig. 5).

A reduction in the droplet size and polydispersity of the dispersed phase promotes the formation of a more compact and stable emulsion structure, as evidenced by an increase in the structure compactness coefficient (Gubsky et al., 2023). Shear flow, which is commonly applied in rheological experiments on food emulsions and emulsion-based products, induces both reversible and irreversible structural changes that are often time-dependent. These changes are reflected rheologically as variations in viscosity. The recovery of the structure to its initial state after the cessation of shear reflects thixotropic behavior.

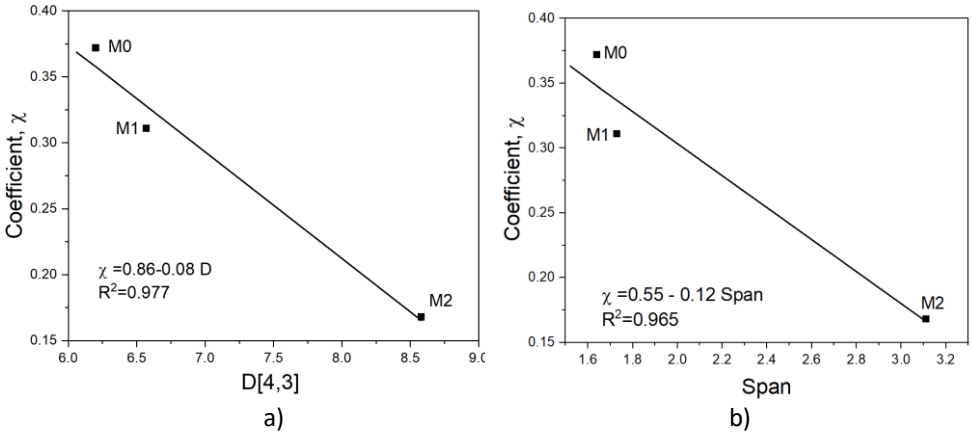

Figure 5. Dependence of the structure compactness coefficient (χ) on D[4,3] (a) and polydispersity factor Span (b) for emulsion samples

Thixotropic behavior is more accurately characterized using a dedicated step-wise three-interval test rather than relying solely on flow curve hysteresis (3ITT) (Fig. 6).

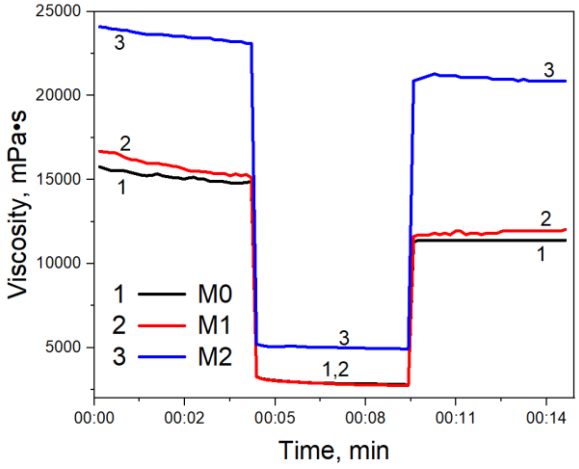

Figure 6. Results of the three-interval thixotropic test (3ITT) for emulsions M0, M1, and M2

The step-wise three-interval test (3ITT) consists of three phases: the first interval simulates the resting state of the emulsion, the second applies an abrupt structural breakdown, and the third monitors the time-dependent recovery of structural parameters. In this study, viscosity was used as the controlled parameter. The thixotropic breakdown coefficients and the structure recovery coefficient, calculated from the 3ITT curves, are presented in Table 3.

Table 3

Thixotropic behavior of the emulsion samples

Sauce	Thixotropic Breakdown coefficient (T_b)	Coefficient of structural recovery (CSR), %
M0	0.189[a]	71.1
M1	0.180[a]	73.1
M2	0.212[b]	87.3

Note: Different letters in the column indicate a statistical difference of the mean relative to the control sauce, according to Dunnett's post hoc test at a significance level of $p<0.05$.

For all investigated emulsions, a sharp drop in viscosity was observed during the second interval, corresponding to structural breakdown and a change in consistency. The relatively high breakdown coefficient (T_b) for emulsion M2 indicates that its structure is easily disrupted under external stress. At the same time, emulsion M2 exhibited the highest structure recovery coefficient (CSR), whereas this coefficient was considerably lower for samples M0 and M1. These results indicate that, despite its susceptibility to breakdown, emulsion M2 recovers its structure relatively quickly. This behavior can be attributed to the larger dispersed phase particle size and higher flocculation tendency of emulsion M2 compared with M0 and M1. These characteristics facilitate the rapid reformation of the spatial structure once the external stress is removed. The combination of easy structural breakdown and quick recovery represents highly desirable properties for mayonnaise-type sauces.

Conclusions

A stable emulsion formulation with a balanced fatty acid composition was developed using blends of sunflower, hempseed, and black cumin oils (30% fat phase) and canned bean aquafaba as a natural emulsifier. Such samples have reduced nutritional value and, due to the use of vegetable proteins, may be of interest for vegan nutrition. The study confirmed the high emulsifying efficiency of aquafaba, attributed to its thermostable hydrophilic proteins and saponins, which promote stable emulsion formation. Ultrasound treatment further reduced droplet size, disrupted flocs, and produced a more homogeneous dispersed system. However, increasing the proportion of hempseed oil broadened the droplet size distribution from 1.64 to 3.11. Mayonnaises containing more than 50% hempseed oil exhibited pronounced flocculation, observable by optical microscopy, and a higher total fat phase surface area (7.3×10^5 m^2/m^3), indicating that higher emulsifier concentrations are required to maintain stability in such formulations.

Rheological analysis showed that all mayonnaise samples exhibited pseudoplastic behavior, regardless of oil phase composition. Formulations with over 50% hempseed oil displayed higher viscosity at low shear rates, lower yield stress, and lower sensory scores. The most favorable balance of sensory and physicochemical properties was observed was achieved with the 70:25:5 sunflower:hempseed: black cumin oil blend, which combined sufficient thixotropy with a high structure recovery coefficient, making it suitable for mayonnaise production requiring easy breakdown and rapid structure regeneration.

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Effect of transglutaminase on quality characteristics of two-structure cooked-smoked sausages

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Abstract

Introduction. This study aimed to determine the effect of substrate interactions in composite protein mixtures and the enzyme transglutaminase on the functional-technological, structural-mechanical, and sensory properties of formed two-structure cooked-smoked sausages, as well as on their nutritional and biological value.

Materials and methods. The substrate protein mixtures differed in the proportions of animal and plant proteins and in the presence of transglutaminase. The sausage mince consisted of semi-fat pork, chicken fillet, chicken mince, and side fat. Experimental samples were produced by replacing 2.0% of semi-fat pork in the formulation with substrate protein mixtures containing transglutaminase.

Results and discussion. A binary protein mixture of porcine blood plasma and soy protein isolate, when treated with microbial transglutaminase (MTGase), effectively forms a monolithic structure in model minced meat systems for cooked-smoked sausages. This effect is attributed to the enzyme's ability to catalyze stable covalent cross-links between proteins, thereby improving textural characteristics and water-holding capacity.

To enhance the functional performance of these ingredients, their behavior in binary composite mixtures was evaluated. A mixture of porcine blood plasma protein and soy protein isolate at a 1:1 ratio in the presence of 0.9% microbial TGase exhibited the most favorable functional–technological properties, including high water-binding (78.85%) and water-holding (52.90%) capacities, along with a 5.21% increase in sausage yield. This formulation also promoted the formation of a uniform monolithic structure, improved elasticity and thermal stability of cooked-smoked sausages, and enhanced their sensory properties.

Sausage samples produced with the specified mixture composition exhibited a dense and uniform structure, along with a harmonious taste and aroma. In contrast, other formulations showed certain drawbacks: samples with the highest levels of protein and microbial transglutaminase were excessively dense, whereas those with lower protein and enzyme contents lacked sufficient elasticity.

The measured water activity of the cooked-smoked sausages ($\leq$ 0.92) indicates their suitability for long-term storage. Additionally, a reduction in the imbalance of amino acid composition, expressed by a 3.65% decrease in the difference coefficient of amino acid score (DCAS), contributed to a 4.16% increase in the biological value of the products.

Conclusion. A composite mixture of porcine blood plasma and soy protein isolate in combination with microbial transglutaminase is recommended for producing formed two-structure cooked-smoked sausages, as it improves structure, sensory quality, yield, and biological value.

Introduction

One of the methods for improving technology to form a stable structure of restructured meat products is the use of the enzyme transglutaminase (TGase) (Ivanov et al., 2021; Mirzaei, 2011). In the production of meat products, the safety of enzyme use lies in their protein nature, and consequently, their denaturation during thermal processing (Feiner, 2010). The temperature range of TGase activity is from 0 to 65 °C, with optimal chemical activity achieved approximately at 55 °C (Tarte, 2015). Denaturation of TGase begins at temperatures of 65 °C and above, and is completely halted at 70–75 °C (Whitehurst and van Oort, 2013).

In the meat processing industry, two main enzymatic preparations containing transglutaminase (TGase) are used: enzymes of bacterial origin (Feiner, 2010) and blood-based systems in which blood is fractionated according to clotting factors and subsequently recombined. In these blood-based systems, a component exhibiting transglutaminase activity is present (Prakasan et al., 2015). However, microbial transglutaminase obtained through industrial cultivation of microorganisms of the genus *Streptoverticillium* is increasingly applied in the food industry, particularly in the fish, meat, dairy, and confectionery sectors (Duarte et al., 2020; Kieliszek and Misiewicz, 2014).

The technological function of TGase is based on the structuring of protein molecules disrupted by mechanical or biochemical processes, promoting the formation of covalent bonds between amino groups and thereby creating a “cross-linking” effect in multi-component meat systems. TGase interacts differently with individual proteins depending on conditions (Ruiz-Carrascal and Regenstein, 2002; Shevchenko et al., 2020). The extent of this reaction is primarily determined by the availability of glutamine and lysine residues, as well as by reaction conditions such as pH and temperature, which must fall within the enzyme’s optimal activity range. Consequently, TGase-containing enzymatic preparations are formulated to provide the enzyme and protein substrate in the appropriate ratio (Castro-Briones et al., 2009).

Because of enzymatic action, high-molecular compounds containing intra- and intermolecular glutamyl-lysine bonds are formed. Covalent bonds formed under the action of TGase between free amino groups and gamma-carboxyl groups of glutamine are resistant to proteolysis. Bonds are formed both within the protein molecule and between its separate molecules. This allows the formation of a homogeneous dense structure in restructured meat products.

TGase also contributes to the deamination of amino acids and the biosynthesis of new ones, which improves the functional-technological properties of meat systems (Ramírez-Suárez and Xiong, 2003). The protein structure formed in this way is stable over a wide temperature range and resistant to further mechanical impacts.

However, for two-structure minced systems of cooked-smoked sausages, the specifics of combining proteins and transglutaminase enzyme in mixtures with specific substrate interaction with microbial transglutaminase are still insufficiently studied. It is important to investigate how individual ingredients (animal and plant proteins, microbial transglutaminase) and mixtures developed based on them affect the functional-technological and structural-mechanical properties of minced systems and provide synergy between recipe components. The use of optimized protein mixtures with specific substrate interaction with microbial transglutaminase will promote the formation of monolithic structure, elasticity, and thermal stability of cooked-smoked sausages.

The development of new functional protein mixtures suitable for enzymatic cross-linking by microbial transglutaminase can improve the sensory, nutritional, and biological properties of sausages, which is especially important under conditions of protein deficiency.

The aim of this study is to investigate the effect of microbial transglutaminase on composite protein mixtures in terms of their functional-technological, structural-mechanical, and sensory properties in formed two-structure cooked-smoked sausages, as well as the resulting nutritional and biological value.

Materials and methods

Materials

Substrate protein mixtures. Composition of substrate protein mixtures: porcine blood plasma protein "AProPork™" (Essentia) – a highly functional animal protein produced by spray-drying the soluble fraction of porcine blood, made from thermostable functional proteins; soy isolate (Archer Daniels Midland Company (ADM)); microbial transglutaminase (MTGase Jiangsu Zipin Biotech / BindPro®) – microbial form of calcium-independent enzyme produced by *Streptoverticillium mobamense*, with activity 50 units/g of powder.

Recipe composition of studied cooked-smoked sausage samples. One of the structural components of cooked-smoked sausages is strips of whole muscle beef, and the other is a minced system of semi-fat pork.

Model minced sausage systems were prepared based on semi-fat pork (40%), chicken fillet (20%), chicken mince (20%), and side fat (20%). During the preparation of the experimental cooked-smoked sausage samples, the developed substrate protein mixtures with various compositions of functional ingredients (Table 1) were added at 2.0%, replacing an equivalent amount of semi-fat pork.

Table 1

Recipe composition of substrate protein mixtures

Components	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
Porcine blood plasma, %	2.0	–	1.6	1.4	1.0
Soy isolate, %	–	2.0	0.4	0.6	1.0
Transglutaminase (MTGase), %	0.70	0.75	0.80	0.85	0.90

Preparation of sausage samples. Model minced systems of cooked-smoked sausages consisted of strips of whole muscle beef tenderloin and minced meat from semi-fat pork, placed longitudinally along the sausage baton, parallel to the whole muscle part. The mince was prepared in a mixer for 8 minutes, formed into a protein sausage casing with a diameter of 55 mm, and thermally processed in a thermo-chamber according to the thermal processing program for cooked-smoked sausages in natural protein casing.

During the preparation of the mince for the experimental cooked-smoked sausage samples, analytically selected mixtures of enzyme and protein or protein mixtures were added in an amount of 2.0% to replace semi-fat pork. Before thermal processing, the samples were subjected to settling for 12 hours at a temperature of 4–6 °C under conditions typical for cooked-smoked sausages and for enzyme activation (at this temperature, the enzyme activity is 50 units/g) (Figure 1).

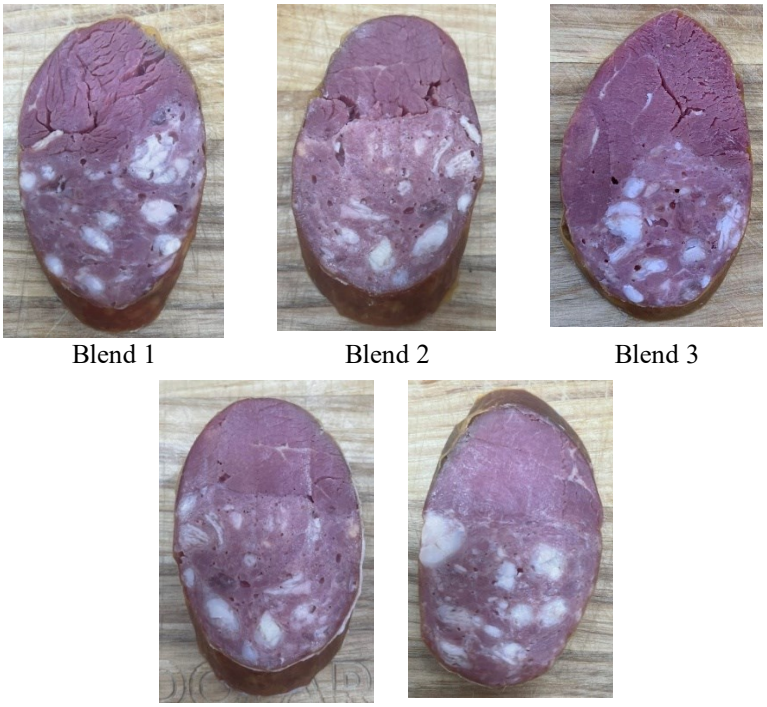

Figure 1. Sausage samples with different compositions of substrate protein mixtures

Thermal processing of experimental sausage samples was carried out until the temperature in the center of the sausage batons reached 70–72 °C. The finished sausage products were then smoked and cooled to a temperature of 12 °C.

Methods for studying the properties of substrate protein mixtures

Determination of pH of the mixture. The pH value was measured using a laboratory pH meter in an aqueous extract prepared in a ratio of mixture: water = 1:10. For this, 5 g of the mixture was placed in a 250 mL conical flask, 50 mL of distilled water was added, and it was extracted for 30 minutes with periodic stirring. After extraction, the extract was filtered through filter paper and the pH of the filtrate was measured with a laboratory pH meter (Puolanne and Kivikari, 2000).

Determination of moisture content. Moisture content was determined according to ISO 1442:1997, applicable for meat and meat products. This method involves drying a meat or meat product sample at a specific temperature to a constant mass. The weight loss of the sample during drying is considered the amount of moisture.

Determination of water-binding capacity (X_1). The water-binding capacity of the test objects was determined by the Grau-Hamm press method modified by V.I. Volovinska and B.Ya. Kelman. The method is based on extracting water from a 300 mg sample by pressing with a weight of 1 kg for 10 minutes. The size of the spot remaining on the filter paper after sorption of the released moisture is outlined with a pencil. The size of the wet spot (outer) is calculated as the difference between the total area of the spot and the area formed by the meat

(product). The water-binding capacity (percentage of bound water relative to total water) was calculated by the formula:

$$X_1 = \frac{(A - 8.4B)100}{A}$$

X_1 is a content of bound water, %, relative to total water;

A is total moisture content in the sample, mg;

B is area of the wet spot, cm².

Determination of water-holding capacity. A sausage sample weighing 0.3–0.5 g was sliced into thin pieces (2–3 mm) from the center of the product to minimize the influence of outer layers. The sample was placed between two sheets of filter paper to absorb moisture released under pressure. A weight or press was applied, typically at 5 kg/cm² for 10 minutes. After pressing, the filter paper was weighed, and the difference in weight before and after pressing was used to calculate the amount of moisture released from the sample (Baur and Ensminger, 1977).

Determination of emulsion stability (X_2). The stability of the emulsion from coarsely ground raw material was determined by heating at 80 °C for 30×60 s and cooling in water for 15×60 s. Then, four calibrated centrifuge tubes with a capacity of 50 ml 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 measured. Emulsion stability was calculated by the formula:

$$X_2 = \frac{V_1}{V_2}100$$

V_1 is a volume of emulsified oil, ml;

V_2 is total volume of the emulsion, ml.

Determination of penetration stress. Penetration stress of sausage products was determined using a digital Brookfield DV1 viscometer by the depth of indenter penetration into the sample at 20 °C. Three measurements were taken on the open surface of the sample at a distance of at least 10 mm from the edge of the product and at maximum distance from other measurement points to avoid deformation interference. The penetration value was then converted to penetration stress.

Determination of sensory properties of sausages. Sensory evaluation was conducted using five criteria: appearance, consistency, color of the cut, odor, and taste. Each parameter was rated on a five-point scale, where 5 = excellent and 1 = unsatisfactory. A panel of experts assessed the products according to these established parameters to determine compliance with quality standards (Fudali et al., 2021).

Determination of water activity. Water activity (a_w) of model minced systems and sausages was measured using a Rotronic Hygro Palm–23 analyzer.

Determination of protein content. Protein content was determined by the Kjeldahl method (ISO 1871:2009).

Determination of biological value. Protein biological value (BV) was determined using the corrected amino acid score, considering the limiting amino acid and "apparent" protein digestibility – PDCAAS, as proposed by FAO/WHO in 1991 according to the formula (Schaafsma, 2000).

Statistical analysis. All experiments were conducted in triplicate or more, and the results were expressed as mean±standard deviation. Statistical evaluation was performed using one-way analysis of variance (ANOVA) in IBM SPSS Statistics (Somers, NY, USA)

Results and discussion

Compositions of substrate protein mixtures

At the first stage, the physicochemical and structural–mechanical properties of gels were studied. These gels contained varying amounts of structuring components, including porcine blood plasma proteins, soy protein isolate, and mixtures of porcine blood plasma proteins and soy protein isolate with microbial transglutaminase (MTGase). Gel-forming properties were evaluated by determining the critical gelation concentration, yield stress, and syneresis after storing the gels at 8±2 °C for 12 hours. The results are summarized in Table 1.

Table 1

Physicochemical and structural–mechanical properties of gels from substrate protein mixtures

Sample	Critical gelation concentration, %	Yield stress, kPa	Moisture separation, % (syneresis)
Blend 1 – Mixture 1 (MTGase 0.70% + blood plasma 2.0%)	0.70±0.02[a]	109.00±0.21[a]	2.90±0.08[a]
Blend 2 – Mixture 2 (MTGase 0.75% + soy protein isolate 2.0%)	0.80±0.01[b]	111.40±0.14[b]	2.80±0.09[a]
Blend 3 – Mixture 3 (MTGase 0.80% + blood plasma 1.6%, soy protein isolate 0.4%)	0.68±0.01[a]	105.50±0.13[c]	2.40±0.09[b]
Blend 4 – Mixture 4 (MTGase 0.85% + blood plasma 1.4%, soy protein isolate 0.6%)	0.85±0.01[c]	110.40±0.14[d]	2.20±0.03[c]
Blend 5 – Mixture 5 (MTGase 0.9% + blood plasma 1.0% + soy protein isolate 1.0%)	0.90±0.01[d]	113.40±0.14[e]	2.00±0.01[d]

Note: Values are the means±standard deviation. Means within the same row with different superscripts are significantly different at $p \leq 0.05$.

It was found that increasing the content of structuring components led to a higher critical gelation concentration, which in turn resulted in an increase in yield stress (Table 1). The yield stress of the different gels ranged from 105.5 to 113.4 kPa, values that are within the acceptable range for sausages in casing. Sample 5 exhibits gel structures resistant to syneresis. An optimal ratio of animal to plant proteins (1:1) ensures uniform formation of a three-dimensional matrix, increasing gel strength and elasticity. This is explained by the synergistic action of plasma proteins and soy protein isolate. Blood plasma contains albumins and globulins, which are good donors of lysine residues, while soy protein isolate contains glutamine residues, which are good acceptors. In the presence of MTGase, ε-(γ-glutamyl)-lysine cross-links form between them, densifying the structure. Thus, the combination of blood plasma and soy protein isolate in the presence of MTGase promotes gelation through protein-protein interactions, such as cross-linking of globulins (Xiong, 2017). The minimal syneresis value (2.0%) also indicates high water-holding capacity of the system, correlating

with higher resistance to deformation. Therefore, the high yield stress of Sample 5 is explained by the most efficient formation of covalent cross-links between blood plasma proteins and soy protein isolate under MTGase action, resulting in a dense, elastic gel with reduced syneresis (Kuraishi et al., 1997; Toldrá and Reig, 2021).

Functional-technological properties of model minced systems and cooked-smoked sausages

At the next stage, studies were conducted to investigate the effect of MTGase on the functional-technological properties of model minced systems during settling. The mince included, in varying amounts as structuring components, the enzyme TGase, proteins (blood plasma, soy protein isolate), and their mixtures (Toldrá and Reig, 2021). The results of these studies are presented in Table 2.

Table 2

Functional-technological properties of model minced systems and cooked-smoked sausages depending on the composition of substrate protein mixtures

Parameter	Substrate protein mixtures				
	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
Settling time (fermentation) 10 h					
Water-binding capacity, %	77.08±3.15^{a}	77.52±3.08^{a}	77.84±3.09^{a}	78.27±3.18^{a}	78.72±3.11^{a}
Water-holding capacity, %	51.14±2.23^{a}	52.12±2.15^{a}	52.37±2.24^{a}	52.41±2.51^{a}	52.84±2.15^{a}
Mass loss during thermal processing, %	22.18±0.98^{a}	21.62±0.91^{a}	21.52±0.87^{a}	21.43±0.81^{a}	20.93±0.85^{a}
Emulsion pH	6.2±0.14^{a}	6.2±0.21^{a}	6.2±0.18^{a}	6.2±0.19^{a}	6.3±0.20^{a}
Settling time (fermentation) 12 h					
Water-binding capacity, %	77.28±3.15^{a}	78.12±3.12^{a}	78.48±3.14^{a}	78.52±3.17^{a}	78.85±3.17^{a}
Water-holding capacity, %	51.16±2.06^{a}	52.19±2.15^{a}	52.51±2.24^{a}	52.48±2.51^{a}	52.90±2.51^{a}
Mass loss during thermal processing, %	21.78±0.81^{a}	20.86±0.89^{a}	20.58±0.84^{ab}	20.46±0.79^{ab}	19.96±0.82^{b}
Emulsion pH	6.2±0.14^{a}	6.2±0.20^{a}	6.2±0.17^{a}	6.2±0.18^{a}	6.3±0.17^{a}

Note: Values are the means±standard deviation. Means within the same row with different superscripts are significantly different at $p \leq 0.05$.

According to experimental data, confirmed by statistical analysis, the functional-technological properties of model minced systems and cooked-smoked sausages remain unchanged. This can be explained by the simultaneous increase in substrate addition (mixture of blood plasma and soy protein isolate) along with increased enzyme content. Therefore, quality indicators remain stable, while the nutritional value of the product increases.

Fermentation time has a statistically significant positive effect on functional-technological properties: it increases water-binding and water-holding capacities and reduces mass loss during thermal processing. The composition of protein mixtures shows a tendency to improve the same parameters: increasing water-binding and water-holding capacities and reducing mass loss during thermal processing. The positive effect of prolonged fermentation

is observed for all types of protein mixtures, which is an important technological conclusion. Completion of the enzymatic "cross-linking" process and settling is indicated by stabilization of pH and reduction of moisture loss, not exceeding 1%, resulting from gel network densification (Table 2).

Study of structural-mechanical properties of model sausage samples

In the production of two-structure cooked-smoked sausages, an important aspect is obtaining a monolithic product with a dense structure. A disadvantage that may occur using classical technology for cooked-smoked sausages is structural separation and detachment of minced elements after thermal processing, as well as an atypical appearance of the cut, negatively affecting both sensory properties and slicing of the finished product.

To assess the degree of influence of substrate protein mixtures on structural-mechanical characteristics of model cooked-smoked sausage samples, water activity and yield were measured in samples with 2.0% of meat raw material replaced by protein mixtures of different composition. Considering the functional properties of selected protein mixtures with different structural conformations, hydration was established at a ratio of 1:4.

The dynamics of changes in shear stress, cutting force, yield, and water activity of model cooked-smoked sausage samples depending on the composition of substrate protein mixtures are presented in Table 3.

Table 3

Structural-mechanical parameters, yield, and water activity of model cooked-smoked sausage

Parameter	Substrate protein mixtures				
	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5
Shear stress, kPa	198.87±8.04^{b}	205.82±8.08^{a}	212.39±8.25^{a}	214.03±8.43^{a}	214.77±8.40^{a}
Cutting force, kPa	206.23±6.31^{b}	209.18±6.0^{ab}	211.16±6.23^{a}	215.12±6.40^{a}	216.02±6.34^{a}
Yield, %	77.22±2.30^{b}	82.14±2.11^{a}	82.22±2.18^{a}	82.35±2.24^{a}	82.43±2.26^{a}
Water activity, a_w	0.914±0.002^{a}	0.913±0.001^{a}	0.912±0.003^{ab}	0.910±0.001^{b}	0.909±0.001^{b}

Note: Values are the means±standard deviation. Means within the same row with different superscripts are significantly different at $p \leq 0.05$.

Analysis of the results (Table 3) indicates that shear stress in the model sausage samples varied depending on the composition of the substrate protein mixtures. An increase in shear stress of 1.88% can be attributed to protein cross-linking, which leads to changes in their physicochemical and structural–mechanical properties. It was established that the formation of covalent bonds induced by MTGase alters the rheological properties of the sausages. Although the overall statistical analysis did not reveal significant differences among all substrate protein mixture groups, pairwise comparisons demonstrated the high efficiency of the modified mixtures. A statistically significant difference was observed between the control mixture Blend 1 (77.22±2.30%) and the most effective Blend 5 (82.43±2.26%). Replacing Blend 1 with Blends 2–5 resulted in an average yield increase of 5.2%, which is a critically important technological parameter.

Analysis of structural-mechanical parameters (shear stress and cutting force) revealed a clear trend of structure reinforcement from Blend 1 to Blend 5. Shear stress increased from

198.87 kPa (Blend 1) to 214.77 kPa (Blend 5), likely due to optimization of the gel-forming properties of the protein matrix through covalent bond formation between primary amines of Gln and Lys residues in protein molecules (Santhi et al., 2017).

Simultaneously, water activity gradually decreased from 0.914 (Blend 1) to 0.909 (Blend 5). This parameter correlates with increased yield and confirms that mixtures Blend 2–5 bind free moisture more effectively. Lower water activity is favorable for improving microbiological stability and extending shelf life of the final product.

Thus, the highest strength properties (shear stress and cutting force) (Table 3) were observed for Blend 5, which contained 0.9% MTGase, 1.0% blood plasma, and 1.0% soy protein isolate in an optimal ratio. Based on the obtained data, Blend 5 is the most technologically efficient mixture, providing the highest product yield, maximum shear stress (indicating the formation of the densest structure), and lowest water activity, creating favorable conditions for longer product shelf life.

Therefore, combining substrate protein mixtures with MTGase is reasonable to improve the structural-mechanical properties of sausages, as covalent bonds formed by transglutaminase are highly resistant to proteolysis and thermal processing (Pfleiderer et al., 2005; Toldrá and Reig, 2021).

Moreover, blood plasma proteins are a better substrate for MTGase due to their easily accessible, flexible, and open chain structure. In comparison, globular whey proteins are less reactive because disulfide bonds stabilize the globular conformation, limiting binding site accessibility. Proteins with a high glutamine content, such as soy proteins, are also good substrates for transglutaminase. Under the action of MTGase, cross-linking between glutamine and lysine residues in meat proteins leads to the formation of ε-(γ-glutamyl)lysine (ε-(γ-Glu)Lys) complexes (Han and Bertram, 2017).

Thus, the effect of MTGase in forming additional bonds between plant and animal proteins contributed to the formation of a denser and more monolithic structure in model cooked-smoked sausage samples (Table 3). These new protein bonds effectively retain moisture in the sausage matrix and increase resistance to deformation forces. MTGase facilitates interactions between meat proteins and hydrophilic groups of the substrate protein mixture, resulting in reduced moisture loss during thermal processing and a 5.21% increase in sausage yield (Lee et al., 2017).

Sausage strength with meat replacement by substrate protein mixtures increased by 1.95%. Therefore, the addition of substrate protein mixtures positively affects overall structural strength and increases yield of cooked-smoked sausages.

The study of structural-mechanical properties showed that using substrate protein mixtures significantly improves strength characteristics of thermally processed sausage samples. This is because the viscosity of the minced component of model sausages increases due to the high protein content in both substrate protein mixtures and meat raw material. Increased adhesion forces between protein molecule surfaces promote the formation of a reinforced protein structure (Mohanan et al., 2020).

Scientific studies indicate that MTGase addition increases apparent viscosity of the mince, regardless of sodium alginate addition or salt concentration. This may result from protein solubilization with covalent bonds formed between Gln and Lys (Chen et al., 2023; Moreno et al., 2008). Microbial transglutaminase catalyzes the formation of isopeptide bonds, ε-(γ-glutamyl)lysine cross-links between glutamine (Gln) residues in peptide chains and ε-amino groups of lysine (Lys) residues in plasma protein and soy protein isolate peptides, enhancing gel textural properties (Li et al., 2018; Wang et al., 2019). Therefore, inclusion of substrate protein mixtures in cooked-smoked sausages significantly improves

texture (elasticity and strength), mechanical resistance, and water-holding capacity of model samples.

Thus, shear stress and cutting force measurements on the Brookfield DV1 viscometer demonstrated that the most optimal ingredient composition is Blend 5. Its use in cooked-smoked sausages contributes to formation of the best structural properties, confirming the high functionality of its composition as a substrate protein mixture for dual-structure cooked-smoked sausages.

In conclusion, analyzing the structural-mechanical characteristics of model sausage samples shows that meat proteins in combination with substrate protein mixtures containing high amounts of Gln and Lys for cross-linking under MTGase influence positively affect overall structural strength of cooked-smoked sausages. This allows prediction of the interaction of structuring components and regulation of quality indicators in meat products (Azaza et al., 2009; Ramos-Diaz et al., 2022; Wei, 2019).

The results of water activity (a_w) studies in cooked-smoked sausages (Fig. 2) showed that model sausage samples with substrate protein mixtures can be classified as group C ($a_w \leq 0.92$) in terms of product stability. Meaning of $a_w \leq 0.92$ indicates that the product is resistant to long-term storage (Ruiz-Carrascal and Regenstein, 2002) because the amount of water biologically available for microorganisms is reduced (Shevchenko et al., 2020).

Amino acid composition of model cooked-smoked sausage samples

To assess the influence of substrate protein mixtures on the biological value of model cooked-smoked sausages, their amino acid composition was compared with that of an "ideal" protein, the mass fractions of which and overall balance fully satisfy the requirements of the human adult body. The results of amino acid composition analysis of the model cooked-smoked sausage samples are presented in Table 4.

Analysis of the amino acid composition of control and experimental sausage samples shows that the biological value index increases by 4.16% when applying the substrate protein mixture Blend 5. The analysis reveals a significant excess of the amino acid SCORE for several amino acids: valine (119.58%), isoleucine (146.42%), and tryptophan (104.5%) (Duarte et al., 2019).

In the control sample, the limiting amino acids were phenylalanine and tyrosine, whereas their content in the experimental samples was slightly higher (74.00 mg/100 g in the control vs. 77.97 mg/100 g in the experimental samples).

A reduction in amino acid imbalance, as indicated by a 3.65% decrease in the DCAS index, suggests more rational protein utilization for anabolic purposes and contributes to a 4.16% increase in biological value compared to the control. These results confirm that the biological value (BV) of dual-structure cooked-smoked sausages improves with the addition of swine blood plasma proteins and soy protein isolate, which provide a better balance of aromatic amino acids in the final product.

Sensory properties of model cooked-smoked sausages

The overall sensory evaluation of the model sausage samples was high. The highest scores were observed for taste, color, and consistency, as illustrated by the quality profiles of the samples (Fig. 2).

Table 4

Amino acid composition of model cooked-smoked sausages (mg/100 g)

Amino acid	Cooked–smoked sausage (control)		Cooked–smoked sausage with 2% lean pork replacement by Blend 5 (experimental)		Reference standard (mg/100 g)
	content	% SCORE	content	% SCORE	
Protein, mg	18050	–	19038	–	1000
Essential amino acids:	417		40.88	113.56	351
Valine	49.11	114.21	51.42	119.58	43
Isoleucine	46.07	139.61	48.32	146.42	33
Leucine	82.16	124.48	82.71	125.32	66
Lysine	86.29	156.89	77.89	141.62	55
Methionine + Cysteine	24.96	99.84	25.31	101.24	25
Threonine	43.55	124.43	44.69	127.69	35
Tryptophan	9.99	99.90	10.45	104.50	10
Phenylalanine + Tyrosine	44.40	74.00	46.78	77.97	60
Histidine	29.97	124.88	30.75	128.13	24
BV	80.34		84.50		100.00

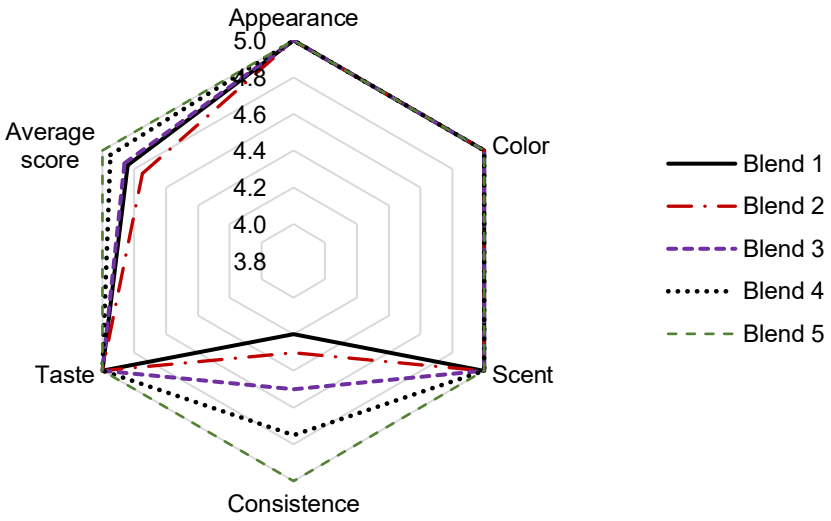

Figure 2. Quality profiles of model cooked-smoked sausage samples depending on the composition of substrate protein mixtures

Modification of proteins with MTGase allows changes in solubility, hydration, and thermal stability, thereby improving structural properties of the mince base, particularly at the junction between structural elements: whole-muscle and minced parts of dual-structure

cooked-smoked sausages. The best sensory properties were observed in Sample 5, which contained 0.9% MTGase, 1.0% blood plasma, and 1.0% soy protein isolate in an optimal ratio within the mince base.

Efficiency of substrate protein mixtures in model cooked-smoked sausages

The use of substrate protein mixtures, particularly Blend 5, effectively stabilizes meat protein systems, reduces technological losses, and prevents structural separation, positively influencing the structural-mechanical properties of sausages. The synergy between plant and animal proteins and microbial transglutaminase enhances elasticity and structural strength and increases the yield of the final product. The developed substrate protein mixture also demonstrates high functional-technological properties, confirmed by positive sensory evaluation of dual-structure cooked-smoked sausages.

The results showed an increase in water-binding and water-retention capacity of mince and thermally processed sausage samples during 12 h settling by 1.57% and 1.74%, respectively, and a reduction in mass loss during thermal processing by 1.82%. Practical application of the substrate protein mixture in the optimal component ratio of Blend 5 in cooked-smoked sausage production allows an increase in shear stress by 1.88% and cutting force by 1.95%. These values align with previous studies (Toldrá and Reig, 2021), which also reported improved rheological and textural characteristics with protein mixtures and/or transglutaminase application.

The yield of final sausages increased by 5.21%. Due to the stability of the water–fat emulsion and preservation of texture, the finished products meet high quality standards, maintaining uniform color, harmonious taste, and aroma.

The use of Blend 5 substrate protein mixture in the proposed composition can be recommended for industrial production of dual-structure cooked-smoked sausages. It improves sensory stability: products have elastic consistency, harmonious taste and aroma, enhancing market competitiveness.

The results confirm the feasibility of using substrate protein mixtures in sausage production. Their application positively affects structural strength and stability, increases mechanical characteristics, promotes monolithic structure, elasticity, and thermal stability of cooked-smoked sausages, and enhances shelf-life stability by reducing water activity to $a_w \leq 0.92$. Reduction of imbalance according to DCAS by 3.65% contributes to an increase in biological value by 4.16%.

As substrate protein mixtures are a promising tool for producing high-quality meat products with improved structural-mechanical properties, the main directions for their implementation in dual-structure cooked-smoked sausages include: controlled dosing (excess causes excessive hardness) and chemical composition modeling to optimize ingredient ratios in mince systems and restructured meat products to positively influence both sensory properties and biological value.

Conclusions

The use of composite protein mixtures, including blood plasma, soy protein isolate, and microbial transglutaminase, improves the structure, elasticity, thermal stability, and sensory properties of dual-structure cooked-smoked sausages. Partial replacement of semi-fat pork with these mixtures enhances gel formation and monolithic structure. Reduced water activity and improved amino acid balance contribute to increased storage stability and biological

value. Overall, the optimized combination of protein components and MTGase provides an effective approach for producing high quality, functional sausages.

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Comparative analysis of the storage of pomegranate varieties under different conditions

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Abstract

Introduction. The objective of this study was to assess the impact of storage temperature, gas composition, and relative humidity on the quality of pomegranate varieties under long-term refrigerated storage conditions.

Materials and methods. The study examined local (Nazik Gabig, Iridane) and new (Gashang, Yeni Guleishe) pomegranate varieties. Phenolic compounds, including anthocyanins, were quantified using GC–MS. The enzymatic activities of ascorbate oxidase, polyphenol oxidase, peroxidase, and catalase were determined using spectrophotometric methods.

Results and discussion. Pomegranate varieties were stored in refrigerated chambers under four conditions: a controlled gas medium (CGM) with 3–4% CO_2 and 2–3% O_2 (variant I), CGM with 1–3% CO_2 and 2–3% O_2 (variant II), normal refrigeration (variant III), and CGM with 3–4% CO_2 and 2–3% O_2, a temperature of from −2 to −4 °C, and 92–95% relative humidity (variant IV). Storage durations were 6, 4, 3, and ≥7 months for variants I–IV, respectively. Natural and microbiological losses were monitored, and sensory evaluation was performed at the beginning, middle, and end of storage using a 10-point scale, considering taste, texture, color, and overall acceptability. Variant IV resulted in the lowest losses and highest sensory scores, demonstrating that the combination of controlled gas composition, low temperature, and high humidity is most effective in preserving fruit quality. These findings indicate that the Iridane and Yeni Guleyshe varieties are particularly suitable for long-term storage and commercial cultivation.

Analysis of anthocyanin composition revealed significant differences among varieties. High levels of delphinidin and cyanidin derivatives, especially in Yeni Güleyşe and Iridane, enhance their biological and functional value, contributing to strong antioxidant activity. Optimized storage conditions thus play a crucial role in maintaining both the sensory quality and health-promoting properties of pomegranate fruits.

Conclusions. Conventional refrigeration conditions led to a sharp decline in sensory properties. The fourth storage variant (temperature from −2 to −4°C, a controlled gas medium with 3–4% CO_2 and 2–3% O_2 under high relative humidity of 92–95% provided the highest sensory evaluation (9.2–9.7 points) and the lowest losses of the fruits (1.1–1.9%).

Introduction

The growing interest in healthy lifestyles and nutrient-rich foods has driven the development of new product lines in the food industry. Pomegranate (*Punica granatum* L.), a fruit native to South Asia and widely cultivated in tropical and subtropical regions, is prized for its high content of bioactive compounds, including phenolics, polyphenols, flavonoids, anthocyanins, essential minerals (such as potassium), and vitamins (C, A, and folic acid) (Giménez-Bastida et al., 2021; Noreen et al., 2025). Global pomegranate production currently reaches approximately 8.1 million tons, cultivated over a total area of 835,950 hectares, and continues to increase annually. Azerbaijan ranks among the leading pomegranate-producing countries worldwide, alongside India, Iran, Turkey, Egypt, the USA, Afghanistan, Tunisia, Spain, Peru, Pakistan, Italy, South Africa, and Mexico (Ezeora et al., 2024).

Regular, year-round consumption of pomegranate is recommended to maximize its health benefits, as its unique composition provides strong antioxidant and immunomodulatory effects, contributing to overall well-being. In addition, pomegranate and its derived products exhibit anticarcinogenic and anti-inflammatory activities (Akhundova et al., 2025; An et al., 2021; Zarfeshany et al., 2014), support cardiovascular health (Saeed et al., 2025), and daily intake has been associated with reductions in body mass index in healthy individuals (Stabnikova and Paredes-López, 2024). Pomegranate juice concentrate has demonstrated strong inhibitory effects against human pathogens such as *Streptococcus mutans* and *Aeromonas hydrophila* (Habib et al., 2023), while ellagitannins in pomegranate extract are converted in the intestines into urolithin A, which benefits the human gut microbiota and supports intestinal health (Bandow et al., 2025; Oseyko et al., 2019).

These multifaceted benefits underscore the potential of pomegranate both as a fresh fruit and in processed forms such as juice, extracts, and functional foods (Zhang et al., 2025).

Pomegranate has more than 500 cultivars distributed worldwide (Kandylis and Kokkinomagoulos, 2020). However, the type of cultivars that have prevailed in certain regions reflects the preferences and taste of the local populations. In general, the same basic pomegranate fruit is known by different names in different regions, and this is mainly because husk and aril color can markedly vary when grown in different regions. These differences mainly affect fruit size, husk color (ranging from yellow to purple, with pink and red most common), aril color (ranging from white to red), seed hardness, maturity, juice content, acidity, sweetness and astringency (Kandylis and Kokkinomagoulos, 2020). The composition of this fruit is characterized by its richness in individual representatives of phenolic compounds with natural antioxidant and antimicrobial properties, particularly flavonoids, biflavonoids, procyanidins, and other monomers, oligomers, and polymers (Suman and Bhatnagar, 2019).

Pomegranate has gained increasing global production and consumption in recent years because of its diverse applications and notable nutritional benefits. However, pomegranate is highly prone to weight loss and spoilage during postharvest handling and storage, which negatively affects consumer acceptability by reducing freshness, taste, and potential health-promoting compounds (Fawole and Opara, 2013). Therefore, establishing storage conditions that maintain fruit quality over extended periods is essential.

The present study aimed to evaluate the effects of different storage conditions - temperature, gas composition, and humidity - on the quality of pomegranate varieties during long-term refrigerated storage.

Materials and methods

Materials

The research objects included local pomegranate varieties widely cultivated in Azerbaijan, namely Nazik Gabig and Iridane, as well as newly developed varieties, Gashang and Yeni Guleyshe, grown at the Goychay Experimental Station of the Azerbaijan Research Institute of Horticulture and Subtropical Plants (Figure 1).

Nazik gabig

Iridane

Gashang

Yeni Guleyshe

Figure 1. Studied pomegranate varieties

Nazik Gabig. Widely cultivated in the Ganja–Gazakh region, the fruits are flattened-round, bright red, with a thin peel, and weigh 200–250 g on average. Harvesting occurs in the second half of October.

Iridane. A local variety grown in Shirvan, Absheron, and Ganja, with round or elongated fruits weighing 220–250 g on average (up to 350–550 g). The thin-skinned fruits have large arils (100 arils weigh 36.5–39 g, occasionally up to 45 g) and high juice yield (52–54%). Both fruit and juice are dark cherry-colored, with 14.4% sugar and 1.84% titratable acidity. Storage ability is moderate, transport tolerance low; mainly used for juice and as a dessert fruit.

Gashang. Cultivated in Shirvan, this medium-height tree has drooping branches and few thorns. Fruits are large (400–500 g), with thick, bright dark crimson peel, thick internal partitions, and large, red arils arranged regularly, with a sweet-sour taste.

Yeni Guleyshe. Grown in Shirvan, Karabakh, and Ganja, the tree has a rounded crown with dense, upright branches. Fruits are globular with a cylindrical neck, thin glossy peel, large dark cherry arils, and small thin seeds. Average fruit weight is 220 g, with sweet–sour taste, sugar content 15.95% and acidity 1.84%. The thin internal partitions yield bright red, high-quality juice, and the fruits are widely consumed locally.

Storage conditions

The pomegranate fruits were pre-sorted, individually cleaned of foreign impurities, and placed in storage containers with a capacity of 8–10 kg before being stored in refrigerated chambers. During the study, the pomegranate fruits were stored in refrigerated chambers under four different conditions.

Pomegranate fruits were stored in refrigerated chambers under four conditions: a controlled gas medium (CGM) with 3–4% CO_2 and 2–3% O_2 (variant I), CGM with 1–3% CO_2 and 2–3% O_2 (variant II), normal refrigeration (variant III), and CGM with 3–4% CO_2, 2–3% O_2, a temperature of −2 to −4 °C, and 92–95% relative humidity (variant IV) (Table 1).

Table 1

Storage conditions of pomegranate fruits

Storage variant	Storage conditions	Atmosphere conditions	Chamber temperature, °C	Relative humidity, %	Internal fruit temperature, °C
I	Refrigeration chamber with controlled atmosphere	3–4% CO_2 and 2–3% O_2	from 0 to +3	80–90	from +3 to +5
II	Refrigeration chamber with controlled atmosphere	1–3% CO_2 and 2–3% O_2	from 0 to +3	80–90	from +3 to +6
III	Refrigerated chamber under standard conditions	—	from 0 to +3	80–90	from +3 to +6
IV	Refrigeration chamber with controlled atmosphere at low temperature	3–4% CO_2 and 2–3% O_2	from –2 to –4	92–95	from 0 to +1

Storage durations were 6, 4, 3, and ≥7 months for variants I, II,II, and IV, respectively.

Methods

The quality parameters of pomegranate varieties were examined across all variants once a month until the end of storage.

Chemical composition of pomegranate fruits. Besides, the changes in key quality indicators, such as total sugars, titratable acidity, phenolic compounds, and vitamin C, were determined (Hasil, 2004).

Determination of phenolic compounds. Using the modern analytical method of Gas Chromatography–Mass Spectrometry (Flamini and Traldi, 2010), the individual representatives of phenolic compounds, including anthocyanins, were quantified in the local Nazik Gabig and new Yeni Guleyshe pomegranate varieties.

Determination of ezymatic activity. During the storage of pomegranate fruits under different conditions, the changes in the activity of enzymes belonging to the oxidoreductase class (ascorbate oxidase, polyphenol oxidase, peroxidase, and catalase) were studied (Hasil, 2004; Nabiyev et al., 2008).

During the storage of pomegranate varieties both natural and microbiological losses were also identified. The degustation of each variety was also carried out separately at the beginning, middle, and end of the storage period (Cefola and Pace, 2016).

Statistical analysis

All experiments were performed in triplicate. The results are expressed as mean ±standard deviation. Statistical analyses were conducted using appropriate software and Microsoft Excel.

Results and discussion

During storage of pomegranate varieties under the first three conditions (variants I–III) in refrigerated chambers, the chamber temperature was maintained at 0 to +3 °C, with relative humidity of 80–90%. Owing to the relatively thick peel of pomegranate fruits, the internal temperature of the fruits in variants I–III remained above 0 °C, which is an important factor for quality preservation. During long-term storage, the internal fruit temperature was regularly monitored at 10-day intervals using a Pocket Test Thermometer.

The results showed that the internal temperature of the fruits ranged from +3 to +4 °C and, in some cases, reached +5 to +6 °C. Such conditions may intensify respiration and fermentation processes, leading to increased nutrient losses. Therefore, the implementation of the fourth storage variant was required to ensure more effective preservation. The primary objective of variant IV was to reduce fermentation activity and limit nutrient depletion associated with respiration. Under this storage condition, the internal temperature of the pomegranate fruits was maintained at 0 to ±1 °C, indicating more favorable storage conditions. Prior to storage under the different variants, the activities of oxidoreductase enzymes, including ascorbate oxidase, polyphenol oxidase, peroxidase, and catalase, were evaluated, as these enzymes play a key role in oxidative processes during fruit storage (Table 2).

Table 2

Enzyme activity (μg/mol) of pomegranate fruits before storage

Storage variant	Enzyme	Local pomegranate varieties		New pomegranate varieties	
		Nazik Gabig	Iridane	Gashang	Yeni Guleyshe
I	Ascorbate oxidase	0.66±0.03	0.55±0.02	0.80±0.04	0.88±0.04
II	Polyphenol oxidase	0.74±0.03	0.72±0.03	0.78±0.03	0.74±0.03
III	Peroxidase	2.10±0.08	1.98±0.07	1.32±0.05	2.21±0.09
IV	Catalase	0.44±0.02	0.36±0.02	0.34±0.02	0.30±0.01

Enzymes are organic compounds present in all living organisms and play a crucial role in sustaining life. Fundamental biological processes—including photosynthesis, respiration, nutrient absorption, and the synthesis and transformation of proteins, lipids, and carbohydrates—occur with the participation of enzymes (Patel et al., 2016). During the storage and processing of pomegranate fruits and other food products, complex biochemical reactions take place, the regulation of which largely depends on enzymatic activity. Therefore, the objective of this study was to investigate enzyme activity and the dynamics of its changes during storage.

One of the key enzymes involved in pomegranate fruit maturation and the enhancement of nutritional value is ascorbate oxidase, an aerobic dehydrogenase belonging to the oxidoreductase class. This metalloprotein catalyzes the oxidation of ascorbic acid to dehydro-L-ascorbic acid. In the storage and processing of plant-based products, including pomegranate fruits, it is essential to establish conditions that reduce or completely inhibit the activity of this enzyme. Otherwise, increased ascorbate oxidase activity leads to a decrease in vitamin C content and accelerates its utilization during respiration (Aslanova et al., 2014).

Polyphenol oxidase has been relatively less studied in pomegranate compared to other fruits and berries. Chemically, polyphenol oxidase is a coenzyme and contains copper as an active group. This enzyme catalyzes a wide range of polyphenols, facilitating the conversion of ortho- and para-diphenols into ortho-quinones.

Catalase breaks down hydrogen peroxide, which forms during the tissue respiration process in fruits and vegetables, including pomegranates, into water and molecular oxygen. Through this catalytic activity, living cells, including human cells, are protected from the harmful effects of hydrogen peroxide.

The primary objective of studying enzyme activity is to regulate metabolic processes that may occur during the long-term storage of pomegranate varieties under different conditions in refrigerated chambers. It is well known that plant-based products, including pomegranates, should be stored under conditions that prevent changes in enzyme activity, keeping them stable or in a permanently inactivated (inhibited) state. An increase in enzyme activity facilitates the consumption of key quality components of pomegranate varieties in the respiration process. To ensure the long-term preservation of food products, including pomegranate varieties, with maintained quality, enzyme activity must be continuously monitored throughout the storage period (Kazimova and Nabiyev, 2022).

Besides, before storage, the main quality indicators of pomegranate varieties, including soluble dry matter, total sugars, titratable acidity, phenolic compounds, and vitamin C, were examined. A review of the literature revealed that pomegranate juice contains more than 400 organic and inorganic compounds (Hasil, 2004). The dry matter of pomegranate juice mainly consists of simple sugars, which are representatives of carbohydrates. It is well known that

sugars are the products of the photosynthesis process. During photosynthesis, not only carbohydrates but also other essential organic and inorganic compounds necessary for life are synthesized. Even oxygen, which is crucial for the survival of all living organisms, is a product of photosynthesis. As a result of the proper functioning of this process, plant-based food products, including pomegranates, can possess high nutritional value.

In modern times, ecosystem disruption, drought, climate change, and stress factors affect the proper progression of the photosynthesis process. As a result, these factors can negatively impact the quality of food products, including fruits and berries. Therefore, it is crucial for people to strive for ecosystem conservation. As mentioned earlier, the dry matter of pomegranate mainly consists of simple sugars. Among these, glucose and fructose are the most abundant (approximately 50%). As pomegranate fruit ripens, the amount of simple sugars in its composition gradually increases. According to literature sources and the results of our research, unripe pomegranate varieties contain lower amounts of glucose and fructose compared to ripe fruits. Therefore, only fully ripened pomegranates should be used for both fresh consumption and long-term storage. Additionally, pomegranate juice contains other sugars such as pentoses, sucrose, pectin substances, and various other compounds.

The main quality indicator of pomegranate varieties is their richness in organic acids. In the composition of pomegranate fruit, organic acids are found in high amounts in the juice, while they are present in smaller quantities in the peel, membranes, and seeds. Pomegranates contain aliphatic polybasic organic acids. These acids are also referred to as natural, non-volatile, and titratable acids. The total acidity of fully ripened pomegranates ranges from 2% to 7%, depending on the variety. In wild pomegranate varieties, total acidity is higher, reaching 5%–12%. In fully ripened pomegranates, citric acid constitutes approximately 75%–80% of the total acidity, while oxalic acid and other acids make up 10%–15%. As noted in the literature, citric acid, along with oxalic acid, contributes to blood purification, blood pressure regulation, and the normalization of blood pressure and cholesterol levels in the body.

The main physicochemical and biochemical parameters of fully ripened pomegranate varieties before storage are presented in Table 3.

Table 3

Physicochemical and biochemical parameters (%) of pomegranate fruits before storage

Parameter	Local pomegranate varieties		New pomegranate varieties	
	Nazik Gabig	Iridane	Gashang	Yeni Guleyshe
Total dissolved solids	17.2±0.2	17.4±0.2	17.6±0.3	17.4±0.2
Total sugar	13.6±0.3	14.4±0.4	15.4±0.5	15.2±0.4
Titrable acidity	2.3±0.05	1.50±0.04	1.66±0.05	1.94±0.06
Phenolic compounds	1.3±0.06	1.3±0.05	1.02±0.04	0.92±0.03
Vitamin C	16.6±0.7	22.1±0.9	17.6±0.8	20.2±0.9

Note: The amount of vitamin C is expressed in mg%.

The total acidity in the studied pomegranate varieties ranged between 2.3% and 1.94% (Table 3). The data also show that the Gashang variety differs from the others in terms of total dissolved solids (17.6%) and total sugar content (15.4%). The highest titratable acidity was observed in the Nazik Gabig variety (2.3%), while the highest levels of phenolic compounds were found in the Nazik Gabig and Iridane varieties (1.3%). Vitamin C content was highest in the Iridane (22.1 mg/%) and Yeni Guleyshe (20.2 mg/%) varieties, distinguishing them from the others.

Pomegranate varieties are rich in water-soluble vitamin C, a biologically active compound essential for metabolic processes, protein and enzyme synthesis. Vitamin C deficiency can lead to colds, fatigue, loss of appetite, and disrupted lipid metabolism, increasing blood cholesterol. As shown in Table 3, the analyzed pomegranate varieties contain high levels of vitamin C, supporting their inclusion in the daily diet for maintaining health.

Total phenolic compounds are important quality indicators in pomegranate varieties. These biologically active substances exhibit strong antioxidant, antimicrobial, antiviral, and antimutagenic effects, support blood circulation, enhance memory, and reduce fatigue. Anthocyanins and their glycosides also aid in eliminating radiation, making pomegranate juice and red wine beneficial for exposed individuals.

The study examined enzyme activity and quality indicators of different pomegranate varieties during storage under various conditions (Table 4).

Table 4

Enzyme activity (%) of pomegranate fruits during storage under different conditions

Enzyme	CGM with 3-4% CO_2 and 2-3% O_2	CGM with 1-3% CO_2 and 2-3% O_2	In refrigerated chamber	CGM with 3-4% CO_2 and 2-3% O_2, -2 to -4 °C
Nazik Gabig				
Ascorbate oxidase	81.1±2.4	62.2±2.1	39.2±1.8	96.0±1.2
Polyphenol oxidase	84.1±2.6	63.6±2.3	40.9±1.9	100.0±0.0
Peroxidase	80.0±2.5	68.6±2.4	14.3±1.1	100.0±0.0
Catalase	70.4±2.3	63.6±2.2	13.6±1.0	95.5±1.3
Iridane				
Ascorbate oxidase	69.0±2.2	57.5±2.0	20.0±1.3	94.0±1.4
Polyphenol oxidase	75.6±2.4	55.5±2.1	33.3±1.6	100.0±0.0
Peroxidase	78.8±2.5	64.6±2.3	18.2±1.2	98.0±1.1
Catalase	72.2±2.3	61.1±2.2	22.0±1.4	95.0±1.3
Gashang				
Ascorbate oxidase	77.5±2.4	72.5±2.3	31.2±1.6	94.5±1.4
Polyphenol oxidase	85.7±2.7	71.4±2.4	45.7±1.9	100.0±0.0
Peroxidase	78.8±2.5	69.4±2.4	25.0±1.5	100.0±0.0
Catalase	72.2±2.3	66.7±2.2	22.0±1.4	98.0±1.1
Yeni Guleyshe				
Ascorbate oxidase	79.5±2.5	63.6±2.2	31.8±1.6	93.4±1.4
Polyphenol oxidase	80.5±2.6	63.9±2.3	38.9±1.8	96.5±1.2
Peroxidase	80.0±2.5	68.6±2.4	14.3±1.1	100.0±0.0
Catalase	75.0±2.4	70.4±2.3	13.6±1.0	100.0±0.0

Table 4 shows that during long-term refrigerated storage, enzyme activity remained partially active under variants I–III. Under variant III (normal refrigeration), peroxidase and catalase activity sometimes exceeded initial levels. In local varieties, peroxidase decreased by 78.8–80% in variant I, 64.6–69.4% in variant II, and 14.3–25% in variant III, with similar trends for catalase. Ascorbate oxidase and polyphenol oxidase activity decreased most under variant I, indicating higher nutrient consumption in variants II and III. In contrast, variant IV

nearly completely inhibited enzyme activity: peroxidase and polyphenol oxidase by 100%, ascorbate oxidase by 96%, and catalase by 95.5%.

Comparison of variants shows that the higher CO_2 concentration in variant I had a stronger inhibitory effect on enzyme activity. During long-term storage under variants I–III, enzymes were not fully inactivated, and in some cases, activity even increased. Internal fruit temperatures ranged from +3 to +4 °C, occasionally reaching +5 °C, due in part to the thicker pomegranate skin. In the variant IV (3–4% CO_2, 2–3% O_2, from −2 to −4°C, with internal fruit temperature maintained at 0±1°C), enzyme activity was effectively suppressed. In the local variety Nazik Gabig, ascorbate oxidase activity decreased by 96%, polyphenol oxidase and peroxidase were completely inactivated, and catalase activity decreased by 95.5%. In the variety Gashang, activity of ascorbate oxidase decreased by 94.5%, polyphenol oxidase and peroxidase were fully inactivated, and catalase decreased by 98% (Table 4). At the end of storage under variant IV, the pomegranate varieties almost completely retained their external appearance, allowing storage not only for 5–6 months but up to 8 months.

The main physicochemical and biochemical parameters of pomegranate varieties during storage under different conditions have also been studied (Table 5).

Table 5 indicates that conditions in variant IV best preserved dry matter and total sugars in both local and new pomegranate varieties, while variant III showed the greatest losses, with variants I and II showing intermediate reductions.

Figure 1 shows that variant IV experienced the smallest decrease in antioxidant phenolic compounds and vitamin C compared to the other variants. This is largely due to stronger inhibition of polyphenol oxidase and ascorbate oxidase, which catalyze these compounds. Reduced enzyme activity in plant-based products, including pomegranate, slows nutrient degradation during storage. The results indicate that limiting enzyme activity in refrigerated pomegranate significantly reduces respiration, and maintaining a gas composition of 3–4% CO_2 and 2–3% O_2, and a temperature of −2 to −4°C best preserves fruit quality.

Before storage, the anthocyanin content of fully mature pomegranate varieties were determined using chromatography–mass spectrometry, with the results presented in Table 6.

Anthocyanins are water-soluble flavonoid pigments responsible for the characteristic color of pomegranate juice and represent the most widely distributed flavonoids in this fruit. Their aglycone forms, known as anthocyanidins, include six major compounds commonly found in fruits: cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin (Stabnikov et al., 2025). Unlike catechins, anthocyanins readily form glucosides by binding with sugars and organic acids, which enhances product stability during storage. At concentrations of 300 mg/L or higher, anthocyanins significantly suppress oxidative enzyme activity and inhibit certain pathogenic microorganisms. Biologically, anthocyanins contribute to cholesterol regulation, cerebral vasodilation, memory improvement, and the removal of radionuclides from the body, supporting the health value of pomegranate products (Bagirzade et al., 2024; Nabiyev and Moslemzadeh, 2008).

Anthocyanin profiling by GC–MS (Table 6) showed marked cultivar-dependent differences. Delphinidin-3-O-glucoside ranged from 15.54 mg/L in Nazik Gabig to 19.48 mg/L in Iridane, while cyanidin derivatives (cyanidin-3-O-glucoside and cyanidin-3,5-O-diglucoside) were the predominant anthocyanins in all cultivars, reaching the highest levels in Iridane and Yeni Guleyshe. In contrast, cyanidin-3-O-rutinoside and pelargonidin-3-O-glucoside were present in the lowest amounts. Overall, Yeni Guleyshe and Iridane exhibited higher total anthocyanin contents than Nazik Gabig and Gashang, enhancing their biological and functional value. These results highlight the importance of optimized cold-storage conditions to preserve anthocyanins during long-term storage.

Table 5

Physicochemical and biochemical parameters (%) of pomegranate fruits during storage under different conditions

Parameter	CGM with 3-4% CO_2 and 2-3% O_2	CGM with 1-3% CO_2 and 2-3% O_2	In refrigerated chamber	CGM with 3-4% CO_2 and 2-3% O_2, -2 to -4 °C
Nazik Gabig				
Total dissolved solids	5.2±0.3	7.0±0.4	12.9±0.6	3.8±0.2
Total sugar	5.1±0.3	7.3±0.4	14.7±0.7	2.6±0.2
Titrable acidity	8.7±0.4	21.0±0.8	30.4±1.1	5.6±0.3
Phenolic compounds	6.9±0.4	10±0.5	16.9±0.7	3.8±0.2
Vitamin C	18.2±0.9	22.7±1.0	40.9±1.4	11.8±0.6
Iridane				
Total dissolved solids	5.7±0.3	8.0±0.4	11.5±0.6	3.2±0.2
Total sugar	5.5±0.3	7.6±0.4	12.5±0.6	1.8±0.1
Titrable acidity	8.7±0.4	10.0±0.5	13.3±0.6	4.8±0.3
Phenolic compounds	7.7±0.4	11.4±0.5	20.8±0.8	4.0±0.2
Vitamin C	21±1.0	27.6±1.1	31.6±1.2	8.5±0.4
Gashang				
Total dissolved solids	4.5±0.3	6.7±0.4	11.9±0.6	3.4±0.2
Total sugar	6.5±0.4	8.4±0.5	13±0.6	3.2±0.2
Titrable acidity	7.8±0.4	10.6±0.5	18.2±0.7	4.4±0.3
Phenolic compounds	8.8±0.4	10.8±0.5	20±0.8	4.9±0.3
Vitamin C	17.1±0.8	19.5±0.9	34.1±1.3	11.2±0.6
Yeni Guleyshe				
Total dissolved solids	5.7±0.3	6.9±0.4	11.5±0.6	2.8±0.2
Total sugar	5.9±0.3	7.2±0.4	13.8±0.6	2.8±0.2
Titrable acidity	9.2±0.4	10.8±0.5	17.5±0.7	4.1±0.3
Phenolic compounds	7.6±0.4	11.9±0.5	23.9±0.9	4.3±0.2
Vitamin C	15.8±0.8	22.4±1.0	40.8±1.4	6.4±0.4

Note: The amount of vitamin C is expressed in mg%

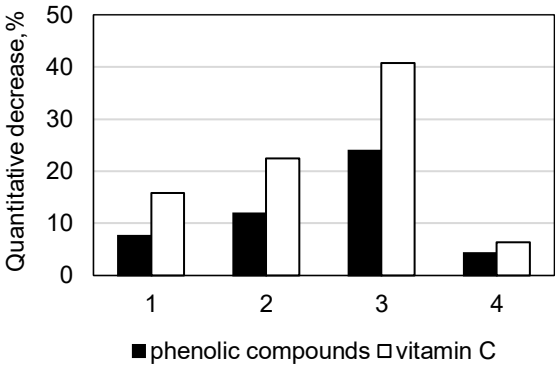

Figure 1. Reduction (%) in the content of phenolic compounds and vitamin C in the *Yeni Guleyshe* pomegranate variety during storage under different conditions

Table 6

Content of anthocyanin (mg/l) in fully ripe pomegranate fruits before storage

Anthocyanins	Nazik Gabig	Iridane	Gashang	Yeni Guleyshe
Delphinidin-3-O-glucoside	15.5365	19.4803	17.4275	18.4803
Delphinidin-3,5-O-diglucoside	6.7350	8.8863	7.6260	9.7833
Cyanidin-3-O-rutinoside	0.4183	0.7560	0.5253	0.8660
Cyanidin-3-O-glucoside	30.4870	37.6190	31.5726	35.5090
Cyanidin-3,5-O-diglucoside	36.6453	41.3137	38.5863	39.4137
Pelargonidin-3-O-glucoside	3.1259	3.5816	3.2019	4.0819
Unidentified	0.8870	1.072	0.9080	1.1722

The study also aimed to investigate the loss rates during the storage of pomegranate varieties under different conditions. It is known that during the long-term storage of fruits and vegetables, including pomegranate fruits, both natural and microbiological losses occur. During the storage period, the conditions in the refrigerated chamber should be optimized to minimize natural and microbiological losses. The storage of pomegranate varieties in the refrigerated chamber was carried out for 6 months under variant I, 4 months under variant II, 3 months under variant III, and for 7 months or more (until the end of May) under variant IV. Natural and microbiological losses during the storage of pomegranate varieties under different variants have been determined.

Detailed data on natural, microbiological, and total losses for each storage variant and cultivar are presented in Table 7. In local varieties, Nazik Gabig and Iridane showed the lowest losses in variant I (2.8% and 2.4%), increasing to ~5% in variant II and ~10% in variant III. Similar trends were observed in the new varieties, Gashang and Yeni Guleyshe, with losses of 3.1% and 2.7% in variant I, 5.4% and 4.8% in variant II, and 11.6% and 10.2% in variant III. Across all cultivars, variant IV had the lowest natural and microbiological losses, demonstrating its superior effectiveness for long-term storage.

Sensory evaluation was performed to assess the quality of pomegranate varieties during storage under different conditions, using a 10-point scale (Table 8).

Table 7

Loss rates (%) during the storage of pomegranate fruits under different conditions

Pomegranate varieties	Variant I			Variant II			Variant III			Variant IV		
	Storage for 6 months			Storage for 4 months			Storage for 3 months			Storage for 7 months		
	NL	ML	TL	NL	ML	TL	NL	ML	TL	NL	ML	TL
Nazik Gabig	1.9	0.9	2.8	3.1	2.1	5.2	6.3	4.2	10.4	1.2	0.6	1.8
Iridane	1.7	0.7	2.4	3.0	2.0	5.0	6.2	4.6	10.6	1.6	0	1.6
Gashang	1.9	1.2	3.1	3.2	2.2	5.4	6.8	4.8	11.6	1.2	0.7	1.9
Yeni Guleyshe	2.0	0.7	2.7	2.4	2.4	4.8	6.6	3.6	10.2	1.1	0	1.1

Note: NL, natural loss; ML, microbiological loss; TL, total loss.

Table 8

Tasting score (points) of pomegranate fruits after storage under different conditions

Pomegranate varieties	Variant I	Variant II	Variant III	Variant IV
	6 months	4 months	3 months	7 months
Nazik Gabig	8.8	8.4	7.6	9.4
Iridane	8.6	7.6	7.0	9.6
Gashang	8.5	8.0	7.1	9.2
Yeni Guleyshe	**9.0**	**8.6**	**8.0**	**9.7**

Sensory evaluation yielded scores of 8.5–9.0 in variant I, 7.6–8.6 in variant II, 7.0–8.0 in variant III, and the highest values, 9.2–9.7, in variant IV. After long-term storage, Iridane and Yeni Guleyshe achieved the top scores of 9.6 and 9.7, respectively. Overall, storage under variant IV (3–4% CO_2, 2–3% O_2, −2 to −4 °C, 92–95% relative humidity) maintained superior sensory quality and minimized natural losses compared with other variants.

Conclusions

The study demonstrated that storage conditions significantly affect the preservation of pomegranate fruit quality. Under conventional refrigeration, all studied local and newly developed cultivars showed pronounced reductions in soluble solids, total sugars, titratable acidity, phenolic compounds, vitamin C, and sensory attributes. In contrast, storage under a controlled atmosphere with reduced temperature (from –2 to –4 °C) and an optimized gas composition (3–4% CO_2 and 2–3% O_2) proved to be the most effective approach, ensuring minimal losses of key quality parameters and slowing physiological and biochemical processes in the fruits. Storage under variant IV resulted in the highest sensory scores (9.2–9.7 points) and the lowest total losses (1.1–1.9%). The results also revealed significant cultivar-dependent differences in anthocyanin composition and content. In particular, the elevated levels of delphinidin and cyanidin derivatives in the Yeni Guleyshe and Iridane cultivars enhanced their biological and functional value. Given the important role of anthocyanins in human health, the application of optimal refrigeration regimes during long-term storage is essential to minimize the depletion of these biologically active compounds through respiratory processes and to preserve the nutritional quality of pomegranate fruits.

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Influence of functional additives on microbiological and physico-chemical characteristics of yogurt

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Abstract

Introduction. Enriching yogurt with functional ingredients is a relevant task; however, their incorporation may significantly affect the sensory and physicochemical properties of the product, necessitating additional study.

Materials and methods. The technological effectiveness of adding (a) the LBLC + SeNPs supplement (biomass of *Lactobacillus bulgaricus* 3511 and *Lactococcus cremoris* 1220 containing selenium nanoparticles synthesized by these strains) and (b) cardamom as a source of technologically active extractive compounds is studied.

Results and discussion. The LBLC+SeNPs supplement substantially affected the activity of the starter culture LAB in yogurt, demonstrating a characteristic U-shaped dose-response relationship within the concentration range from 2.25 to 13.75 µg Se/100 mL. At a selenium content of 6.88 µg/100 mL, activation of the starter culture LAB was observed, whereas exceeding this concentration led to inhibition of their activity. The values of active acidity and syneresis of the yogurt samples correlated with LAB activity throughout fermentation, indirectly confirming the interrelationship among these indicators. Within the tested selenium concentration range, the selenium-containing supplement significantly influenced the apparent viscosity and thixotropic behavior of yogurt, increasing the values of these parameters nearly twofold compared with the control; this effect is attributed to selenium's impact on LAB metabolism.

The extractive compounds of dried cardamom, added at an initial level of 0.1–0.5%, caused a slight suppression of LAB activity during fermentation. The effect of cardamom on the apparent viscosity of fermented milk was minor; however, it enhanced thixotropy, which is technologically important in the production of drinking yogurt. When applied at rational dosages, both functional additives positively contributed to the overall quality of the final product during 28 days of storage, including the prevention of excessive post-acidification. Further research should focus on combining the functional and technological properties of the selenium-enriched biopreparation and cardamom extractives to formulate a new type of nutritionally enhanced yogurt.

Conclusions. The incorporation of the studied functional additives into yogurt significantly improves the overall quality characteristics of the product.

Introduction

Selenium is an essential trace element required for maintaining human health (Dinh et al., 2018; Niu et al., 2024; Skrotska et al., 2025). It is a key component of numerous selenoproteins involved in immune function, thyroid hormone metabolism, antioxidant protection, reproduction, and cardiovascular health. Adequate selenium intake helps prevent various disorders, including cardiovascular diseases, infertility, muscle degeneration, cognitive decline, and increased susceptibility to infections (Brigelius-Flohé, 2018). Its well-known antioxidant role is linked to glutathione peroxidase, which protects cells from oxidative damage (Hariharan and Dharmaraj, 2020; Zoidis et al., 2018). Although selenium deficiency poses serious health risks, its safe intake range is narrow, making careful dietary control essential. Recommended daily intake for adults generally ranges from 30 to 75 μg/day, depending on national guidelines (IMFNB, 2000; Kieliszek and Blazejak, 2016; Stabnikova et al., 2022).

In the food industry, selenium-enriched microbial products are primarily developed for the production of functional foods. These selenium-fortified products not only meet the growing consumer demand for nutrition and wellness but also enhance the added value of food items. Common applications include the use of selenium-enriched yeast and lactic acid bacteria in the manufacture of bakery and dairy products, as well as in the development of selenium-rich functional beverages (Du et al., 2024; Luo et al., 2025; Stabnikova et al., 2008, 2022; Shu et al., 2020; Wang et al., 2025).

It is known that Se can affect the physicochemical characteristics of food systems. In particular, Du et al. (2024) established the mechanism by which selenium-enriched yeast affects dough rheology. The addition of sodium selenite to yeast cultures (to produce selenium-enriched yeast) has been found to inhibit the yeast's energy metabolism and the synthesis of glutathione, glycerol, and linoleic acid, ultimately leading to dough softening. It was shown that as the amount of selenium accumulated in yeast cells increases, their enzymatic activity and gas-forming capacity decline, resulting in slower fermentation and proofing of the dough (He et al., 2023; Stabnikova et al., 2008). Therefore, the impact of accumulated Se on the activity and viability of microorganisms employed in fermented food production requires further detailed investigation.

Selenium accumulated by yeasts and bacteria offers advantages when incorporated into fermented dairy products due to its low toxicity and high bioavailability (Kieliszek et al., 2023). It has been demonstrated that certain lactic acid bacteria (LAB), including *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Bifidobacterium longum*, are capable of biotransforming selenite (a toxic form) into bioavailable, less toxic selenium nanoparticles, and Se–amino acids (non-toxic forms) (Martínez et al., 2020). There is experience in enriching dairy products with selenium nanoparticles in the form of biomass of lactic acid bacteria that synthesized them during cultivation in media containing inorganic selenium (Stabnikov et al., 2025; Stabnikova et al., 2023).

Csapó et al. (2015) produced yogurt from milk obtained from cows fed selenium-enriched yeast (Selplex-2300). The milk, containing 53.0 ± 2.80 μg/kg of selenium, was pasteurized, cooled, and inoculated with a starter culture of *Lactobacillus delbrueckii* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* for fermentation. The resulting yogurt had a high selenium content of 58.5 ± 0.40 μg/kg.

A selenium-enriched yogurt was developed using the high–selenium-tolerant strain *Lactiplantibacillus plantarum* NML21, which is capable of converting inorganic selenium in the form of sodium selenite (Na_2SeO_3) into organic forms (Wang et al., 2025). The application of selenium-enriched *L. plantarum* NML21 in yogurt production made it possible to obtain a

selenium-enriched functional product with high antioxidant activity and excellent physicochemical properties.

Guo et al. (2023) investigated quality changes of yogurt fermented with selenium-enriched lactic acid bacteria during 21 days of refrigerated storage. Compared with the control, selenium-enriched yogurt showed a slower increase in titratable acidity (approximately 10–15% lower by the end of storage), significantly reduced syneresis (by about 20–30%), and higher apparent viscosity and water-holding capacity throughout storage. In addition, viable LAB counts remained 0.5–1.0 $\log_{10}$ CFU/g higher than in the control at day 21, indicating improved microbial stability.

Osman et al. (2020) reported that during 10 days of storage, yogurt samples containing 0.2–0.6 mg/L of selenium exhibited a slight decrease in titratable acidity, an increase in viscosity, and a reduction in syneresis. A selenium concentration of 0.4 mg/L was identified as the threshold for producing yogurt without flavor or odor defects.

Thus, the available information on the effect of selenium on yogurt quality parameters is somewhat contradictory and requires clarification. The nature of this effect likely depends on the composition of the selected selenium preparation, its dosage and method of application, the type of lactic acid bacteria, and the duration and conditions of storage of the fortified yogurt, all of which should be considered in each specific case (Wang et al., 2021). Therefore, fortification of yogurt with selenium preparations requires detailed study to determine its specific impact on physicochemical, rheological, and microbiological parameters, including during storage.

To impart unique sensory properties to yogurts with Se and to offset potential flavor and aroma defects, natural flavor additives can be used. There is some experience in producing yogurts with natural flavor additives, including spices such as cinnamon, cardamom, thyme, and ginger. These additions can enhance organoleptic properties, provide antioxidant benefits, and generally maintain probiotic viability during storage.

Thus, probiotic yogurts have been developed with spice oleoresins (cardamom, cinnamon, and nutmeg) and probiotics (*Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis)*. The best sensory properties were obtained with cardamom oleoresin. The presence of spice oleoresins did not affect the probiotic populations in the yogurt during 4 weeks of storage (Vijayalakshmi et al., 2014). Yogurt samples with cardamom water extract at concentrations of 100–250 μg/L showed reduced pH, decreased peroxide value, and significantly improved sensory properties (Ismael et al., 2024). The suitability of combining yogurt with cardamom was also confirmed by Santos et al. (2025). Cardamom imparts a unique flavor to yogurt without negatively affecting the viability of lactic acid bacteria during storage. Furthermore, encapsulated cardamom oil is known to extend yogurt shelf life and stabilize its sensory and antibacterial properties for up to 21 days (Teymuri-Yeghaneh et al., 2025).

Therefore, it is worth investigating the potential use of cardamom in yogurt as a flavoring and aromatic ingredient, with a view to its possible combination with selenium. The aim of the present study was to investigate the effects of the LBLC+SeNPs supplement (lactic acid bacteria biomass with biosynthesized selenium nanoparticles) and cardamom as a flavoring additive on the physicochemical, rheological, and microbiological properties of yogurt.

Materials and methods

Materials

For yogurt production, the Iprovit starter culture, containing *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, was used.

Experimental yogurt samples enriched with selenium were prepared using a dietary supplement consisting of lactic acid bacteria biomass (*Lactobacillus bulgaricus* 3511 and *Lactococcus cremoris* 1220) together with selenium nanoparticles (LBLC+SeNPs) biosynthesized by these bacteria. The LBLC+SeNPs supplement was added to the yogurt as a powder suspension in 0.15 M NaCl, taking into account the Recommended Dietary Allowance (RDA) for selenium of 55 μg/day. The supplement was added at 0, 25, and 50% of the RDA, corresponding to selenium contents of 2.25, 6.88, and 13.75 μg/100 ml of yogurt, respectively.

To study the effect of natural flavor additives on yogurt properties, ground cardamom (origin: India; composition: proteins – 11%, fats – 7%, carbohydrates – 68%) was used as the additive.

Preparation of yogurt samples

Yogurt samples with a fat content of 1% were prepared from standardized milk: control sample without additives; sample 1 (2.25 μg Se/100 ml); sample 2 (6.88 μg Se/100 ml); sample 3 (13.75 μg Se/100 ml); sample 4 (0.1% cardamom); sample 5 (0.3% cardamom); sample 6 (0.5% cardamom).

Yogurt samples were prepared in 200 ml volumes. The dietary supplement with SeNPs was added to standardized milk before pasteurization, followed by stirring for 1 minute.

Dry cardamom was added to the standardized milk before pasteurization, during which, with vigorous stirring, the water-soluble extractive compounds of cardamom are extracted into the aqueous phase of the milk, eliminating the need for preliminary preparation of an aqueous cardamom extract (Ismael et al., 2024). Pasteurization was carried out at a temperature of 87±2 °C for 2–3 minutes. To separate the milk from the cardamom segments, the hot mixture was filtered through four-layer cheesecloth. The mixtures, cooled to a temperature of 40±1 °C, were inoculated with pure cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (10^7 colony-forming units (CFU)/ml) and fermented until active acidity reached pH 4.8 or lower. Before fermentation, the dry lyophilized starter culture was activated for 2 hours at 40±1 °C in heat-treated milk. During fermentation, the active acidity of the yogurt samples was determined every hour.

The resulting yogurt samples were cooled to 4±2 °C. After 24 hours, the active acidity and degree of syneresis were determined. The viscosity and thixotropic capacity of the yogurt samples were determined after 14 days of storage, which is the average shelf life of the product. The concentration of lactic acid bacteria in the samples was determined after 1, 7, 14, and 28 days of storage.

Methods

Active acidity (pH) was measured potentiometrically using an ADWA AD1200 ATC laboratory pH/mV/ISE/temperature meter.

The number of lactic acid bacteria cells in yogurt samples was determined by serial tenfold dilutions. The yogurt sample was diluted with sterile saline (0.85% NaCl) and then plated on solid MRSA (De Man-Rogosa-Sharpe agar) medium (CondaLab, Spain). To create anaerobic conditions, the Petri dishes were overlaid with an additional layer of MRSA medium cooled to 45 °C. Colony counts and subsequent conversion to colony-forming units (CFU/ml yogurt) were performed after incubation at 37 °C for 48 hours.

Syneresis of milk protein yogurt curds was determined by centrifugation. After mixing, 25 mL of yogurt in a calibrated tube was centrifuged using a Sigma 2-6E laboratory centrifuge (Germany) for 20 min at 1,000 rpm and 20 °C. The volume of separated whey was measured and expressed as mL per 100 g of product (Polischuk et al., 2020).

The effective viscosity of yogurt samples was measured using a Kinexus Pro+ rotational rheometer (Malvern Instruments Ltd, United Kingdom). For the study, the upper geometry C25 DIN L0142 SS and lower geometry PC25 DIN C0350 AL were selected. Prior to measurement, yogurt samples were mixed for 30 s, poured into the measurement cylinder, the upper geometry was lowered, and the sample was equilibrated to 10 °C for 5 min. Effective viscosity was determined during a shear rate sweep in the forward direction (0.1–100 s^{-1}) and in the reverse direction (100–0.1 s^{-1}). Viscosity and thixotropic behavior of control and experimental yogurt samples were evaluated on the 14th day of storage, corresponding to the average shelf-life period when the product exhibits its most pronounced rheological characteristics.

The degree of structural recovery of yogurt samples, upon decreasing the shear rate to 0.1 s^{-1} at the end of rotational viscosity measurements, was determined using a calculation method, with the effective viscosity at the beginning of the measurement taken as 100% (Liang et al., 2022).

Statistical analysis

All assays were performed in triplicate. Means±standard deviation or mean values were reported. Statistical analysis of data was done using a one-way analysis of variance (ANOVA) and the differences between the means of samples were analyzed by Tukey's test at a significance level of 0.05.

Results and discussion

Activity and viability of lactic acid bacteria during yogurt fermentation and storage

Yogurt samples were fermented and stored at a temperature of 4±2°C for 2 hours. The cell concentration of lactic acid bacteria *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in fresh yogurt were determined (Figure 1).

Addition of Se-containing biopreparation had a significant effect on lactic acid bacteria cell counts in yogurt samples, exhibiting a characteristic U-shaped dose–response relationship. A similar dose–response relationship was reported by Yang et al. (2017). When the threshold selenium concentration is exceeded, bacterial growth may be suppressed due to the formation of reactive oxygen species and damage to cell membranes. In the present study, sample 1 contained a very low selenium dose and showed LAB counts comparable to those of the control, indicating no pronounced effect of selenium on the microbiological background of yogurt. In contrast, sample 2, with a selenium content of 6.88 μg/100 mL, demonstrated significant activation of *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, with cell numbers increasing 1.8-fold compared with the control (223×10^8 vs. 123×10^8 CFU/mL). This stimulation of LAB growth may be attributed to selenium involvement in the synthesis of antioxidant enzymes, thereby reducing oxidative stress in bacterial cells during fermentation (Zan et al., 2024). However, a further increase in selenium content in sample 3 resulted in a sharp decline in LAB counts, indicating inhibition of bacterial activity. Thus, determining the optimal dosage of the

bioadditive for yogurt fortification is essential, as selenium concentration directly affects the development of starter culture lactic acid bacteria.

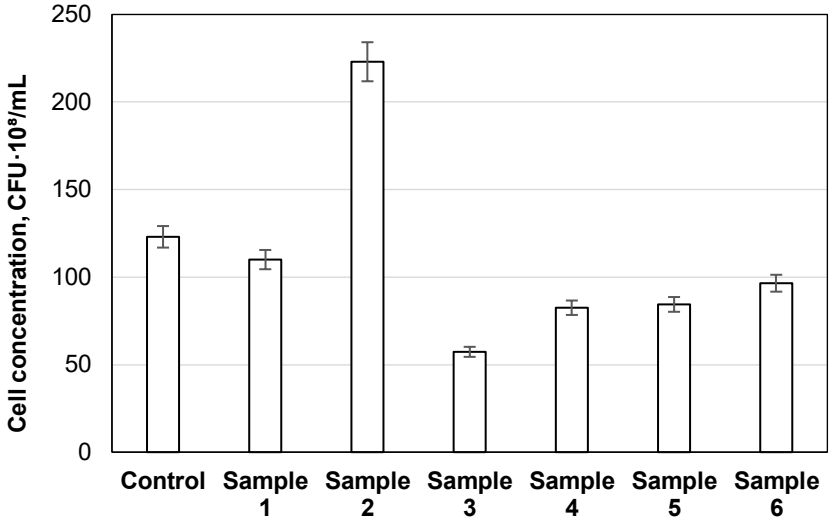


Figure 1. Lactic acid bacteria cell concentration in fresh yogurt

Regarding yogurt samples 4–6, a moderate inhibition of LAB activity by cardamom extractive compounds at the selected dosage was observed, as evidenced by a 1.3–1.5-fold reduction in bacterial cell counts compared to the control sample. Nevertheless, the overall LAB population in samples 4–6 remained sufficiently high, ranging from 82.5×10^8 to 96.5×10^8 CFU/mL, which is considered technologically acceptable for fermented dairy products.

Available literature data on the effects of cardamom extracts on lactic acid bacteria are inconsistent. In particular, Ismael et al. (2024) reported no inhibitory effect of aqueous cardamom extract on LAB at low concentrations and noted the formation of a product with high sensory quality and stable pH values. In contrast, the findings of Sobhy et al. (2023) demonstrated pronounced antibacterial activity of 1,8-cineole, the main component of cardamom essential oil, which may suppress not only pathogenic microorganisms but also other bacterial species, depending on the dosage and the composition of the growth medium. Therefore, further investigation of the effects of biologically active compounds of cardamom is required in each specific application, including their influence during product storage.

Additionally, the degree of syneresis and active acidity of the yogurt samples were studied (Table 1).

The values of active acidity of the yogurt samples generally correlate with the activity of lactic acid bacteria and indirectly confirm the relationship between these parameters. Regarding syneresis, the water-holding capacity is also associated with LAB activity, as these microorganisms are capable of producing viscous exopolysaccharides (EPS) (Brüls et al., 2024). The macromolecules of these biopolymers can significantly modify the microstructure of yogurt gels, in particular by binding water, reducing whey separation, and, under certain conditions, forming complex structured gels with milk proteins. However, this effect depends on the molecular structure and quantity of EPS produced (Jurášková, 2022). Therefore, the rheological characteristics of yogurt samples containing functional additives were also investigated in this research.

Table 1

Degree of syneresis and active acidity of yogurt samples

Yogurt samples	Degree of syneresis, mL/ 100 mL	Active acidity, pH
Control	30.3^{ab} ±1,7	4.68^{ab}±0.17
with LBLC + SeNPs		
Sample 1	19.0^{c}±1.1	4.72^{ab}±0.20
Sample 2	16.1^{d}±0.9	4.48^{b}±0.13
Sample 3	22.3^{bc}±0,9	4.78^{a}±0.15
with cardamom		
Sample 4	24.8^{b}±1.0	4.74^{ab}±0.20
Sample 5	28.5^{ab}±1.2	4.71^{ab}±0.21
Sample 6	31.2^{a}±1.5	4.70^{ab}±0.18

Note: Values are the means±standard deviation. Means within the same column with different superscripts are significantly different at $p \leq 0.05$.

Changes in the concentration of viabile LAB cells in yogurt samples during storage for up to 28 days is shown in Figure 2a, b, and their final amounts are shown in Figure 3.

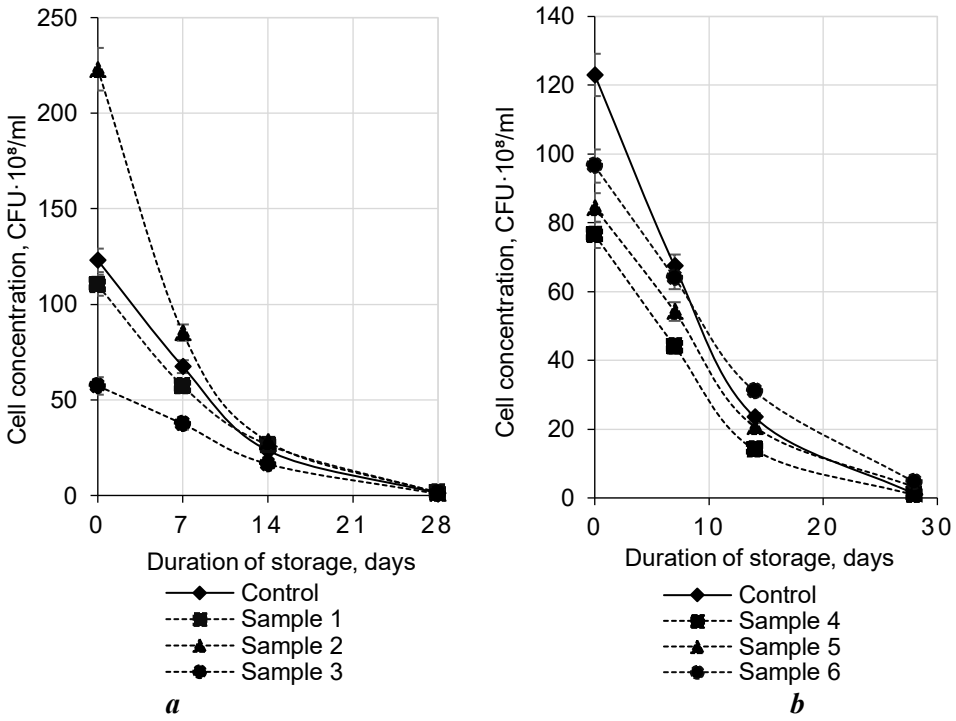


Figure 2. Changes in concentration of lactic acid bacteria cells during storage of yogurt samples with selenium (a) and cardamom (b)

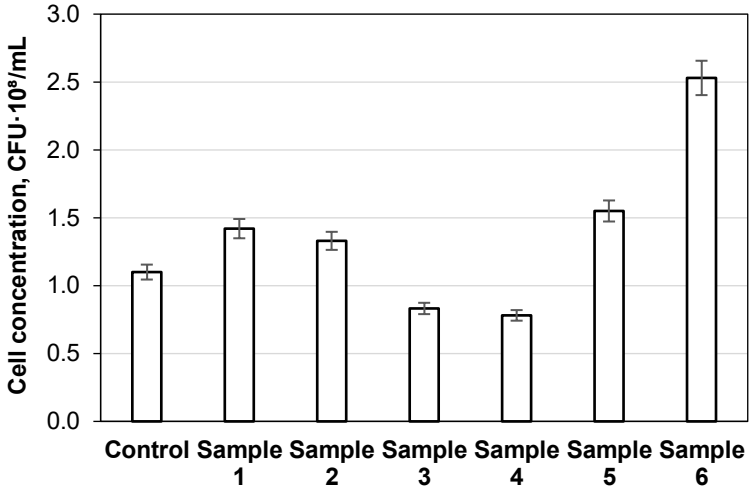


Figure 3. Lactic acid bacteria cell counts in yogurt samples on the 28th day of storage

According to Figures 2a and 3, the number of LAB cells in the yogurt samples containing the biopreparation LBLC + SeNPs gradually approaches that of the control, despite a substantial initial difference. This convergence is most pronounced between days 14 and 28, particularly in yogurt sample 1. Sample 2 exhibits the greatest change, whereas sample 3 shows the least variation in LAB counts in the yogurt during prolonged storage.

For the yogurt samples containing cardamom (samples 4–6), a gradual, nearly linear decrease in LAB cell counts is observed throughout the entire storage period (Figures 2b and 3).

According to international requirements (Codex Alimentarius, STAN 243-2003), yogurt must contain at least 10^7 CFU/g of viable microorganisms (in our case *L. bulgaricus* and *S. thermophilus*) at the time of consumption. All yogurt samples fully complied with these requirements throughout the selected storage period, despite the observed partial decline in LAB cell viability, especially between days 14 and 28.

However, different effects of functional additives on LAB cell concentrations in yogurt samples on day 28 (Figure 3) can be observed, particularly for samples 2 and 6. The maximum LAB count recorded in freshly prepared yogurt sample 2, supplemented with a selenium-containing preparation to achieve a Se concentration of 6.88 μg/100 mL, indicates stimulation of bacterial growth; however, after 7–14 days of storage, LAB counts approach those observed in the control sample.

At the same time, the freshly prepared Sample 6, containing the maximum level of cardamom (0.5%), exhibited the highest LAB content, indicating that compounds present in this spice support bacterial viability. This effect was similarly observed by Ismael et al. (2024).

The values of active acidity and syneresis of the yogurt samples generally correlate with the activity of lactic acid bacteria and indirectly confirm the relationship between these parameters. Functional additives exhibit different effects on the fermentation of the milk mixture and on the content of viable lactic acid bacteria cells in yogurt samples during fermentation. To activate LAB during fermentation and thus shorten the duration of the

technological cycle, it is advisable to use a biopreparation LBLC + SeNPs providing a selenium content of 6.88 μg/100 mL. Cardamom at concentrations of 0.1–0.5% does not exert a significant effect on fermentation. The determined doses of functional additives are considered rational for preventing excessive post-acidification of yogurt containing cardamom.

Influence of functional additives on the rheological characteristics of yogurt

The rheological characteristics of yogurt depend on the composition of the milk base, particularly the content of total solids, protein, fat, and carbohydrates (Yu et al., 2016), as well as on the technological processing conditions applied to it (Ilić et al., 2024; Prócel et al., 2025; Sözeri Atik et al., 2024). A significant effect on yogurt viscosity and the structure of the protein gel is exerted by lactic acid bacteria, which are capable of producing viscous exopolysaccharides (EPS) (Brüls et al., 2024). The functional additives used in this study may indirectly influence EPS production through their specific effects on LAB activity or may suppress bacterial viability by day 14 of storage, which requires further investigation.

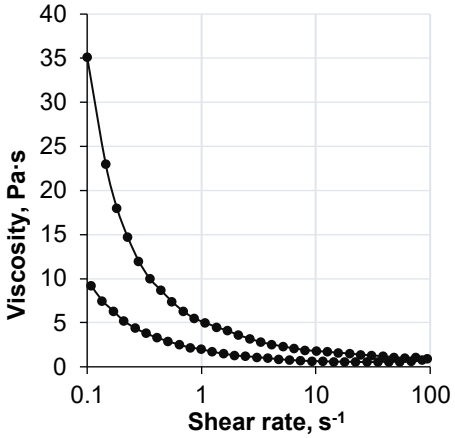
Figure 4 presents the changes in the effective viscosity of the control yogurt and samples containing the lowest and highest levels of the selenium-containing biopreparation (samples 1 and 3) and cardamom (samples 4 and 6) during forward measurement over a shear rate range from $\gamma = 0.1$ s^{-1} to $\gamma = 100$ s^{-1}, as well as during backward measurement with decreasing shear, allowing assessment of the thixotropic behavior of each sample.

To gain a more detailed insight into the behavior of the studied food systems during rotational measurement, the most important values of their rheological characteristics were analyzed (Table 2).

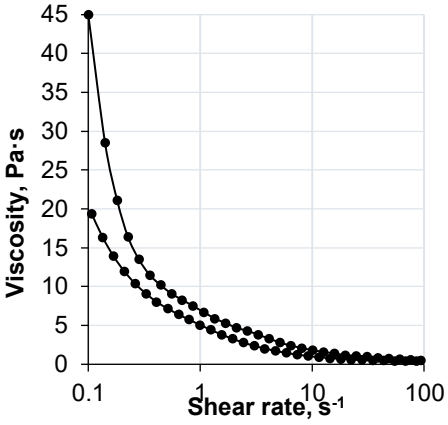
According to Figure 4 and Table 1, the most structured yogurt samples were 2 and 3, whose effective viscosity at the beginning of measurement (65.1172 and 68.2143 mPa·s, respectively) was almost twice that of the control sample (35.1276 mPa·s). This effect can be attributed to the influence of selenium on the metabolism of lactic acid bacteria. In particular, Krausova et al. (2020) found that selenium, especially when used to "selenize" LAB strains, enhances EPS production, which strengthens the protein gel structure. Changes in the viscosity characteristics of all yogurt samples containing the selenium biopreparation LBLC + SeNPs are linked to the effect of this element on LAB activity (Figs. 1 and 3).

The cardamom-containing samples (4–6) exhibited viscosities close to those of the control sample, indicating a minimal effect of this spice on lactic acid bacteria activity. The effect of cardamom on yogurt's rheological characteristics depends on its form (powder, extract, or essential oil) (Ismael et al., 2024). Future studies should examine how different forms of cardamom influence the microbiological and viscosity properties of yogurt.

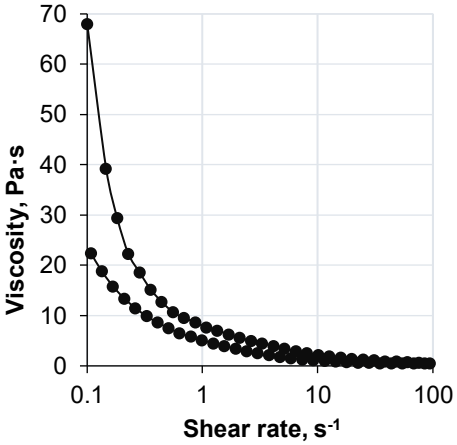
A comparative analysis of the thixotropic behavior of the yogurt samples was conducted using a criterion classifying food systems as exhibiting low or medium thixotropy. After disruption under high shear, yogurt gels typically recover up to 30% of their structure (Lee and Lucey, 2010). This value was therefore adopted as the threshold for assessing an adequate level of thixotropic behavior in the samples.



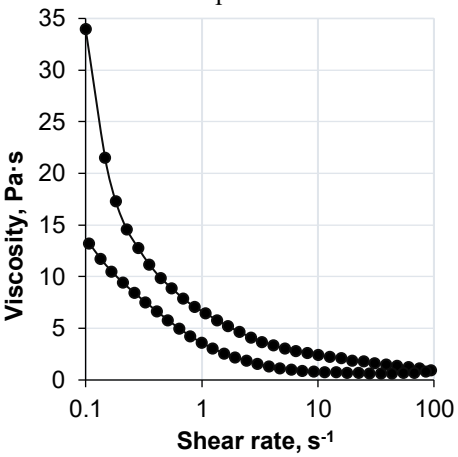
Control



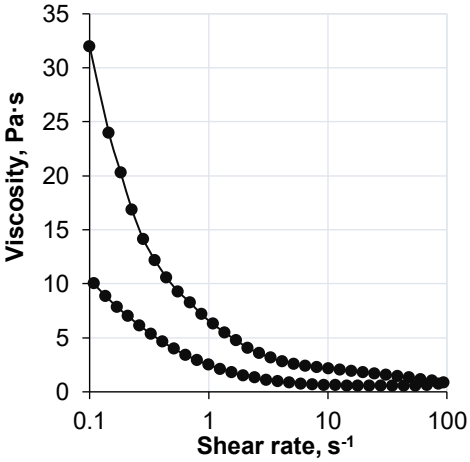
Sample 1



Sample 3



Sample 4



Sample 6

Figure 4. Change in effective viscosity of yogurt during rotational measurement

Table 2

Rheological characteristics of yogurt samples

Sample	Effective viscosity (mPa·s) under varying shear rate gradient			Degree of structure recovery, %
	$\gamma = 0.1\ s^{-1}$ (forward run)	$\gamma = 100\ s^{-1}$	$\gamma = 0.1\ s^{-1}$ (backward run)	
Control	$35.1276^{c} \pm 1.9312$	$0.5326^{c} \pm 0.0173$	$9.2309^{e} \pm 0.2543$	26.34
with LBLC + SeNPs				
Sample 1	$45.0034^{b} \pm 2.0052$	$0.4513^{de} \pm 0.01644$	$19.3601^{b} \pm 1.0525$	43.02
Sample 2	$65.1172^{ab} \pm 2.2107$	$0.5293^{cd} \pm 0.0203$	$23.5216^{a} \pm 1.5753$	36.12
Sample 3	$68.2143^{a} \pm 1.9954$	$0.4780^{d} \pm 0.0199$	$22.4233^{ab} \pm 1.2410$	32.87
with cardamom				
Sample 4	$34.7599^{cd} \pm 1.2954$	$0.6541^{a} \pm 0.02621$	$13.2591^{c} \pm 1.0385$	38.14
Sample 5	$33.4921^{cd} \pm 1.5930$	$0.5966^{b} \pm 0.0199$	$12.8738^{cd} \pm 0.8941$	38.44
Sample 6	$32.7921^{cd} \pm 1.9921$	$0.5628^{bc} \pm 0.0203$	$10.0300^{d} \pm 0.4603$	31.34

Note: Values are the means±standard deviation. Means within the same colunm with different superscripts are significantly different at $p \leq 0.05$.

A low degree of structure recovery (26.34%) was observed for the control sample. The high thixotropic behavior was demonstrated by samples 1, 2, 4, and 5, which contained moderate amounts of functional additives (no more than 2% for the selenium biopreparation and no more than 0.3% for cardamom). Exceeding these doses likely reduces the production of viscous exopolysaccharides by lactic acid bacteria, which, through numerous low-energy interactions, are capable of rapidly restoring the disrupted protein gel structure. In particular, Han et al. (2016) found that EPS macromolecules increase the relaxation time of structural bonds, thereby altering the thixotropy curve. It is also known that EPS reinforce the protein–casein gel by interacting with casein micelles or filling voids within the gel, which increases initial viscosity and provides better mechanical stability (Buldo et al., 2016). Thus, it can be concluded that the functional additives used in the yogurt positively influence the rheological characteristics of the final product, specifically by structuring the gel and enhancing its ability to spontaneously recover its structure after disruption, an important factor in the production of drinking yogurt obtained by tank fermentation.

The appearance of the yogurt samples after 1 day of storage at 4 ± 2 °C is shown in Figure 5.

Figure 5 visually demonstrates the significant impact of functional additives on the consistency of stirred yogurt. A thick, homogeneous texture was observed in sample 2, containing the selenium biopreparation LBLC + SeNPs at a Se concentration of 6.88 µg/100 mL, as well as in sample 5, with cardamom extract added at 0.3%.

Thus, based on a combination of physicochemical and microbiological indicators, the selected functional additives were shown to substantially improve yogurt quality. These findings are of practical significance and support further research on combining the functional-technological properties of LBLC + SeNPs and cardamom to develop a new type of fortified yogurt.

Control

Sample 1

Sample 2

Sample 3

Sample 4

Sample 5

Sample 6

Figure 5. Appearance of yogurt samples

Conclusions

The selenium-containing biopreparation LBLC + SeNPs affects lactic acid bacteria (LAB) activity in yogurt in a U-shaped dose-dependent manner, with the optimal effect at 6.88 μg/100 mL, while higher concentrations inhibit bacterial activity. Cardamom extracts slightly reduce LAB activity, including during storage. The addition of LBLC + SeNPs improved yogurt viscosity and thixotropic behavior, whereas cardamom mainly enhanced thixotropy. Using these functional additives at appropriate doses enhanced overall yogurt quality by maintaining LAB viability, improving gel recovery, and preventing excessive post-acidification.

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Intensification of gibberellin synthesis by surfactant producers *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017

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Abstract

Introduction. The surfactant-producing strains *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 can synthesize gibberellin phytohormones, although generally at low levels. One approach to enhance gibberellin production is the introduction of biosynthetic precursors into the cultivation medium, which may stimulate metabolic flux toward hormone formation.

Materials and methods. Bacteria were cultivated in liquid media supplemented with 100–500 mg/L erythritol, which was introduced either during the lag phase or at the onset of the stationary growth phase. Phytohormone concentrations were quantified using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Extracellular surfactants were extracted with a methanol–chloroform mixture, and the activity of key enzymes involved in surfactant and gibberellin biosynthesis was measured in cell-free extracts using spectrophotometric assays.

Results and discussion. In the presence of 300–500 mg/L erythritol as an exogenous precursor, and regardless of the carbon source used in the culture medium (refined oil, ethanol, or biodiesel production waste), the concentration of biologically active gibberellins GA_3 and GA_4 synthesized by *Acinetobacter calcoaceticus* and *Rhodococcus erythropolis* increased significantly by approximately 1.5- to 16-fold relative to cultures grown without erythritol. This pronounced enhancement indicates that erythritol effectively increases metabolic flux through the gibberellin biosynthetic pathway.

Under such conditions, a 1.4–1.7-time increase in the C-methyl-D-erythritol-4-phosphatecytidylyl transferase activity, a key enzyme of these phytohormones biosynthesis in the methyl-erythritol-4-phosphate pathway, was observed in the cells of both strains. The presence of erythritol in the cultivation medium of strains IMV B-7241 and Ac-5017 didn`t affect the surfactant synthesis.

Conclusions. The data obtained provide a basis for the development of an efficient, integrated technology for the co-synthesis of surface-active compounds and phytohormones, with potential applications in plant production.

Introduction

Recently, there has been a growing interest among biotechnology researchers in so-called integrated microbial technologies (Linda et al., 2024; Katagi et al., 2024; de Siqueira et al., 2024). Their appeal lies in the fact that several valuable metabolites can be obtained during the implementation of a single technological process, which allows significantly reducing the cost of target products, increasing their competitiveness in the market and the overall efficiency of biosynthesis.

Previous studies (Pirog et al., 2019) demonstrated that the surfactant-producing strains *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 are capable of synthesising phytohormonal compounds, including auxins, cytokinins, and gibberellins, on various substrates such as sunflower oil and biodiesel production waste. In addition, the positive effects of the strains' exometabolites on crop yield have been reported (Piatetska and Pirog, 2023). However, the concentrations of phytohormones produced by strains IMV B-7241 and IMV Ac-5017 were relatively low, substantially limiting the effectiveness of the complex preparation for crop production.

It has been shown that the synthesis of auxin-type phytohormones can be enhanced by supplementing the culture medium of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 with exogenous tryptophan, a key precursor of auxin biosynthesis (Pirog et al., 2020). Based on these findings, it was hypothesised that a similar strategy, namely, the addition of an exogenous precursor of the target product biosynthesis, could also be effective in stimulating gibberellin synthesis.

In eukaryotes, including industrial producers of gibberellins, these phytohormones are synthesised via pathways involving mevalonic acid (Salazar-Cerezo et al., 2018). Until the late 1990s, the mevalonate pathway was considered the only mechanism for the biosynthesis of isoprenoid precursors, including gibberellins. However, in the 1990s, the methyl-erythritol-4-phosphate (MEP) pathway for isoprenoid biosynthesis was discovered, which functions in bacteria, green algae, and higher plants (Rohmer et al., 1993).

The MEP pathway begins with the condensation reaction of pyruvate and D-glyceraldehyde-3-phosphate to form 1-deoxy-D-xylulose-5-phosphate, which is subsequently converted to 2-C-methyl-D-erythritol-4-phosphate (Rohmer et al., 1993). Since gluconeogenesis reactions are required for the synthesis of MEP pathway intermediates during cultivation on non-carbohydrate carbon sources, it has been suggested that the addition of exogenous erythritol, a possible precursor for gibberellin biosynthesis, to the medium may be accompanied by an increase in the synthesis of these phytohormones.

In this context, the aim of this study was to identify the key enzymes involved in the methylerythritol 4-phosphate pathway in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, and to evaluate the effect of exogenous erythritol on the synthesis of gibberellins and surfactants during cultivation of these strains on non-carbohydrate substrates.

Materials and methods

Research objects

The research objects were strains of oil-oxidising bacteria isolated from oil-contaminated soil, identified as *Acinetobacter calcoaceticus* K-9 and *Rhodococcus erythropolis* KU-8 and registered in the Depository of Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine under the numbers IMV B-7241 and IMV Ac-5017, respectively.

Cultivation conditions

Cultivation of *R. erythropolis* IMB Ac-5017 was carried out in a liquid mineral medium (g/L): $NaNO_3$ – 1.3, NaCl – 1.0, $Na_2HPO_4 \cdot 12H_2O$ – 0.6, KH_2PO_4 – 0.14, $MgSO_4 \cdot 7H_2O$ – 0.1, $FeSO_4 \cdot 7H_2O$ – 0.001, pH 6.8–7.0. The source of carbon and energy was ethanol, as well as refined oil at a concentration of 2% (volume fraction) (Pirog et al., 2022a, b).

A. calcoaceticus IMB B-7241 was cultivated in a medium with the following composition (g/L): $(NH_2)_2CO$ – 0.35, $MgSO_4 \cdot 7H_2O$ – 0.1, NaCl – 1.0, Na_2HPO_4 – 0.6, KH_2PO_4 – 0.14, pH 6.8–7.0. The medium was supplemented with yeast autolysate – 0.5% (by volume) and a solution of trace elements – 0.1% (by volume). A solution of trace elements with the following composition (g/100 ml): $ZnSO_4 \cdot 7H_2O$ – 1.1, $MnSO_4 \cdot H_2O$ – 0.6, $FeSO_4 \cdot 7H_2O$ – 0.1, $CuSO_4 \cdot 5H_2O$ – 0.004, $CoSO_4 \cdot 7H_2O$ – 0.03, H_3BO_3 – 0.006, KI – 0.0001, EDTA – 0.5. pH 6.8–7.0. Biodiesel production waste (biofuel plant, Zaporizhzhia region) and refined sunflower oil at a concentration of 2.0% (volume fraction) were used as a carbon source (Pirog et al, 2022).

Erythritol was added as a 1% solution at the beginning of the process or at the end of the exponential growth phase at a concentration of 100−500 mg/L.

As an inoculum, a culture in the exponential growth phase was used, grown in a medium of the above composition with 0.5% (volume fraction) of the corresponding substrate. The amount of inoculum (10^4–10^5 cells/ml) was 5–10% of the volume of the nutrient medium. Bacteria were cultivated in 750 ml flasks with 100 ml of medium on a rocker (320 rpm) at 28–30 ºC for 168 h.

Determination of surfactants and phytohormones concentration

After the *R. erythropolis* IMB Ac-5017 and *A. calcoaceticus* IMB B-7241 cultivation in a medium with refined oil, the residual oil was removed from the culture liquid by three-fold extraction with hexane (ratio 1:1). The biomass was separated by centrifugation (5000 g) for 25 min.

The surfactant isolation was carried out using modified method of Bligh and Dyer (1959), after extraction with a chloroform and methanol mixture (2:1) from the supernatant of the culture liquid as described in our work (Pirog et al., 2024). The concentration of extracellular surfactants (g/l) was determined by the gravimetric method.

Extracellular phytohormones, gibberellins, were isolated from the supernatant of the culture liquid by extraction with ethyl acetate at a pH of 2.5. The obtained extracts were evaporated in a vacuum at 40–45 °C. The dry residue was dissolved in 80% ethanol and transferred to microtubes. The obtained extracts were stored at a temperature of 24 °C. Preliminary purification and concentration of phytohormonal extracts (accumulating thin-layer chromatography) were carried out on plates with silica gel brand «Silufol UV254» (Chemapol, Czech Republic) in a mixture of solvents introduced sequentially: chloroform, 12.5% aqueous ammonia, and ethyl acetate: acetic acid (20:1). The qualitative and quantitative composition of gibberellins was analysed by high-performance liquid chromatography using an Agilent 1200 liquid chromatograph equipped with G6120A mass detector (Agilent Technologies, USA).

The separation of aliquots of extracts was carried out on an analytical column (Zorbax Eclipse Plus C18, 4.6 mm × 250 mm, 5 μm) (Agilent Technologies, USA). The column thermostat temperature was maintained at 30 °C, and the injection volume was 20 μL. Elution was carried out in the system acetonitrile – water+acetic acid in a gradient mode: 0 min: acetonitrile/0.1% solution of acetic acid in deionized water (30/70) – 20 min:

acetonitrile/0.1% solution of acetic acid (70/30) – 30 min: acetonitrile/0.1% acetic acid solution (100/0) at a constant flow rate of 0.5 ml per minute. The duration of the column equilibration after analysis (post-run) was 15 min. Standards of gibberellins GA_3 and GA_4 (Sigma-Aldrich, Germany) were used for identification.

Gibberellins were detected using a diode array detector with signal recording at wavelength 210 nm. The molecular weight was determined using a single-quadrupole mass spectrometric detector. Ionisation was performed in the combined mode (electrospray and chemical ionisation at atmospheric pressure), resulting in the formation of negative ions. Ion detection was carried out in the SCAN and SIM (selected ion monitoring) modes, with a mass-to-charge (m/z) range of 200-500. GA_3 and GA_4 were identified by comparing the retention times, molecular mass values of ions, and spectral characteristics of the obtained peaks. The quantitative content of GA_3 and GA_4 was determined by the method of external calibration using the SIM mode for ions 345 and 331 m/z (monitoring of the 345 and 331 m/z values according to the time table).

HPLC/MS analysis of gibberellin extracts of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 was performed at the Center for the Collective Use of Chromatography and Mass Spectrometry of the National Academy of Sciences of Ukraine at M.G. Kholodny Institute of Botany of the NAS of Ukraine.

Enzymatic analyses

Trehalose phosphate synthase (EC 2.4.1.15) activity was analysed by the formation of uridine diphosphate, which was determined spectrophotometrically by the oxidation of NADH at 340 nm in coupled reactions with pyruvate kinase and lactate dehydrogenase.

The activity of 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase (EC 2.7.7.60), one of the key enzymes of the MEP pathway of gibberellin biosynthesis, was determined by the rate of lactate formation, which was determined by the oxidation of NADH at 340 nm in a conjugate reaction with pyruvate kinase and lactate dehydrogenase (Kuzuyama et al., 2000).

Statistical processing

All experiments were conducted in triplicate or more, and the results were expressed as mean±standard deviation.

Results and discussion

Activity of the methyl-erythritol-4-phosphate pathway key enzyme in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 isoprenoid biosynthesis

The enzyme 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase catalyses the formation of 4-(cytidine5'-diphospho)-2-C-methyl-D-erythritol from cytidine triphosphate (CTP) and 2-C-methyl-D-erythritol-4-phosphate, a key intermediate in the methyl-erythritol-4-phosphate pathway of isoprenoid biosynthesis (Diamanti et al., 2022; Kuzuyama et al., 2000). The presence of 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase activity in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 cells grown on different substrates (Table 1) may indicate the functioning of the MEP pathway in these bacteria.

Table 1

Activity of the methyl-erythritol-4-phosphate pathway key enzyme during the growth of *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017 on different substrates

Strain	Growth substrate	2-C-methyl-D-erythritol-4-phosphate-cytidylyltransferase activity, nmol/min·mg protein
Acinetobacter calcoaceticus	Refined sunflower oil	5263±263
	Biodiesel production waste	667±33
Rhodococcus erythropolis	Refined sunflower oil	278±13
	Ethanol	103±5

The data presented in Table 1 served as the basis for further studies to determine the effect of erythritol on the gibberellins synthesis in surfactant producers.

Gibberellin synthesis in erythritol-supplemented cultures of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017

Today, it has been proven that the ability to synthesise phytohormones of gibberellin nature is inherent in many living organisms. Various species of plants, bacteria, fungi and yeast produce more than 130 forms of gibberellins (Hernández Rodríguez et al., 2024). At the same time, only some of them, in particular GA_1, GA_3, GA_4 and GA_7, are characterised by high biological activity, while other forms are physiologically inactive and serve as intermediates in the biosynthesis of active gibberellins. Our previous studies (Leonova et al., 2020) showed that A. calcoaceticus IMV B-7241 and R. erythropolis IMV Ac-5017 produce GA_3 and GA_4.

In experiments on the effect of erythritol on the gibberellin synthesis, surfactant producers were grown in a medium with refined oil, biodiesel production waste, and ethanol.

Our previous studies (Pirog et al., 2020) have established a positive effect of exogenous tryptophan (a precursor of the phytohormone auxins biosynthesis) on the auxins formation during the cultivation of A. calcoaceticus IMV B-7241 on biodiesel production waste and R. erythropolis IMV Ac-5017 on ethanol. Thus, there is a potential opportunity to increase the synthesis of both auxins and gibberellins on these substrates with the simultaneous introduction of tryptophan and erythritol into the medium. This, in turn, will significantly enhance the efficiency of integrated technologies for surfactant and phytohormone production, as well as the practical application of these exometabolites in plant production.

The erythritol concentration choice (100-500 mg/L) and the moment of its introduction into the medium (lag phase and the beginning of the stationary phase) was because the concentration of exogenous precursors is usually 10–20% of the concentration of the main growth substrate, and phytohormones are secondary metabolites, the synthesis of which begins in the stationary phase of growth (Cen et al., 2020).

Table 2 shows the biologically active gibberellin synthesis in the presence of erythritol in the culture medium of A. calcoaceticus IMV B-7241.

The data presented in Table 2 show that during the cultivation of strain IMV B-7241 on biodiesel production waste, the concentration of GA_3 increased with an increase in the precursor content in the medium.

Table 2

Effect of erythritol on the biologically active gibberellins synthesis by *Acinetobacter calcoaceticus* IMB B-7241

Substrate	Erythritol concentration, mg/L	Erythritol addition (growth phase)	Gibberellin concentration, % of control	
			GA_3	GA_4
Refined sunflower oil	100	lag phase	116	79
		beginning of stationary phase	187	84
	200	lag phase	406	80
		beginning of stationary phase	909	87
	300	lag phase	911	178
		beginning of stationary phase	1590	182
	400	lag phase	938	165
		beginning of stationary phase	1500	178
	500	lag phase	476	115
		beginning of stationary phase	586	115
Biodiesel production waste	100	lag phase	21	87
		beginning of stationary phase	55	96
	200	lag phase	25	101
		beginning of stationary phase	64	115
	300	lag phase	33	159
		beginning of stationary phase	97	170
	400	lag phase	43	174
		beginning of stationary phase	429	169
	500	lag phase	60	156
		beginning of stationary phase	984	158

Note: Control – concentration of gibberellins in medium without erythritol.

The amount of GA_4 practically didn`t depend on the moment of erythritol introduction, unlike GA_3, the concentration of which was higher in the case of the precursor introduction at the beginning of the stationary growth phase. The optimal concentration of erythritol for the GA_3 formation was a concentration of 500 mg/L (an increase in synthesis of almost 10 times compared to that without the precursor). An increase in GA_4 synthesis by 156–174% was observed at erythritol concentrations from 300 to 500 mg/L. Interestingly, regardless of the application time, lower concentrations (100–300 mg/L) of erythritol had a more positive effect on GA_4 formation (increase in synthesis of 87–170%) than GA_3 (increase in synthesis of 21–97%). At the same time, when applied in the stationary phase of growth at higher concentrations (400–500 mg/L), the precursor had a more positive effect on the GA_3 formation (increase in the amount by 429–984%) (Table 2).

During the cultivation of *A. calcoaceticus* IMV B-7241 on refined oil, the GA_3 and GA_4 amounts increased with increasing erythritol concentration from 100 to 400 mg/L. In comparison, at 500 mg/L of the precursor, the synthesis of both gibberellins decreased (see Table 2). The synthesis of GA_4 (as well as on biodiesel production waste) did not depend on the moment of adding erythritol to the medium with refined oil. At the same time, the concentration of GA_3 was higher when the precursor was added in the stationary phase of growth compared to its addition at the beginning of the strain IMV B-7241 cultivation process. The optimal erythritol content in the medium for the formation of GA_3 was 300–400 mg/L (an increase in synthesis by 911–938 and 1500–1590% when the precursor was added in the lag and stationary phases, respectively) (Table 2).

It should be noted that the effect of adding erythritol to a medium with refined oil or biodiesel production waste on the formation of GA_4 was practically the same: in the presence of the precursor (depending on its concentration), an increase in the synthesis of this phytohormone by 79–182% was observed compared to the indicators without erythritol. Other patterns were found for the GA_3 formation in the presence of the precursor in a medium with various substrates: the introduction of different concentrations of erythritol into a medium with refined oil and biodiesel production waste was accompanied by an increase in the synthesis of GA_3 by 116-1590 and 21-984%, respectively (Table 2).

In our opinion, the substrate-dependent effect of the precursor on the GA_3 synthesis can be explained as follows. The order of gibberellin synthesis from 7-hydroxykaurenic acid is as follows (Kamiya, 2025):

gibberellin GA_{14} → gibberellin GA_4 → gibberellin GA_7 → gibberellin GA_1 → gibberellin GA_3.

Since GA_4 is an intermediate metabolite in the synthesis of GA_3, it is quite likely that the composition of biodiesel production waste contains components that inhibit the activity of enzymes responsible for the transformation of GA_4 into GA_3. Such inhibitors of enzyme activity can be monovalent cations (potassium and or sodium), or alcohols (ethanol, methanol).

Table 3 presents data on the erythritol effect in the cultivation medium of *R. erythropolis* IMV Ac-5017 on the synthesis of biologically active gibberellins.

Table 3

Effect of erythritol on the biologically active gibberellin synthesis by *Rhodococcus erythropolis* IMB Ac-501

Substrate	Erythritol concentration, mg/L	Erythritol application time (growth phase)	Gibberellin concentration, % of control	
			GA_3	GA_4
Refined sunflower oil	100	lag phase	125	131
		beginning of stationary phase	105	113
	200	lag phase	161	156
		beginning of stationary phase	135	129
	300	lag phase	262	234
		beginning of stationary phase	158	130
	400	lag phase	380	361
		beginning of stationary phase	211	151
	500	lag phase	177	109
		beginning of stationary phase	150	58
Biodiesel production waste	100	lag phase	121	116
		beginning of stationary phase	132	129
	200	lag phase	130	125
		beginning of stationary phase	143	138
	300	lag phase	152	136
		beginning of stationary phase	186	157
	400	lag phase	171	164
		beginning of stationary phase	233	206
	500	lag phase	305	287
		beginning of stationary phase	468	407

Note: Control – concentration of gibberellins in medium without erythritol.

Regardless of the growth substrate nature in the cultivation medium of *R. erythropolis* IMB Ac-5017, the increase in the content of the gibberellin biosynthesis precursor was accompanied by an increase in the concentration of the formed gibberellins GA_3 and GA_4. The maximum increase in the phytohormone amount during the cultivation of the strain IMB Ac-5017 on ethanol (361-380% of the control) was achieved in the presence of 400 mg/L of erythritol, and on refined oil (407-468% of the control) – in the presence of 500 mg/L of the precursor in the medium (see Table 3). It should be noted that the level of gibberellin synthesis depended not only on the erythritol concentration, but also on the moment of its introduction into the cultivation medium of *R. erythropolis* IMB Ac-5017.

Thus, the concentration of synthesised phytohormones was higher in the case of adding an exogenous precursor in the lag phase of the producer's growth on ethanol compared to that when adding erythritol at the beginning of the stationary phase (125–380 and 105–211%, respectively). In the process of growing the strain IMV Ac-5017 on refined oil, the gibberellins synthesis was higher when adding erythritol at the beginning of the stationary growth phase than at the start of the process (129-407 and 116-287%, respectively) (see Table 3). It should be noted that regardless of the erythritol concentration and the moment of its introduction into the cultivation medium of *R. erythropolis* IMB Ac-5017 with both ethanol and refined oil, an almost identical increase in the synthesis level of both biologically active gibberellins GA_3 and GA_4 was observed.

At the next stage, the key enzyme of gibberellin biosynthesis activity was determined in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 cells grown on various substrates in the presence of erythritol (Table 4).

Table 4

Effect of erythritol on the key enzyme of gibberellin biosynthesis activity in *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017

Strain	Substrate	Erythritol concentration, mg/L	2-C-methyl-D-erythritol-4-phosphatecytidyl transferase activity, nmol/min·mg protein
Acinetobacter calcoaceticus IMB B-7241	Refined sunflower oil	0	5263±263
		300	7519±375
	Biodiesel production waste	0	667±33
		500	1071±53
Rhodococcus erythropolis IMB Ac-5017	Refined sunflower oil	0	278±13
		500	484±24
	Ethanol	0	103±5
		400	159±7

Note: Erythritol was added to the culture medium at the beginning of the stationary phase of growth of strains IMV B-7241 and IMV Ac-5017.

The data presented in Table 4 indicate that the exogenous precursor is involved in the gibberellins synthesis in the methyl-erythritol-4-phosphate pathway: 2-C-methyl-D-erythritol-4-phosphatecytidylyl transferase activity in cells of both strains grown under conditions of maximum increase in the gibberellin phytohormones synthesis (see Tables 2 and 3) was 1.4–1.7 times higher than during the cultivation of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 in a medium without erythritol.

In our work (Pirog et al., 2025), it was noted that, unlike a fairly large number of publications on the auxin biosynthesis intensification in the presence of the precursor tryptophan, there is no information about the effect of erythritol on the formation of gibberellins by microorganisms. In our opinion, one of the reasons for this state of affairs is the fact that at present the main highly efficient producers of gibberellins are the fungi *Gibberella fujikuroi* (now reclassified as *Fusarium fujikuroi*) and *Fusarium moniliforme*, in which the synthesis of these phytohormones is carried out via mevalonic acid (Salazar-Cerezo et al., 2018), and not in the methyl-erythritol-4-phosphate pathway, as in bacteria. In addition, the fungi *F. fujikuroi* and *F. moniliforme* are industrial producers of gibberellic acid (GA_3); therefore, primary scientific research today focuses on increasing the efficiency of biotechnology based on these strains.

Thus, at present, the main approaches to intensification of gibberellin synthesis by the fungi *F. fujikuroi* and *F. moniliforme* are the conditions for cultivating producers optimisation and their improvement by metabolic and genetic engineering methods (Cen et al., 2020; 2023; Hernández Rodríguez et al., 2024; Peng et al., 2020; Shani et al., 2024; Wang et al., 2023).

Previously (Pirog et al., 2025), we found that the introduction of 300-400 mg/L erythritol into the cultivation medium of the surfactant producer *Nocardia vaccinii* IMV B-7405 was accompanied by a 2–14 times increase in the concentration of biologically active gibberellins GA_3 and GA_4 compared to the synthesis indicators in the medium without the precursor. This work is a logical continuation of our previous studies on enhancing integrated microbial technologies for plant production. In addition, these studies suggest that introducing exogenous precursors of the biosynthesis of the target product into the cultivation medium of the producer is a simple and highly effective method for intensifying the synthesis of practically important metabolites.

Effect of erythritol on surfactant synthesis in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017

At the final stage, the level of surfactant synthesis was determined during the cultivation of producers in a medium with erythritol (Table 5).

The data in Table 5 show that the presence of erythritol in the cultivation medium of both strains with different growth substrates didn`t affect the indicators of surfactant synthesis.

Surfactants as well as phytohormones are classified as secondary metabolites. The producer's cultivation conditions determine the composition and properties of these compounds. Therefore, it is impossible to guarantee that preparations synthesised in the presence of erythritol will possess the biological properties necessary for their effective use in plant production, in particular, antimicrobial activity against phytopathogenic bacteria. The main component of the surfactant complex of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, responsible for antimicrobial activity, is aminolipids (Pirog et al., 2020). The results of enzymatic studies showed that during the cultivation of both strains under conditions that ensure maximum gibberellins synthesis, the key enzyme activity of surface-active aminolipids was 1.4–3.6 times higher than during cultivation in a medium without a precursor (Table 6). The activity of trehalose phosphate synthase, the key enzyme of glycolipid (trehalose mycolate) biosynthesis in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, was practically the same under the growth conditions of both strains in the presence and absence of erythritol.

Table 5

Surfactant synthesis by *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017 in the presence of erythritol

Strain	Substrate	Erythritol addition (growth phase)	Erythritol concentration, mg/L	Surfactant concentration, g/L
Acinetobacter calcoaceticus IMB B-7241	Refined sunflower oil	–	0	1.74±0.09
		lag phase	300	1.63±0.08
		beginning of stationary phase	300	1.81±0.09
	Biodiesel production waste	–	0	2.84±0.14
		lag phase	500	2.71±0.13
		beginning of stationary phase	500	3.08±0.15
Rhodococcus erythropolis IMB Ac-5017	Ethanol	–	0	1.89±0.09
		lag phase	400	1.62±0.08
		beginning of stationary phase	400	1.71±0.09
	Refined sunflower oil	–	0	1.22±006
		lag phase	500	1.05±005
		beginning of stationary phase	500	1.12±0.05

Table 6

Erythritol effect on key enzyme of surface-active glyco- and aminolipid biosynthesis activity in *Acinetobacter calcoaceticus* IMB B-7241 and *Rhodococcus erythropolis* IMB Ac-5017

Strain	Substrate	Erythritol concentration. mg/L	Activity, nmol/min·mg protein	
			$NADP^+$-glutamate dehydrogenase	trehalose phosphate synthase
Acinetobacter calcoaceticus IMB B-7241	Refined sunflower oil	0	2608±130	19±0.8
		300	5630±280	23±1.2
	Biodiesel production waste	0	708±35	33±1.6
		500	986±49	39±2.2
Rhodococcus erythropolis IMB Ac-5017	Refined sunflower oil	0	556±27	23±3
		500	1250±62	16±0.8
	Ethanol	0	82±4	10±0.5
		400	298±14	10±0.5

Note: Erythritol was added to the culture medium at the beginning of the stationary phase of strains IMV B-7241 and IMV Ac-5017 growth.

Currently, there are few reports describing the ability of surfactant-producing microorganisms to synthesise gibberellin phytohormones (Abdelmoteleb et al., 2022; Chen et al., 2021; Hao et al., 2019). For example, Abdelmoteleb et al. (2022) isolated three strains of *Bacillus subtilis* that simultaneously synthesised the lipopeptide iturin and produced 0.53–1.65 mg/L of the auxin phytohormone indole-3-acetic acid and 1.64–1.97 mg/L of gibberellin GA_3. Similarly, Chen et al. (2021) demonstrated that *Bacillus atrophaeus* B44 is capable of concurrent production of an aminolipid complex with antifungal activity and the biologically active gibberellin GA_3 at concentrations of 7.7–23.1 mg/L.

In 2024, Ding et al. (2024) reported that *Bacillus amyloliquefaciens* MG-2 simultaneously synthesised the lipopeptides fengycin, iturin, and surfactin, along with a complex of auxin-type phytohormones, cytokinins, and the biologically active gibberellins GA_1 and GA_3, as determined by high-performance liquid chromatography.

Bacteria of the genus *Bacillus*, as shown in previous studies (Abdelmoteleb et al., 2022; Chen et al., 2021; Ding et al., 2024; Hao et al., 2019), are predominantly plant-associated microorganisms that naturally synthesise phytohormones and lipopeptides. In contrast, *A. calcoaceticus* IMB B-7241 and *R. erythropolis* IMB Ac-5017 are free-living soil bacteria, and information on the ability of such microorganisms to synthesise a surfactant complex together with phytohormones of three classes (auxins, cytokinins, and gibberellins) is currently lacking. A further advantage of *A. calcoaceticus* IMB B-7241 and *R. erythropolis* IMB Ac-5017 is their capacity to produce metabolites of practical relevance for plant production using inexpensive substrates. Moreover, the surfactants synthesised by these strains exhibit antimicrobial activity against phytopathogenic bacteria.

Conclusions

Thus, the results demonstrated that phytohormone gibberellin biosynthesis in the surfactant-producing strains *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 occurs via the methylerythritol 4-phosphate (MEP) pathway. Supplementation of the cultivation medium with erythritol—an intermediate in isoprenoid biosynthesis within this pathway—led to a marked increase in the synthesis of biologically active gibberellins. In the presence of 300–500 mg/L of this exogenous precursor across various growth substrates, the concentrations of GA_3 and GA_4 produced by strains IMV B-7241 and IMV Ac-5017 increased by 1.5–16-fold relative to cultures without erythritol.

These findings provide a foundation for the development of a highly efficient, integrated technology for the simultaneous biosynthesis of surfactants and phytohormones for application in plant production.

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Hydrodynamic characteristics of mixed flow in liquid-gas ejectors

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Abstract

Introduction. The aim of this study is to identify the regularities governing mixed-flow formation and to determine the influence of ejector design elements on hydrodynamic characteristics.

Materials and methods. Ejection processes in ejectors with a cylindrical mixing chamber and active nozzles (jet and centrifugal-jet nozzles) are studied experimentally on a hydraulic stand and using numerical modeling tools in the academic version of the ANSYS 2020 R2 program with a maximum number of calculation cells of 512,000.

Results and discussion. It was found that the vacuum generated in a conventional jet sulfitator with a geometric characteristic of 126 and a standard jet nozzle is relatively low, reaching only 20–25 Pa. In contrast, replacing the standard nozzle with a centrifugal jet nozzle markedly increased rarefaction: at a distance of 20 mm from the nozzle outlet, the vacuum reached 425–555 Pa. Replacing the jet nozzle with a centrifugal jet nozzle while maintaining the same geometric characteristic of the ejector resulted in stable operation of the sulfitation station. Further analysis identified a rational range of the ejector geometric characteristic of m = 25–40 when using a centrifugal jet nozzle, which ensured stable rarefaction in the range of 78–100.4 kPa and reliable gas-phase ejection. Under a liquid pressure of 0.225 MPa at the nozzle, the ejection coefficient reached 2.5, which is sufficient to achieve the required technological pH of the liquid during the diffusion process.

At a liquid pressure in the nozzle channel of the nozzle of 0.25 MPa, its maximum speed reaches 20 m/s along the ejector axis. Due to the loss of energy for dispersion and resistance to the movement of droplets in a gas environment in the mixing chamber, the liquid speed at the ejector outlet decreases to 13.8 m/s. Two zones of mixed flow formation in the ejector have been established. In the first zone at a distance of up to 1.5 diameters of the mixing chamber, primary formation occurs with the formation of a homogeneous flow. In the second zone at a distance of up to 3 diameters of the mixing chamber, phase redistribution occurs and a ring flow regime is established. Based on these results, a rational design of a sulfitator for sugar production liquids was proposed to improve controllability and process stabilization. The working nozzle must provide sufficient surface area for phase contact, which is achieved using centrifugal-jet type nozzles, and the geometric characteristic of the ejector should remain within the identified range of 25–40 to ensure optimal vacuum generation and ejection efficiency.

Conclusions. Experimental and numerical (CFD) studies of ejection processes in liquid-gas sulfitators allowed us to establish the regularities of mixed flow formation and recommend rational size ratios when designing ejectors.

Introduction

The advantages of ejectors make them widely used in various industries as effective equipment for processes requiring high rates of phase interaction. One such application is the sulfitation of liquids in sugar production. These advantages, however, are realized only when the dimensions of the ejector are appropriately selected, taking into account hydrodynamic behavior and technological requirements; otherwise, the equipment may fail to perform effectively.

In the sugar industry, ejectors have been used as sulfitators without sufficient study of the formation of mixed flows and the influence of key structural elements on hydrodynamic characteristics. In practice, this has resulted in unfavorable environmental conditions during sulfitation, difficulty in maintaining regulated technological parameters of the solutions, increased reagent consumption, and higher energy costs. Therefore, the relevance of the present research is evident.

A mandatory technological process in sugar production is the sulfation of liquids and sugar solutions, the main tasks of which are: (a) disinfection of water from microorganisms and lowering its pH for the extraction of sucrose from beet chips in a diffusion apparatus; (b) processing of sugar solutions to reduce the content of non-sugars and improve the color of the final product to normalized values (Saska et al., 2010). This process is carried out in classical sulfitators of irrigation or bubbling types and jet (ejection) sulfitators.

Jet sulfitators consist of a liquid-gas ejector and a cyclone separator for phase separation (Ponomarenko et al., 2014). They offer several advantages over irrigation- and bubbling-type devices, including more intensive mass transfer between components, significantly lower specific metal content, simpler design, and reduced sulfur dioxide emissions into the atmosphere. However, during their operation, a number of design shortcomings were identified, which are explained by inadequate research of ejection processes. Such shortcomings are: lack of gas phase ejection in some operating modes, insufficient quality of liquid processing, SO_2 emissions into the production premises and into the atmosphere.

The practical limitations of ejector equipment can be addressed through a comprehensive scientific study of the ejection processes occurring in the mixing chamber and by determining the optimal hydrodynamic and design parameters. The choice of nozzle type is particularly relevant: in a typical jet sulfitator, a perforated disk is used as the working nozzle to disperse the liquid, effectively functioning as a jet nozzle (compact liquid jet) (Cramers et al., 2001). Since sulfitation is a heat and mass transfer process that requires a large interfacial area for efficient operation, such a nozzle proves to be inefficient.

For such processes, the use of centrifugal-jet type nozzles is recommended, which are characterized by a sufficiently high flow rate (0.7-0.9) and a spray torch filled with liquid droplets (Kandakure et al., 2005; Sliusenko and Ponomarenko, 2021). However, despite the fact that such nozzles have been studied experimentally (Balamurugan et al., 2007; Chen et al., 2019), transient processes during droplet formation have been described (Dos Santos et al., 2019), and data on determining their diameters have been provided (Balamurugan et al., 2007; Dos Santos et al., 2019), there is no unified idea of the mechanism of spray torch formation.

Establishing the patterns of mixed flow formation is necessary when designing ejectors, since the efficiency of their operation significantly depends on the design. The main elements of ejectors that affect the hydrodynamic characteristics are: (a) the design of the receiving chamber, which is manifested in the hydraulic resistance of the ejected (passive) flow entering the mixing chamber (Bergman et al., 2011); (b) the ratio of the main structural dimensions (Lefebvre and McDonell, 2017) (the value of its main geometric characteristic m is the ratio of the area of the mixing chamber to the area of the nozzle).

Thus, in Ashgriz et al. (2011) and Kandakure et al. (2005) it is noted that the highest ejection coefficient (k, the ratio of the passive flow rate to the active flow rate) for an ejector with a compact liquid jet is achieved at m = 4. Balamurugan et al. (2007) and Jain et al. (2014) experimentally demonstrated that the maximum k is at the value of the main geometric characteristic m = 10, while Chen et al. (2019) and Liu (2014) noted that optimal mixing of components in the ejector is achieved at m = 20-25. It should be emphasized that in a typical sulfitator, the geometric characteristic is m=126, which is significantly higher than these optimal values and may explain its insufficient operational efficiency.

Materials and methods

A comprehensive study of the ejection processes of jet devices with a cylindrical mixing chamber and a compact (jet nozzles) and dispersed liquid jet (centrifugal jet nozzles) was carried out by conducting full-scale experiments on a hydraulic stand and numerical modeling of processes in the academic version of the ANSYS 2020 R2 program with a maximum number of calculation cells of 512,000.

Nozzle designs

The studies used jet-type nozzles (Figure 1), which are typical in industrial sulfitators (Allauddin et al., 2024).

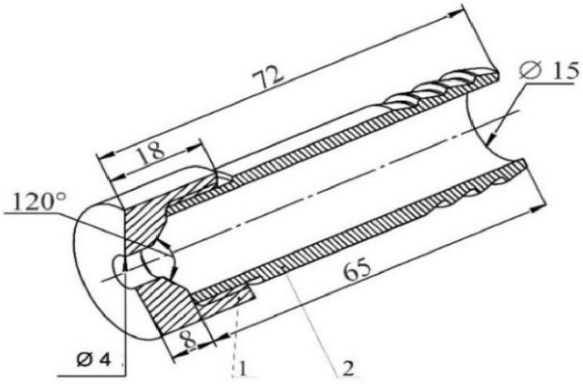


Figure 1. Jet nozzle: 1 – nozzle; 2 – body

The jet nozzle provides a compact liquid jet with a spray angle of 2-4 degrees, is the working nozzle of a classic ejector. The dimensions of the experimental nozzle are indicated in the drawing. The roughness of the nozzle opening is Ra = 0.16, which corresponds to the grinding operation. The flow rate of such a nozzle is quite high and is in the range of 0.93-0.98, which is its advantage.

Another type of nozzles that were studied are centrifugal jet type nozzles (Ponomarenko et al., 2024) (Figure 2).

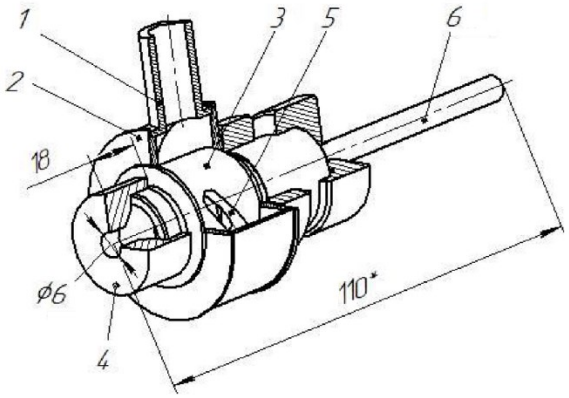


Figure 2. Centrifugal jet nozzle:
1 – inlet pipe; 2 – housing; 3 – twisting chamber;
4 – nozzle; 5 – inlet channel; 6 – plunger with rod

The spray torch lies in the range of 20–600, and is formed by liquid droplets that are formed when it exits the nozzle opening at a short distance. A feature of the nozzle is that the slotted inclined channels 5 for supplying liquid to the swirl chamber of the nozzle 3 are placed so that both edges of the channel adjoin it tangentially, but on different sides of the axis. Such an entry of liquid into the swirl chamber contributes to the formation of a turbulent flow that moves in the direction of the nozzle 4 and flows out of it in the form of a dispersed jet.

Ejector design

Ejection processes were studied in jet devices of two types: with a compact and dispersed liquid jet. In the first case, the ejector had a geometric similarity with the ejector of a typical water sulfitator for the diffusion process. The criterion for the similarity of ejectors is the equality of the value of their main geometric characteristic m. The main dimensions of the studied ejector: nozzle diameter d_c = 4 mm, mixing chamber diameter D = 45 mm, which corresponds to the geometric characteristic of the water sulfitator ejector m = 126. An ejector with d_c = 4 mm and mixing chamber diameter D = 22 mm was also studied. Geometric characteristic m = 30.25.

The design of the ejector and the receiving chamber is shown in Figure 3.

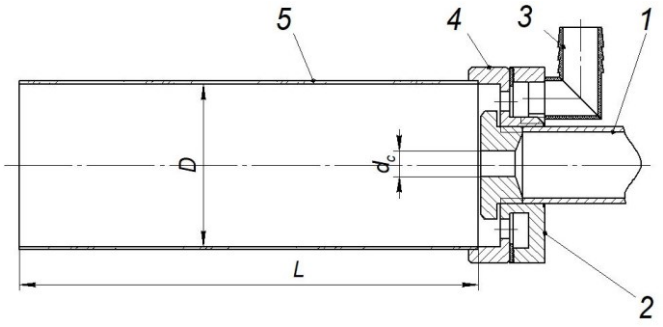


Figure 3. Design of an ejector with a cylindrical mixing chamber:
1– nozzle, 2 – cover, 3 – gas supply pipe,
4 – receiving chamber body, 5 – mixing chamber

This design of the ejector allows you to change the type of nozzle and install glass-mixing chambers of different diameters, which allows for experimental studies of ejection processes and visualization of the hydrodynamics of flows in ejectors with different geometric characteristics while maintaining the design of the receiving chamber. Table 1 shows the characteristics of the investigated ejectors and pressure ranges.

Table 1

Parameters of the investigated ejectors

Description of the ejector	Nozzle diameter, d_c, mm	Mixing chamber diameter, D, mm	Geometric characteristic m	Mixing chamber length, l, mm	Range of tested pressures, P, MPa
Compact liquid jet ejector	4	45	126	152	0.05–0.25
Ejector with dispersed liquid jet	4	45	126	152	0.05–0.25
Ejector with dispersed liquid jet	4	22	30.25	152	0.05–0.2

Design of an experimental setup for studying ejection processes

The design of the experimental setup is shown in Figure 4. The setup consists of a pump, a pipeline system, a liquid tank, shut-off and control valves, and control and measuring instruments. The liquid (water) flow rate was determined using a rotary flow meter (accuracy class 1.5). A sample pressure gauge (accuracy class 1.5) was used to control the liquid pressure in the nozzle. The gas (air) flow rate was measured using a rotary meter (accuracy class 1.5). A differential pressure gauge was used to determine the vacuum in the ejector receiving chamber.

The studies were conducted in a water-air environment at a phase temperature of 20 °C and a relative humidity of 85%. Changes in the ejector operating modes were carried out by adjusting the liquid pressure in the nozzle, which was controlled by a pressure gauge. During the studies, the readings of the liquid and gas flow meters at the beginning and end of the experiment, the liquid pressure in the nozzle and the vacuum in the receiving chamber were recorded.

Methodology and conditions of CFD modeling of ejection processes

For CFD modeling of ejection processes in ejectors with compact and dispersed liquid jets and different values of the main geometric characteristic m, the ANSYS CFX software package (ANSYS 2020 R2 with a maximum number of computational cells of 512,000) was used.

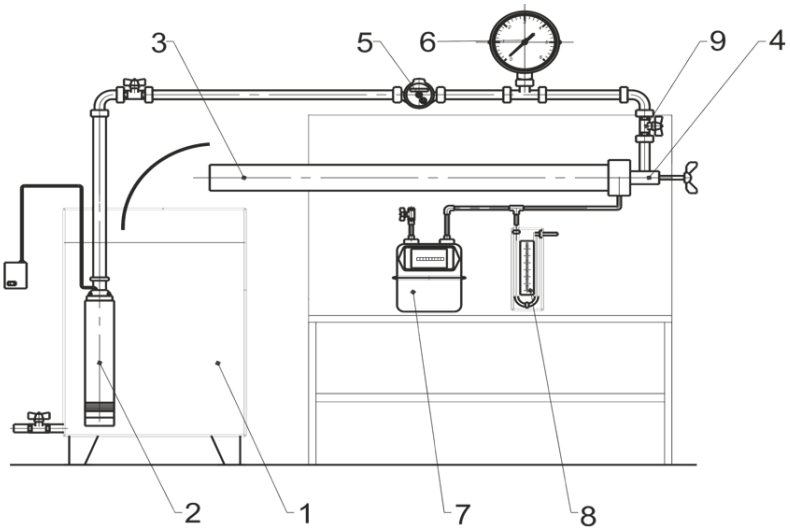


Figure 4. Design of the experimental setup: 1 – tank with liquid; 2 – pump; 3 – mixing chamber; 4 – nozzle with receiving chamber; 5 – liquid flow meter; 6 – pressure gauge; 7 – gas flow meter; 8 – differential pressure gauge

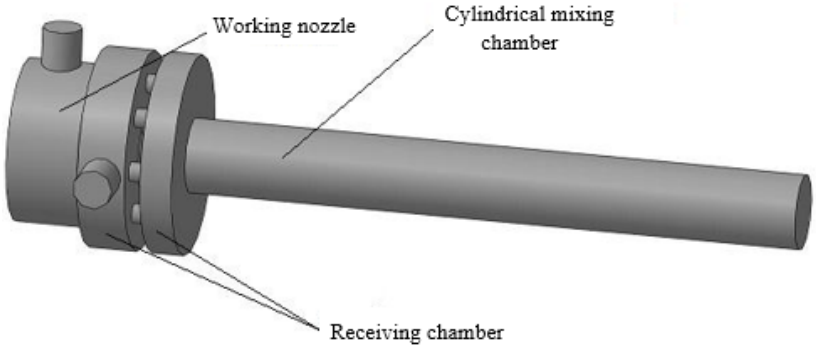


Figure 5. 3D model of the ejector

Figure 5 shows a 3D model (computational domain) of the investigated ejector. The boundary conditions for the simulation are (Figure 5): liquid inlet into the nozzle "Inlet_liquid", gas inlet into the receiving chamber "Inlet_gas", wall "Solid_wall" and outlet of the two-phase mixture from the mixing chamber "Mixture_Outlet".

Table 2 and Table 3 provide a list of parameters for CFD modeling of ejection processes.

Table 2

Basic parameters of CFD modeling of ejection processes

Indicator	Value
Calculation grid	Unstructured with a seal in the nozzle area and annular gap at the gas phase inlet to the mixing chamber. Maximum cell size is 4 mm, minimum size is 2 mm.
Task type	Non-stationary (Ansys, 2020)
Duration of computer calculation	0.5 s
Step between iterations	0.001
Criterion for convergence of results	0.0001
Calculation accuracy	Double precision
Work environments	Water and air
Turbulence model	*k-ε* (Dos Santos et al., 2019)
Multiphase flow model	Euler's "Mixture" model (Ansys, 2020; Ishii and Hibiki, 2011)
Initial simulation conditions	Zero speed components, Pressure P = 101325 Pa, Temperature t = 20 ºC The volume fraction of air is 1, i.e. 100% in the entire calculation area

Table 3

Boundary conditions of CFD modeling of ejection processes

Boundary condition name	Type of boundary condition	Parameters
Inlet liquid	Inlet	Mass flow rate of liquid: M = 0.2 kg/s Turbulence model parameters: k = 0.05, ε = 10 Volume fraction of component: water – 1, air – 0
Inlet gas	Inlet	Pressure P = 0 Pa Turbulence model parameters: k = 0.05, ε = 10 Volume fraction of component: water – 0, air – 1
Solid wall	Wall	No-slip wall
Mixture_Outlet	Outlet	Pressure P = 0 Pa

Results and discussion

Rarefaction in jet sulfitator

Comprehensive studies of ejection processes in a jet apparatus with geometric characteristics m = 126 (d_c = 4 mm, D = 45 mm) and a jet nozzle (compact liquid jet), which

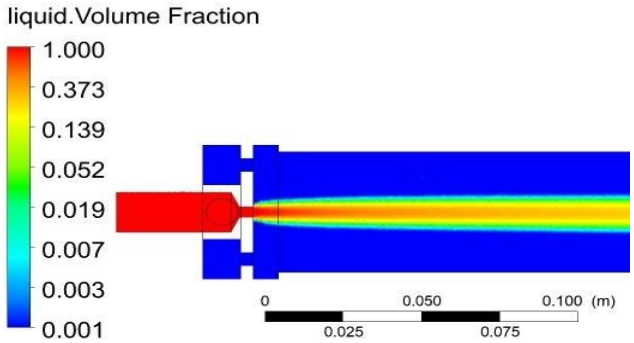
corresponds to the operation of a typical water sulfitator ejector for the diffusion process, were carried out. Figure 6a shows the liquid distribution in the ejector in the form of a compact jet along the ejector axis obtained using CFD modeling.

Confirmation of such liquid distribution is visual observation of the operation of an experimental ejector with the same geometric dimensions and a transparent mixing chamber (Figure 6b).

It should be expected that when an ejector with such geometric dimensions operates, the vacuum in the receiving chamber will be minimal or absent. This statement is based on the fact that to create an ejection effect, it is necessary to create a closed geometric region bounded by the walls of the mixing chamber and the outer contour of the spray torch (Zheng et al., 2017). If this condition is not met, then only the gas phase circulates within the mixing chamber (Mifsud et al., 2019). This conclusion is confirmed by the practice of operating a jet sulfitator (Ponomarenko et al., 2022), which shows that the wrong choice of geometric dimensions of the main elements of the ejector leads to loss of equipment performance. An unsuccessfully implemented idea of an ejector device can lead to the abandonment of this type of equipment.

Flow hydrodynamics modeling in the ANSYS CFX software package allows obtaining both a quantitative and qualitative picture of the pressure distribution in the cross-section of the mixing chamber (Kuś et al., 2024). Such data were obtained at a distance of 20 mm from the injector nozzle (Figure 7).

The calculations performed for this ejector show that the vacuum in this cross section is only 20–25 Pa. Such a vacuum is not enough for the equipment to operate effectively.



a

b

Figure 6. Liquid distribution in an ejector with a jet nozzle and m = 126: a – CFD modeling; b – experiment

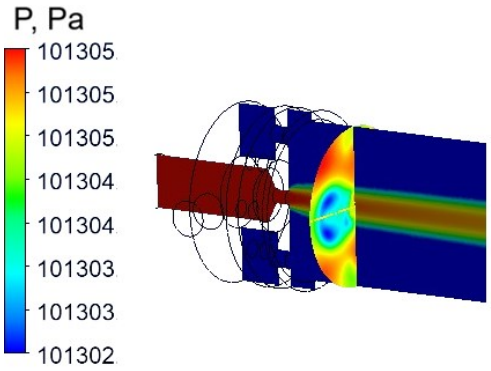


Figure 7. Gas phase pressure across the cross section of the mixing chamber

Eector with centrifugal jet nozzle

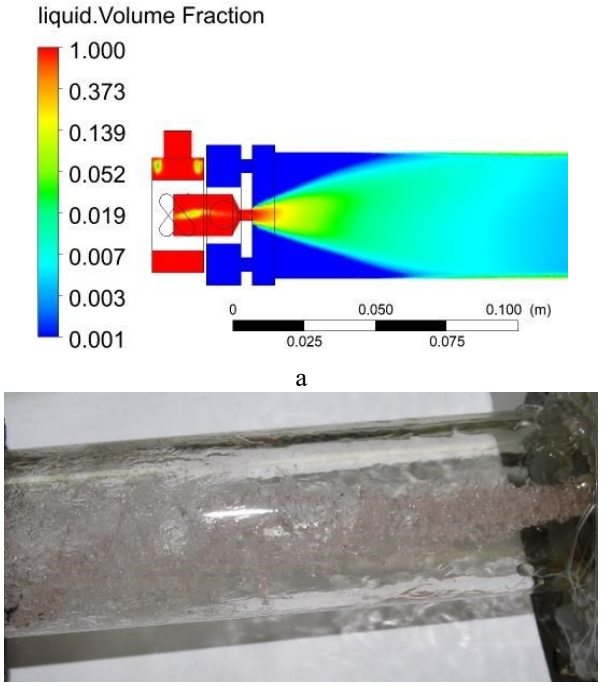
It should be noted that sulfitation is a heat and mass transfer process for the effective course of which requires a sufficient interfacial contact surface of the phases (Balamurugan et al., 2007), and according to Fick's law (Mauro, 2021), the amount of transferred substance is directly proportional to the size of this surface.

The jet nozzle does not provide the creation of a significant area of phase contact (Lyons et al., 2010), since mass exchange processes occur only on the outer side of the liquid flame (Zhou and Prosperetti, 2020). Therefore, it is proposed to change the type of nozzle from jet to centrifugal-jet. This will improve the course of the sulfitation process by significantly increasing the contact surface due to liquid dispersion. There are extremely few studies on the impact of such a replacement in the design of the ejector on ejection processes, they need to be clarified and deepened. The implementation of these tasks will allow solving the practical problem of optimizing the design of an industrial jet-type sulfitator.

Replacing the jet nozzle with a centrifugal jet nozzle with a dispersed liquid jet allowed us to radically change the pattern of liquid distribution in the ejector mixing chamber (Figure 8).

The liquid spray torch reaches the walls of the mixing chamber, which ensures the formation of a guaranteed zone of reduced pressure, and therefore the ejection of the gas phase. The results of CFD modeling and experimental research, shown in Figure 8, are in good agreement.

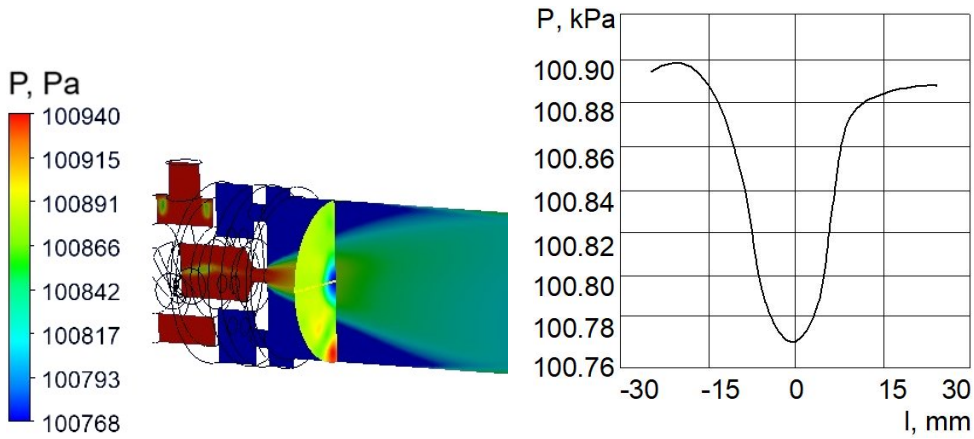
Figure 9a presents a visual picture of the pressure distribution in the cross section at a distance of 20 mm from the nozzle opening of the jet nozzle, and Figure 9b shows its graph. The results of the numerical experiment show the presence of rarefaction along the axis of the spray torch within 20-30 Pa, which decreases along the cross section of the torch, and on its surface the pressure is equal to the pressure in the ejector mixing chamber. This result is confirmed by experimental data (Chegini et al., 2025). It should also be noted that the rarefaction along the axis of the spray torch decreases with distance from the nozzle and disappears at a distance of about 150 mm. The obtained data on rarefaction in the spray flame near the nozzle explain the ejection of the gas phase into it.



a

b

Figure 8. Liquid distribution in an ejector with a centrifugal jet nozzle and geometric characteristic m = 126: a – CFD modeling; b – experiment



a b

**Figure 9. Gas phase pressure across cross section of the mixing chamber:
a - visualization of pressure distribution during CFD modeling at a distance of 20 mm;
b – pressure distribution in radial direction**

Comparative characteristics of ejectors with jet and centrifugal jet nozzles

Comparison of the rarefaction along the axis of the spray jet for the jet and centrifugal-jet nozzles is presented in Figure 10. The obtained numerical values of the rarefaction in the cross section for the centrifugal-jet nozzle lie in the range of 425-555 Pa, which exceeds the rarefaction indicators for the jet nozzle (20-25 Pa) by an average of 20 times. The explanation for such a significant difference in the rarefaction along the axis of the spray jet lies in the features of its formation. During the centrifugal-jet outflow of liquid from the nozzle, there are two liquid flows: axial and centrifugal, which, when interacting, form a common flow. The second feature of such interaction is the rapid expansion of the spray jet and the formation of liquid droplets at a close distance from the nozzle (Vambol et al., 2020). In this case, the liquid droplets attach the corresponding part of the gas phase, and since the axial flow velocity is higher than the velocity of the liquid droplets moving along the peripheral trajectory, this results in a rarefaction, and it is greater along the axis of the torch. It is possible to predict that the higher the liquid spray pressure, the greater the axial velocity and the greater the rarefaction will be achieved.

When the liquid flows out of the nozzle in a jet, the spray angle is within 2-5°, i.e. the entire liquid moves almost at the same speed, dispersion on the drop occurs at a greater distance from the nozzle, which leads to the creation of a much smaller rarefaction.

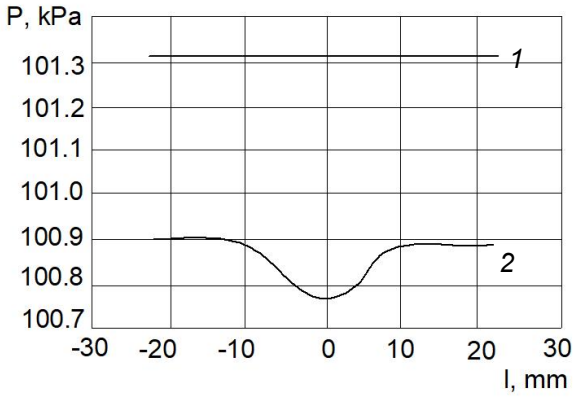


Figure 10. Comparative values of rarefaction for jet (1) and centrifugal jet (2) nozzles in radial direction

Thus, replacing the jet nozzle with a centrifugal jet nozzle with the same geometric characteristics of the ejector allowed stabilizing the operation of the sulfitation station.

Determination of rational characteristics of ejectors

As our research has shown (Figure 11), the value of the main geometric characteristic of the ejector m of the water sulfitator for the diffusion process in the sugar industry (m = 126) does not correspond to the optimal value at which the operation of the jet apparatus with the highest ejection coefficient is achieved. It was found that its rational value is in the range of 25–40.

Therefore, studies of ejection processes in ejectors with a centrifugal jet nozzle were continued in the zone of optimal values of geometric characteristics. At the same time, thanks to CFD modeling, results were obtained that are quite difficult to obtain experimentally.

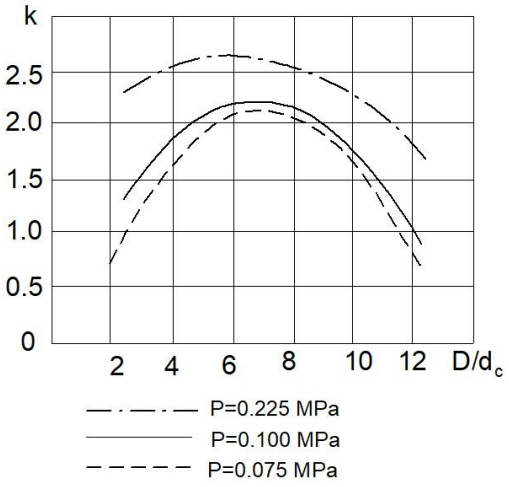


Figure 11. Dependence of volumetric ejection coefficient k on geometric dimensions of ejector D/d_c at different liquid pressures P

Patterns of mixed flow formation

Figure 12 shows the distribution of static pressure in the ejector at a mass flow rate of 0.2 kg/s, which corresponds to its pressure in the nozzle of 0.25 MPa with a nozzle diameter of 4 mm and a mixing chamber diameter of 22 mm (m = 30.25).

Under the conditions of the experiment, the static pressure in the nozzle is 250 kPa. When the liquid exits the nozzle, the static pressure drops sharply (Figure 12b) because of the conversion of potential compression energy into kinetic energy of the flow.

Analysis of the results obtained allowed us to establish that a zone of reduced pressure of 78–100.4 kPa appears in the receiving chamber and in the area 1.5D from the beginning of the mixing chamber. This guarantees the ejection of the gas phase and will definitely allow us to achieve stable operation of the sulfidation station (Kiên et al., 2025). This is the zone of primary formation of the mixed ejector flow, when the liquid spray jet expands and reaches the walls of the mixing chamber. A zone of reduced pressure is formed (Figure 9b), which is confirmed by the graph of Figure 12b.

The length of the low-pressure zone along the ejector axis is insignificant, which is the result of the reformatting of the mixed flow due to the impact of the droplets on the ejector wall (Siddiqui and Khan, 2025). Then, some of the liquid droplets move along the wall of the mixing chamber in the form of a liquid film, and the other part of the droplets changes the trajectory of movement, moves to the ejector axis, and again hits the opposite wall. Such a complex liquid flow contributes to the reformatting of the mixed flow, which is confirmed by Figure 13b.

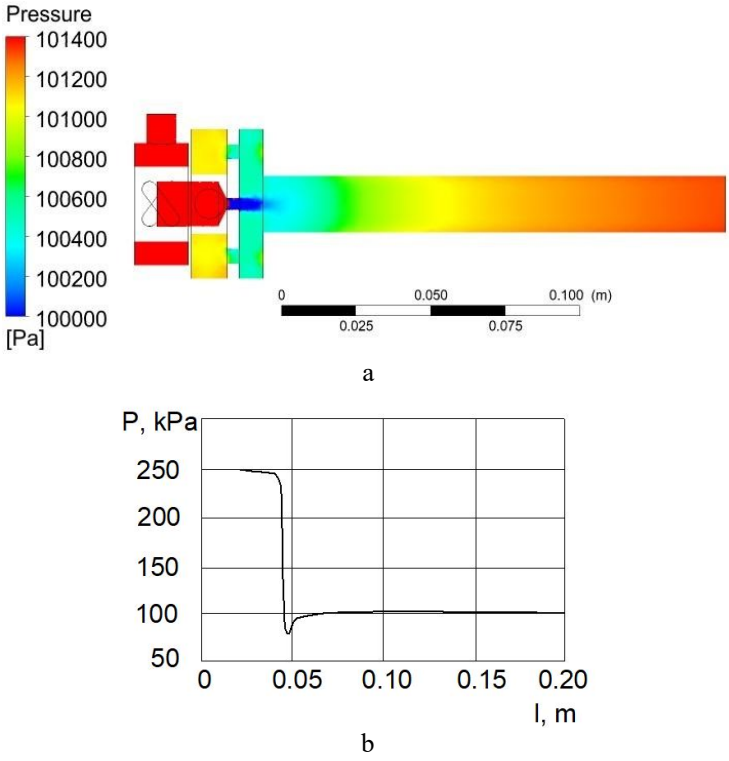


Figure 12. Static pressure distribution in the ejector:
a – static pressure on the ejector elements;
b – static pressure distribution graph along ejector axis

Figure 13 shows the distribution of the volume fraction of the liquid along the length of the ejector. At a distance of about 1.5D (section I-I) from the nozzle cut, the liquid droplets are evenly distributed over the cross section of the mixing chamber. Between zones I-I and II-II, the mixed flow is re-formed due to the impact of drops on the wall of the mixing chamber, and after the section II-II, a stable flow formation occurs. Along the axis of the mixing chamber, the volume fraction of the liquid is minimal, and near the wall, it is maximal, which is clearly visible from the graph in Figure 13b. The movement of the liquid in the mixing chamber corresponds to the annular flow regime.

The distribution of the liquid velocity along the ejector axis was obtained (Figure 14). According to the obtained graph, the maximum liquid velocity in the nozzle at the specified nozzle research parameters reaches 20 m/s (distance 0.05 m from the origin of coordinates, which is located on the inner wall of the nozzle). Due to the significant loss of energy for liquid dispersion at the outlet of the centrifugal jet nozzle (Ismailov et al., 2025), gas ejection and the initial formation of a mixed flow, there is a sharp decrease in the liquid velocity to 16 m/s and subsequently, due to the resistance to the movement of droplets in a gas medium (Basmanov et al., 2025) along the length of the mixing chamber, the liquid velocity at the outlet of the ejector decreases to 13.8 m/s.

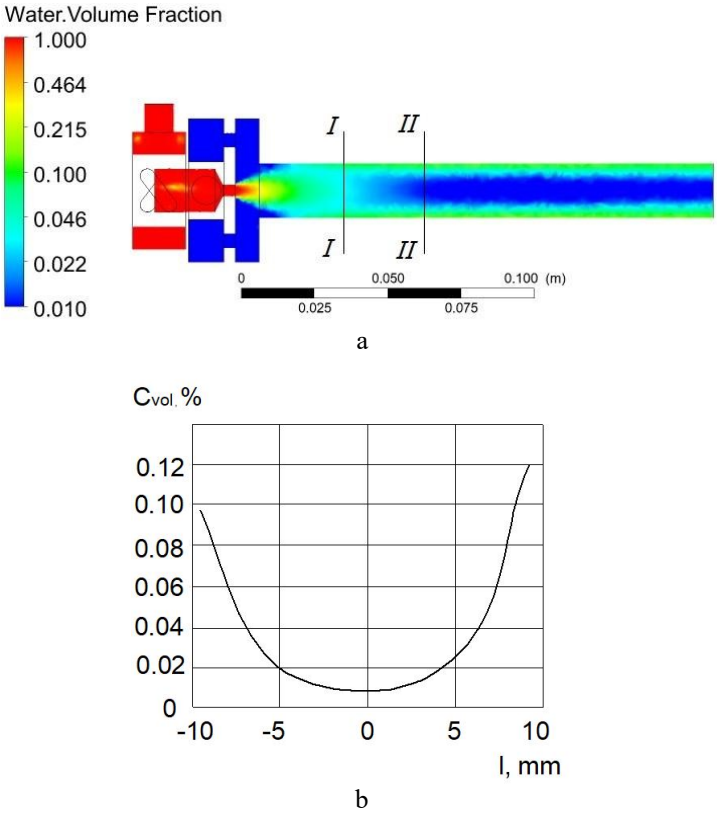


Figure 13. Distribution of the volume fraction of liquid in the ejector:
a – longitudinal section;
b – cross-section of mixing chamber at distance of 3D from its beginning

Since the Euler model "Mixture" (Ansys, 2020; Ishii and Hibiki, 2011) allows us to visualize the distribution of each phase, we will use this opportunity and present the distribution of the volume fraction of gas in the ejector (Figure 15).

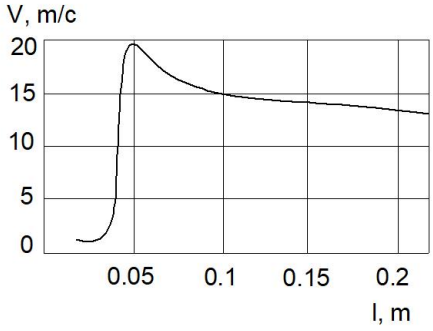


Figure 14. Liquid velocity distribution along the ejector axis

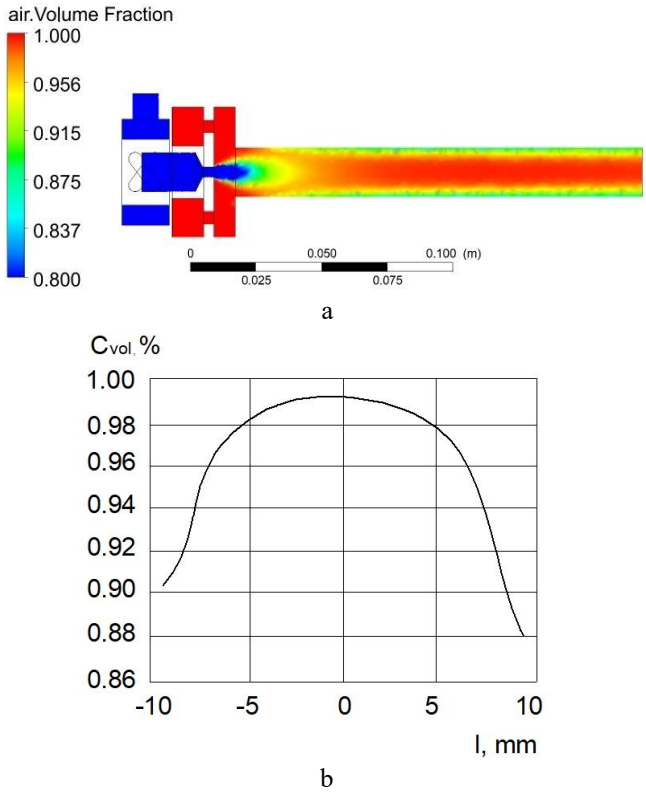


Figure 15. Distribution of the volume fraction of gas in the ejector:
a – longitudinal section;
b – cross-section of the mixing chamber at a distance of 3D from its beginning

The obtained results of gas distribution in the ejector correlate with the previously presented results of liquid phase distribution. At a distance of 1.5–3 D, gas ejection into the middle of the liquid flow occurs, and at a distance of more than 3D along the ejector axis, the volume fraction of gas is maximum (~90%), and when approaching the wall it gradually decreases to ~10%. Gas ejection into the middle of the sprayed liquid flow is explained by the presence of rarefaction. Analysis of phase distribution graphs in cross-sections of the mixing chamber shows the presence of a slight asymmetry of the flows relative to the axis, which is explained by the following reasons and can be eliminated by adjusting the dimensions of the ejector structural elements:

- deformation of the liquid jet under the influence of turbulence and rarefaction occurring in the ejector (Bhaduri et al., 2025);
- uneven distribution of the gas phase in the ejector receiving chamber (Jingyang et al., 2023);
- insufficient length of the nozzle swirl chamber, which leads to asymmetric distribution of liquid droplets in the spray jet (Zhu et al., 2014).

The obtained results are confirmed by the results of studies by other scientists. During experimental and theoretical studies of the ejector operation, it was found (Guangming et al.,

2010) that it can operate only in one optimal state with fixed geometric dimensions. We have established such an optimal operating mode for ejectors with a centrifugal jet nozzle in the range of geometric characteristic values 25–40.

As for the studies of mass transfer in the ejector, it was found that the geometric characteristics of the ejector (nozzle diameter, mixing chamber length, ratio of nozzle and mixing chamber diameters) significantly affect mass transfer (Cramers et al., 2001). Mass transfer processes depend on the physical properties of the liquid and the geometric parameters of the ejector, which are interconnected (Cramers et al., 2001). The volumetric mass transfer coefficient increases with an increase in the fraction of the gas phase (volumetric ejection coefficient) and the length of the mixing chamber. When studying the water-air ejector (Balamurugan et al., 2007), the optimal ratio of the area of the ejector throat to the area of its nozzle was established, which is 10. This value of the geometric characteristic is explained by the design features of the ejector. The mass transfer coefficient and the area of the interface surface increase with increasing velocity in the nozzle. The proposed correlation for predicting mass transfer agrees with experimental data ±20%. Ejectors used as mass transfer devices are particularly effective in the case of rapid or instantaneous chemical reactions (Cramers et al., 2001).

Studies of pressure distribution along the mixing chamber (Balamurugan et al., 2007; Li and Li, 2012) showed that a "shock" occurs at the beginning of the mixing chamber. That is, sharp changes in pressure, density and velocity lead to an intensive exchange of impulses of turbulent vortices. For ejectors of the gas-liquid system, the length of the "shock" section can be up to 6 diameters of the mixing chamber, which depends on the design of the ejectors. Such results confirm the presence of a zone of spray flame formation near the nozzle; however, the length of this zone is individual for ejectors of different designs.

Conclusions

A comparison of experimental and CFD studies on the operation of liquid-gas ejectors during the sulfitation of sugar solutions using jet and centrifugal-jet nozzles was carried out, confirming the reliability of CFD modeling for predicting hydrodynamic characteristics. When a centrifugal-jet nozzle was used in a typical jet sulfitator, the vacuum at a distance of 20 mm from the nozzle reached 425–555 Pa, approximately 20 times higher than the 20–25 Pa observed for a standard jet nozzle. This has been demonstrated for the first time under sugar solution sulfitation conditions. The rational range of the ejector geometric characteristic for a centrifugal-jet nozzle was found to be m = 25–40, ensuring stable rarefaction of 78–100.4 kPa and guaranteed ejection of the gas phase; previously, a value of m=126 was used in sugar industry devices, which proved ineffective. These results can be applied to the development of new jet sulfitator designs with increased efficiency, contributing to reduced SO_2 emissions and improved quality of the final product.

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Mascot effect: Analysing brand character influence on purchase intentions in Thailand's food market

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Abstract

Introduction. The aim of this research was to examine the influence of brand mascots on consumer purchase intentions in the Thai food sector. The study integrated the Theory of Planned Behavior (TPB) with the construct of emotional engagement.

Materials and methods. This study employed an empirical approach based on primary data collected through an online survey. The target population comprised consumers familiar with mascot-driven brands. 368 valid responses were obtained. Data were analyzed using confirmatory factor analysis and structural equation modeling to evaluate model fit.

Results and discussion. The study's respondents were primarily male (48.9%), within the 25–34 age bracket (31.3%), held a Bachelor's degree (36.1%), and were self-employed (24.5%). This demographic profile reflects a young, economically active, and marketing-responsive consumer segment. All structural model fit indices demonstrated acceptable adequacy (e.g., CMIN/DF = 2.310), while reliability and validity metrics met the required thresholds, with the minimum Average Variance Extracted (AVE) = 0.548 and minimum Composite Reliability (CR) = 0.759, confirming strong measurement quality. The empirical results revealed that Emotional Connection ($\beta = 0.489$) was the strongest positive predictor of Purchase Intention, underscoring its pivotal role in consumer decision-making. In contrast, Attitude exerted a significant negative effect ($\beta = -0.175$), suggesting that rational evaluations alone may not always align with affective or behavioral outcomes. Furthermore, Emotional Connection significantly mediated the effects of Mascot Characteristics, Subjective Norm, and Perceived Behavioral Control, reinforcing its central role as an emotional bridge between perception and intention. These findings confirm that emotional engagement serves as a crucial determinant of consumer behavior, particularly in culturally expressive markets such as Thailand, where affective symbolism and brand storytelling strongly influence purchase motivation.

Conclusions. The findings show that food brand mascots significantly influence consumer attitudes, intentions, and behavior. Emotional resonance, rather than purely cognitive evaluation, was found to drive purchase intention. Accordingly, marketers in the Thai food sector should develop mascots that convey authenticity, relatability, and emotional warmth aligned with consumer values and cultural expectations, thereby strengthening brand loyalty and long-term engagement.

Introduction

In the current global, dynamic and highly competitive market environment, businesses are using every innovative way to capture consumers' attention. Among the emerging strategy being used to market a brand to consumers are mascots (Malik and Guptha, 2014; Palladino et al., 2022). A brand mascot is considered a suitable strategy of presenting a brand to the customer because of its unique characteristics, such as distinctive personalities, visual identities, and narrative potential (Kim, 2018; Min et al., 2019). As indicated by Bisht (2021) brand mascot serves as a powerful brand ambassador, capable of influencing consumer behavior through visual identity and emotional story telling. Brand mascots are defined as characters that are used to represent a company or product. This means the characters may be human, animal or cartoons and are commonly seen in ads for the brand. Besides supporting the brand's image, they play a role in giving brand human qualities, helping it stick in people's minds and making it more relatable (Reddy et al., 2024).

The usage of mascots has essentially evolved into a critical marketing tool for mass creation of corporate identity and public interest in a brand; whereas, they place a brand on the radar in the view of the market by increased brand awareness through visual engagement opportunities (Ahmad et al., 2024). In the food sector, mascots are especially impactful, as food purchasing decisions often involve emotional, habitual, and social components. Such names of mascots are The Bar B Gon for Bar B Q Plaza, Zon Zon for Cafe Amazon, Chickira for Chick-A-Boom, the Nong Noey for KFC and Ronald McDonald for McDonald's (Ares et al., 2022; Bohn and Wittenzellner, 2024; Keller et al., 2012; Kraak and Story, 2014; Reddy and Sathish, 2024; Vermeir and Roose, 2020). Nowadays, considering the media glut and the scant attention of consumers, such characters would be of greatest value in advertising campaigns. They make the brands easier to identify, recall, and understand for customers. Gupta et al. (2021) put forward that a transformed form is characters associated with brands, which would have been made a permanent long-term inexpensive medium for passing on the imagery and values of the brand to audiences. Thailand, where food is not just a need but, by itself, directly a part of identity, also has mascots that entice consumption and facilitate brand recall (Ganapathy et al., 2024; Wattanarak et al., 2021; Wu et al., 2025). The main objective of using brand mascot is to create positive emotions, views and attitudes towards a brand and its products. The positive image then would create attraction and possible buying behavior on the intended products or services (Yadav et al., 2015). Due to its potential influence on buyers' behavior, they are being applied in various sectors, from manufacturing to education.

Earlier studies have shown how mascots play a significant part in making the minds of customers lean towards the brand because of its anthropomorphic characteristics (Reddy et al., 2024), when the brand identity aligns with self-identity with respect to intended consumers that influence consumer attitudes and intentions toward the brand (Palladino et al., 2022). In another study, Sarkar et al. (2019) found that one effective way of creating human-like perceptions of a brand is through advertising, which can foster an emotional bond with consumer segments and enhance brand affinity. Such advertising is particularly effective because it can generate a deep-seated and lasting impression, strengthening consumers' emotional attachment to the brand. Despite all of the earlier studies, there are not enough empirical research results on how mascots can affect what customers buy especially in the food sector. Previous studies suggest that the presence of a mascot enhances brand visibility (Oh and Kim, 2025; Septianto and Paramita, 2021); therefore, it is important to examine whether positive affect toward mascots can also stimulate consumers' purchase intentions. It matters more now because companies are relying on experiences and emotions to get customers interested. Taking these into consideration, the main aim of this study is to investigate how mascots help shape consumers' purchase aspiration from food brands in Thailand.

The Theory of Planned Behavior (TPB) has gained considerable traction as a model for understanding decision-making and human behavior. The TPB postulates that perceived behavioral control induces the behavior of a person and subjective norms and personal attitudes (Cheng, 2019). Also mentioned within literature is the need for emotion wherein the link between consumers and the brand has formed an emerging area of focus. The study offers ideas on how mascots can be used for marketing.

Literature review

A brand mascot is a visual or verbal character that represents a brand and functions as a symbolic embodiment of its identity. Typically presented as animated or illustrated figures, mascots serve as recognizable representatives of brands. According to Deligoz and Ünal (2021), brand mascots are primarily used to strengthen brand identity and to create emotional connections between brands and their target consumers. Well-known examples such as Chikira, Nong Noey, and Ronald McDonald illustrate how mascots extend beyond promotional tools to symbolize what a brand stands for. By personifying brand attributes, mascots become more relatable, evoke emotional responses, and may motivate consumer action. These perceived characteristics reflect the set of qualities consumers associate with the mascot.

Features of these characters are their good looks, relatability and being one of a kind; these are the details that people link to the brand. Admittedly, Reddy et al. (2024) aver that mascots infuse a human touch to brands; thereby enabling personal and close association between the consumer and the brand. This, in turn, establishes the most critical point for the differentiation of any brand from others. In Chen's (2021) view, mascots that are easy to look at and express emotions have a greater effect on people's attitude and the way they make purchases. Such characteristics in advertising have been noticed to improve consumer trust and indirectly influence whether someone decides to buy from the brand. Some also believe that mascots partake in building the first impression consumers have, as well as their first feelings (Wang et al., 2025; Wu et al., 2025). This has an influence on their intention to engage with the product of purchase from the brand. From these arguments, the following hypothesis was developed:

H1: *Mascot characteristics have a positive and significant influence on the purchase intention*

Ajzen's (1991) Theory of Planned Behavior (TPB) provides a framework for understanding the factors that guide consumers in their purchasing decisions. The theory posits that behavior is influenced by three key components: attitude, subjective norms, and perceived behavioral control. Attitude reflects how favorably or unfavorably consumers evaluate a particular brand. Previous studies have indicated that the presence of an appropriate and appealing brand mascot can positively influence consumer attitudes, leading them to develop stronger preferences for the brand than they otherwise would (Deligoz and Ünal, 2021; Shimpi, 2021). Using mascots can encourage people to adopt a good outlook on a brand because they amuse the public and reflect the things customers' value (Oh and Kim, 2025). If the mascot is not important or properly represents the brand, it could create a negative perception in the minds of customers (Reddy and Sathish, 2024; Tanjaya, 2025).

Subjective norm implies that the perceived social pressure that affects engagement of non-engagement in a particular behavior. In the context of mascot, it denotes the influences on or not from a brand with mascot. Other people such as facility of peers' orientation regarding the brand using mascot would influence the perception and purchase intention regarding the brand concerned. Literature has demonstrated that social pressure such as social endorsement of brand mascot strengthens subjective perspectives of a brand, and the resultant purchase intention (Attah et al., 2024; Osei-Frimpong et al., 2022; Thai and Wang, 2020).

Perceived behavioral control implies consumer beliefs regarding how easy or difficult it is to carry out a particular activity, such as purchasing from a brand (Bortne et al., 2025). According to Sathish (2023) the perspective of consumers regarding a brand mascot, for instance, its affordability, accessibility, and ease of purchase influence the resultant consumer behavior. Mascots have a life far beyond the façade of advertising lure to persuade the consumer. Brand mascots serve as organizational totems to the extent that they can rally members of an organization around a concrete presentation of its unique character. Thus, mascots assist in condensing the consumers' understanding of the organization's identity in a context far removed from the organization's own milieu (Cayla, 2013). As illustrated by Arunrangsiwed and Pairoa (2016), when a mascot is used to guide the consumers' journey and generate customer services interactions, it has a great influence on how consumers perceive the brand. Mascot symbolism creates positive views towards brands. From these arguments, the literature findings, the following three hypotheses were developed:

H2: *Attitude towards mascot has a positive and significant influence on the purchase intention*

H3: *Subjective norm has a positive and significant influence on the purchase intention*

H4: *Perceived behavioral control regarding mascot has a positive and significant influence on the purchase intention*

In addition to the concepts borrowed from the TPB, this study explored the literature of emotional connection. Such a link refers to the feelings and connection a person has with a certain brand (Bigné et al., 2023; Ghorbanzadeh and Rahehagh, 2021; Niharika and Yadav, 2023). According to Nasr-Esfahani et al. (2022), the bond is usually formed when a brand communicates in a humanlike way with mascots. Researchers say that following a mascot creates positive feelings in customers which leads to the formation of a close relationship with the brand (Sayin and Gürhan-Canlı, 2024; Shimpi, 2021). Being emotionally involved is a better predictor of buying a product with symbolic aspects, like a company mascot, than analyzing the facts about it. From these arguments, the following hypothesis are developed:

H5: *Emotional connection has a positive and significant influence on the purchase intention*

H6: *Emotional connection mediates the effect of Mascot characteristics, attitude, subjective norm, and perceived behavioral control has a positive and significant influence on the purchase intention.*

Materials and methods

Conceptual framework and hypothesis

The study has four independent variables, namely mascot characteristics, attitude, subjective norm, and perceived behavior control. The mediating variable was emotional connection, while the dependent variable is the purchase intention. The conceptual framework is visualized in Figure 1.

A quantitative cross-sectional research design was utilized to scrutinize the influence of food brand mascots on the consumers' purchase intention in Thailand's food market sector. The study relied on the TPB framework, with inclusion of emotional connection as a mediating variable. The study population was Thailand food consumers above 18 years, who have been appropriately exposed to and/or interacted with brands that utilize a distinct mascot in their marketing and branding activities. These consumers were considered to have developed emotional connections, attitudes towards a particular food brand(s) using mascot.

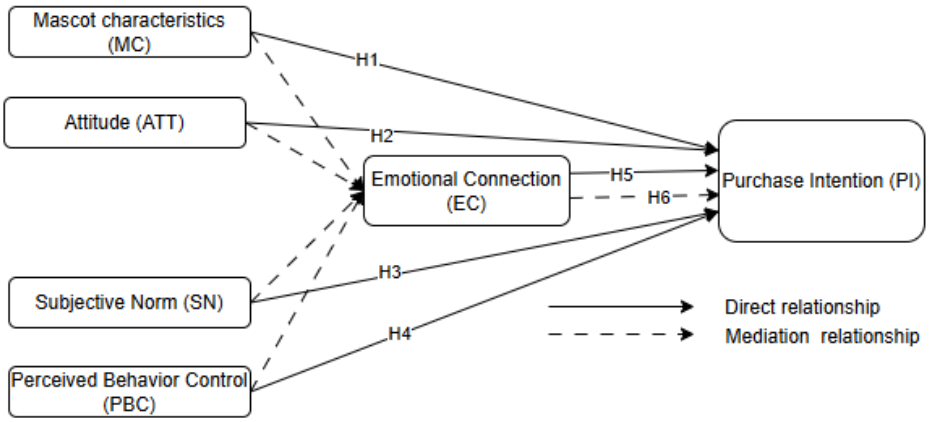

Figure 1. Conceptual framework of the study
Source: own processing based on literature

Materials – Sample collection

The study adopted a non-probability purposive sampling technique, where only the relevant respondents were included in the study. These were the respondents with sufficient familiarity with brands that use mascot in the food market, such as The Bar B Gon mascot (from Bar B Q Plaza), The Zon Zon mascot (from Café Amazon Coffee in Thailand), Chikira mascot (from Chick-A-Boom), Nong Noey mascot (daughter of KFC founder), and Ronald McDonald (from McDonald's). The participants were recruited through online platforms including email invitations, targeted marketing forums and social media. To determine the appropriate sample size, the study adopted the sample-to-item ratio, which determines the sample size based on the number of items in the study. A ratio of 10-to-1 ratio was considered suitable (Brown and Greene, 2006). This study questionnaire had 26 items, which resulted in a minimum of (26*10) 260 respondents. The sample size was therefore a minimum of 260 respondents.

To establish validity and reliability, the measurement scales of the questionnaire were adapted to suit the setting. Items were generated from the literature, after having identified the dimensions of the mascot characteristics from both the literature of Dellgoz and Ünal (2021) and Shimpi (2021). As for the Theory of Planned Behavior, operational definitions were hence defined; thus the measurement of attitude towards mascot as on a semantic differential scale followed literature from Ajzen (1991) and Spears and Singh (2004).; subjective norms were operationalized using scales of Taylor and Todd (1995) and Izquierdo-Yusta et al. (2022); and perceived behavioral control utilized items from Dangaiso (2023), and La Barbera and Ajzen (2021). Furthermore, the construct of emotional connection was assessed using scales adapted from research on brand relationships (Bigné et al., 2023; Ghorbanzadeh and Rahehagh, 2021). Finally, the dependent variable, purchase intention, was operationalized using a scale from Dodds et al. (1991) and Liu (2025).

The data collection was conducted using a self-administered online questionnaire. The questionnaire was developed on Google forms in English language. The questionnaire

comprised on demographic questions and constructs questions. All the constructs were measured using multiple-item scales, which were developed with reference to the previous literature. Each construct was measured using a 5-point Likert scale, with response options ranging from strongly disagree to strongly agree. The questionnaire was hosted on google forms, and respondents invited through various means including emails, and social media channels such as Facebook and Twitter. The study collected a total of 412 responses. Upon review and adjustment of inappropriate responses and missing data, the study was left with valid sample responses of 368. The data was collected from August 2025 to September 2025.

Data analysis was conducted using the SPSS software for preliminary descriptive statistics analysis. To evaluate the model fitness, reliability, and validity of the model, confirmatory factor analysis (CFA) was adopted. Path analysis was adopted to evaluate the hypothesis of the relationship among the variables. Bootstrap was used for moderation analysis, to evaluate the indirect effects through emotional connections. Appropriate ethical considerations were made as the research involved human participants. Informed consent was presented to all the respondents, informing them of their voluntary participation, and opting out of the survey. Additionally, the data collected was treated with complete confidentiality and used only for the purpose of this study.

Results and discussion

The study first conducted analysis of the demographic characteristics of the respondents. The results are summarized in Table 1.

Table 1

Descriptive statistics of demographics

Variables	Categories	Frequency (n)	Percent (%)
Gender	Male	180	48.9
	Female	168	45.7
	Others	20	5.4
Age	18-24	71	19.3
	25-34	115	31.3
	35-44	90	24.5
	45-54	56	15.2
	55+	36	9.8
Educational Level	High School and Below	54	14.7
	Diploma or Equivalent	89	24.2
	Bachelor's Degree	133	36.1
	Master's Degree	56	15.2
	Doctoral Degree	36	9.8
Employment Status	Student	59	16.0
	Full Time Employment	54	14.7
	Part Time Employment	59	16.0
	Self Employed	90	24.5
	Unemployed	66	17.9
	Retired	40	10.9

Source: own processing from field data

The results indicated that males comprised the majority of respondents (48.9%), while females accounted for 45.7%. Regarding age, the largest group was 25–34 years (31.3%), followed by the 18–24 age group (24.5%), with the smallest proportion being those aged 55 and above (9.8%). In terms of education, respondents with a bachelor's degree represented the majority (36.1%), followed by those holding a diploma or equivalent qualification (24.2%). With respect to employment status, the largest group was self-employed individuals (24.5%), followed by the unemployed (17%), while retirees represented the smallest proportion (10.9%).

Model fitness evaluation

The second analysis that was conducted was an evaluation of the model fitness, reliability and validity. Various fitness indices were tested as summarized in Figure 2 and Table 2. From the results presented, it is found that all the required thresholds regarding the fit indices were met (Bacon and Bacon, 2001). This implies that model demonstrates solid structural validity, and it is well-specified and appropriately represents the data.

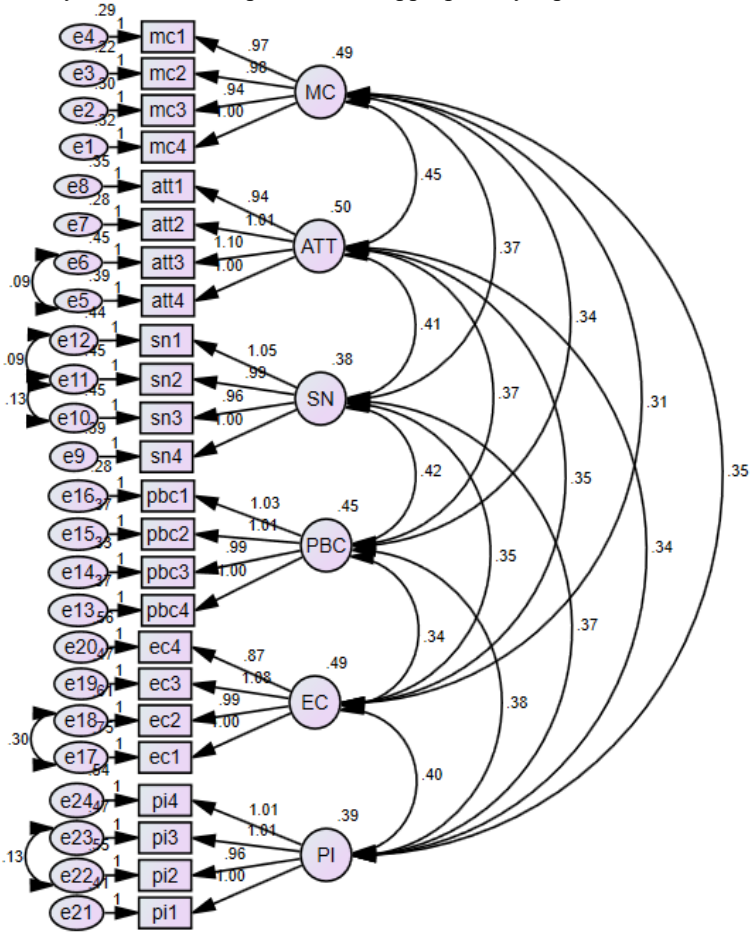

Figure 2. Model fitness evaluation
Source: own processing in SPSS

Table 2

Model fitness evaluation

Fitness Index	CMIN/DF	RMR	RMSEA	NFI	IFI	TLI	CFI
Values	2.310	0.045	0.060	0.901	0.941	0.930	0.941
Required Threshold	<3.0	<0.080	<0.080	>0.90	>0.90	>0.90	>0.90
Decision	✓	✓	✓	✓	✓	✓	✓

Source: own processing in SPSS

The other analysis that was conducted was the evaluation of the reliability and validity of the data. The reliability was evaluated using Cronbach's alpha and composite reliability (CR), while validity was evaluated using the average variance extracted (AVE) and standardized factor loadings. The results are summarized in Table 3.

The required threshold of Cronbach's alpha is 0.7 while the required threshold for CR is also 0.7. From the results obtained both Cronbach's alpha and CR valued met this required threshold. As a result, the reliability and internal consistency were confirmed. For the AVE, the required threshold was is 0.5, and as well, the required threshold for standardized loadings is also 0.5. As seen in the values, the threshold was met, leading to conclusion that the constructs explains more than 50% of the indicators variances (Cheung et al., 2024).

Table 3

Reliability and validity analysis results

Latent Variables	Observed Variables	Standardized Loadings	CR	AVE	Cronbach's Alpha
ATT	att1	0.745	0.848	0.583	0.850
	att2	0.804			
	att3	0.756			
	att4	0.747			
EC	ec1	0.628	0.764	0.548	0.770
	ec2	0.667			
	ec3	0.742			
	ec4	0.634			
MC	mc1	0.78	0.867	0.619	0.868
	mc2	0.823			
	mc3	0.768			
	mc4	0.776			
PBC	pbc1	0.792	0.844	0.575	0.846
	pbc2	0.744			
	pbc3	0.756			
	pbc4	0.741			
PI	pi1	0.698	0.759	0.641	0.761
	pi2	0.628			
	pi3	0.676			
	pi4	0.651			
SN	sn1	0.701	0.780	0.670	0.780
	sn2	0.676			
	sn3	0.661			
	sn4	0.702			

Source: own processing in SPSS

Empirical Analysis

Having established satisfactory model fit, as well as construct reliability and validity, the study proceeded to empirical analysis to test the proposed hypotheses. Structural equation modeling (SEM) was employed for this purpose. The analysis was conducted in two stages: assessment of direct effects and evaluation of mediation effects. The results of the direct effects analysis are presented in Table 4.

Table 4

Direct effects for empirical results

Hypothesis	Path Relationships			Estimate	S.E.	C.R.	p-value
H1	MC	→	PI	.267	.040	6.598	***
H2	ATT	→	PI	-.175	.043	-4.018	***
H3	SN	→	PI	.178	.068	2.632	.008
H4	PBC	→	PI	.303	.051	5.956	***
H5	EC	→	PI	.489	.096	5.099	***

Note: *** = 99% confidence level; ** = 95% confidence level; PI = purchase intention; MC = mascot characteristics; ATT = attitude; SN = subjective norms; PBC = perceived behavioral control; EC = emotional connection
Source: own processing in SPSS

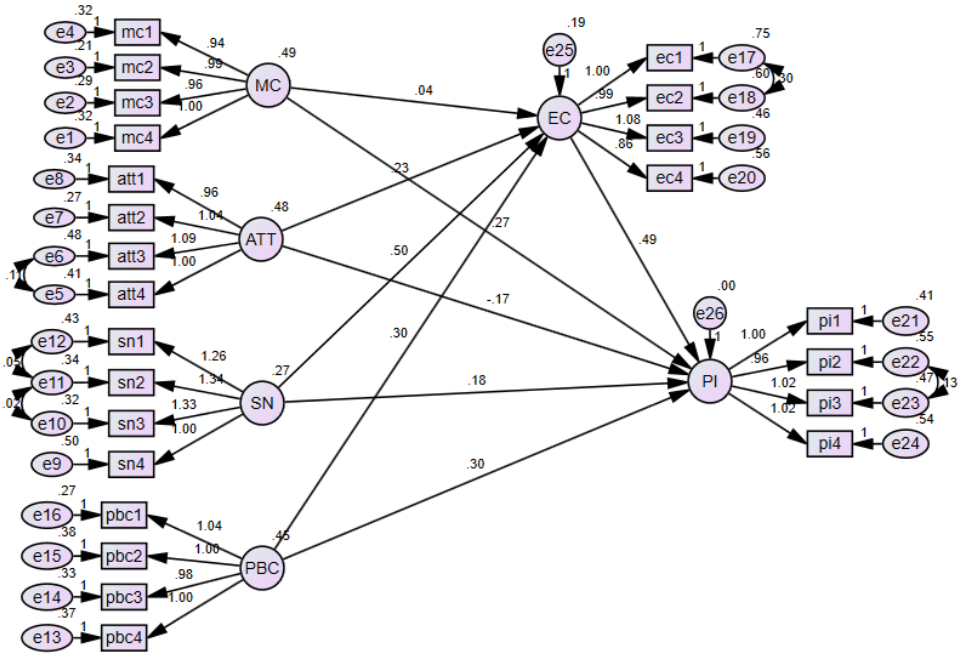

Figure 3. Direct effects for empirical results
Source: own processing in SPSS

The results of direct effects indicated that mascot characteristics have a positive and significant influence on the purchase intention (β = 0.267, p<0.05), hence confirming hypothesis 1. The attitude towards mascots has a negative and significant influence on the purchase intention (β = -0.175, p<0.05), hence rejecting the second hypothesis.

The third hypothesis was confirmed by the findings that subjective norm has a positive and significant influence on the purchase intention (β = 0.178, p<0.05). Additionally, perceived behavioral control regarding mascot was found to have a positive and significant influence on the purchase intention (β = 0.303, p<0.05). This confirmed hypothesis 4. Lastly, the emotional connection was found to have a positive and significant influence on the purchase intention (β = 0.489, p<0.05).

The other analysis that was conducted was the mediation analysis. The mediating effect of emotional connection between the independent variables (mascot characteristics, attitude, subjective norm, and perceived behavioral control) and purchase intention was evaluated. The results are presented in Table 5. The results indicated that the emotional connection significantly mediated the effect mascot characteristics, subjective norm, and perceived behavioral control on purchase intention, but did not mediate the effect of attitude on purchase intention.

Table 5

Mediation analysis of emotional connection

Hypothesis	Path relationships					Total effects	Direct effects	Indirect effects
H6	PBC	→	EC	→	PI	0.450**	0.303**	0.147**
	SN	→	EC	→	PI	0.422**	0.178	0.244**
	ATT	→	EC	→	PI	-0.065	-0.175**	0.110
	MC	→	EC	→	PI	0.289**	0.267**	0.022**
	EC	→	EC	→	PI	0.489**	0.489**	0.000**

Note: *** = 99% confidence level; ** = 95% confidence level; PI = purchase intention; MC = mascot characteristics; ATT = attitude; SN = subjective norms; PBC = perceived behavioral control; EC = emotional connection

Source: own processing in SPSS

The study pertains to food brand mascots that can be said to speak to commitment with regard to their effect on consumer purchase intention and use TPB as its base and emotional connection as a mediating variable. It takes different perspectives on how mascots are leveraged and, in the ever-evolving marketplace, how they influence selection for brand purchase. Interesting results were obtained regarding the relationships between food brand mascots, emotional and behavioral constructs, and consumers' purchase intentions. Emotional connection with mascot was found to have the highest significant positive influence on the purchase intention of the consumers.

The results are largely consistent with previous research on the influence of brand mascots on consumer behavior. Hypothesis 8 was confirmed, indicating that mascot characteristics have a significant positive effect on purchase intention. This finding aligns with Reddy et al. (2024), who emphasized that mascots infuse a human touch that fosters personal associations with brands. Similarly, Chen (2021) reported that visually appealing and emotionally expressive mascots positively influence consumer attitudes and purchase decisions, reflecting the direct effect observed in this study. Overall, the positive relationship supports the view that well-designed mascots create favorable first impressions and emotional responses that enhance purchase intention (Wang et al., 2025; Wu et al., 2025).

The confirmation of Hypothesis 3 indicates that subjective norms have a significant positive effect on purchase intention, consistent with prior research. Studies by Attah et al. (2024), Osei-Frimpong et al. (2022), and Thai and Wang (2020) demonstrate that social pressure and social endorsement of brand mascots shape consumers' subjective evaluations and subsequently strengthen their purchase intentions. These findings reinforce the critical role of social influence in consumer decision-making for brands employing mascots.

The positive and significant influence of perceived behavioral control has been supported in the literature, and this was confirmed by Hypothesis 4. Sathish (2023) argued that consumers' perceptions of a brand mascot's affordability, accessibility, and ease of purchase affect their behavior. The findings of the present study further suggest that the greater a consumer's sense of adequacy and control when interacting with a brand represented by a mascot, the stronger their intention to make a purchase.

Hypothesis 5 confirms that emotional connection has a strong positive influence on purchase intention, fully aligning with the study's theoretical framework and prior empirical evidence. Previous research has consistently emphasized the importance of emotional ties in consumer–brand interactions (Bigné et al., 2023; Ghorbanzadeh and Rahehagh, 2021; Niharika and Yadav, 2023). Nasr-Esfahani et al. (2022) highlighted human-like communication through mascots as a mechanism for strengthening consumer bonds, while Sayin and Gürhan-Canlı (2024) and Shimpi (2021) demonstrated that mascots evoke positive emotions that enhance brand attachment. The strong effect observed in the present study underscores the pivotal role of emotional connection in purchase decisions, particularly for symbolic brand elements such as mascots.

Perceived behavioral control towards mascot was found to also have a significant influence on the purchase intention. It implied the ease or difficulty of purchasing a product associated with a mascot is a critical aspect of consideration. Mascot is therefore found to be easy to make the consumers decision making process through intuitive branding, identification and clear messaging (Yoon, et al. 2016). This emphasizes the importance of inculcating the aspect of accessibility, confidence and affordability when designing the brand mascot. One interesting thing that caught attention during investigation was the characteristics of the mascot. Key elements in a mascot, for example, being attractive, having an identity others recall and being easy for people to relate to influence the decision to make a purchase. Deligoz and Ünal (2021) highlight similar points and show that mascots play a vital role in how consumer behavior is shaped. Additionally, Reddy and Sathish (2024) indicated that a brand's mascot helps to persuade consumers and guide their decisions when buying.

The results indicate that mascots effectively trigger emotional connections with consumers, increasing their propensity to purchase from the brand (Shimpi, 2021). These findings are consistent with Reddy and Sathish (2024), who identify mascots as emotional branding tools that foster brand loyalty and nostalgia. Similarly, Hoolwerff (2014) reported that consumers who form emotional bonds with brand mascots exhibit stronger brand commitment. Together, these insights underscore the importance of designing mascot representations that evoke affection, trust, and empathy among target consumers.

The emotional connection as the mediating effect per Hypothesis 6 for mascot's characteristics, subjective norm, and perceived behavioral control on purchase intention seems to be further supported in the literature. This shows that while these factors directly affect purchase intention, their impact is augmented when a person has created a strong emotional bond with the mascot of the brand. The fact of mascots mustering organizational members and concentrating their understanding of an organization into a single cue (Cayla, 2013) and leading consumer journeys (Arunrangsiwed and Pairoa, 2016) states the deeper connection these emotions can fashion.

How individuals perceive what others think about mascot plays a big role in making them want to purchase it. In other words, the way peers, family or leader figures react to the mascot is seen to influence whether consumers decide to buy the brand's product. According to the findings mentioned, Sartore-Baldwin and McCullough (2019) suggest that brands using mascots can influence consumers' intentions to make purchases in social media settings. Additionally, emotional connection was identified as an important and significant factor affecting the relationship. Therefore, mascot may draw in customers by helping them feel supported. According to the study, components of a mascot help form a bond with consumers that positively influence their desire to buy the product.

An important divergence from the anticipated findings was concerned with accepting and rejecting the second hypothesis, which proposed that the attitude towards the mascot would exert a positive and significant influence upon purchase intention. The results of this study found, contrary to our hypothesis and contrary to some literature (Deligoz and Ünal, 2021; Shimpi, 2021), a negative and significant influence ($\beta=-0.175$, $p<0.05$). This mark makes this finding rather intriguing to probe deeper. Some studies suggest a suitable mascot can lead to favorable brand attitudes (Oh and Kim, 2025; Reddy et al., 2024), whereas our finding might suggest that in some conditions a negative attitude towards a mascot could directly deter purchase intention without the mediation of emotional connection that could have produced a positive impact on intention. Alternatively, it is possible that a highly distinctive one, perhaps too polarizing for an average perception, could induce strong negative attitudes among some sections of the populace, hence overtaking whatever good intentions accrued toward purchase intention. The finding may throw some nuance into the relationship between attitudes toward mascots and purchase intention, an attitude that may not convert into actual purchase behavior but one which will actively block the purchase behavior once in the negative.

Consequently, emotional attachment was found to moderate the relationships between mascot characteristics, subjective norms, and perceived behavioral control, but it did not moderate the relationship between attitude and purchase intention. This lack of moderation suggests that attitude toward the mascot may influence purchase intention through a pathway that does not involve emotional connection, or that negative influences may strongly counteract any potential positive mediating effects. These findings highlight a promising area for further exploration in future research.

Implications from the results

From this research, both practical and theoretical recommendations were developed. To start, this research looked at the concept of brand mascot in the food sector together with the TPB. Thus, the findings should be found quite important by organizations and advertisers who use or intend to use brand mascots in their marketing, especially in the food industry. Most studies conducted thus far have investigated the TPB concepts separately. This study adds to the literature by focusing on how the involvement of emotions in relation to mascot can affect the impact of the TPB concepts. Furthermore, it presents a serious challenge to what TPB is generally expected to do. This might be mostly due to some complexities in how mascots influence how customers view food brands. It provides many suggestions for brand managers, advertising experts and marketing experts. Firstly, the study suggests that they create mascots that appeal strongly to people's feelings. This happens because of the strong emotional attachment to buying the product. Consequently, it is important for a mascot to form an emotional bond with its audience. That means composing stories with people who can talk and feel realistic. It is also recommended to use the power of social influence. Such

factors are important due to their effects on what people plan to buy. Hence, marketers might want to try initiatives such as sharing with others or using testimonials and partnering with influencers. Besides, it's a good idea for marketers to use mascots to spark emotional responses in their audiences. Along with using the mascot as a design element, it could form a connection with the target audience.

The validity of the construct "purchase intention" is thereby made more difficult, and considerable importance must now be placed on careful design decisions and consistent portrayals of mascots. Indeed, marketers believe that an appealing mascot should be easy for the average person to identify with, and possess the requirements for the brand personality. According to Min et al. (2019) and Kim (2018), these are also different personalities and appearances, as well as storytelling abilities of the mascot that determine the success. Other forms are subjective norms that can be applied as examples of social influence. To achieve this purpose, it is necessary for companies to build environments capable of producing positive word of mouth and social actions in support of their mascots, employing tools, for example, social media campaigns, influencer marketing, and community development. Massive social buzz around the mascot could significantly elevate purchase intentions.

The perceived control behavior, by positively affecting the ease of interaction and access to a brand, generates more influence toward the brand. In order to ground the mascot into consumers' perception of all brand activities involving the mascot, the mascot must exist at diverse consumer interaction points- i.e., online interactivity, clear communication of messages, and easy access to product-related information linked to the mascot.

A very big lesson to learn is that these connections can be emotional, and these emotions affect consumers' decision when buying. The connection between the mascot and the consumer must be a very close and very deep emotional connection to the point that it suffices for recognition purposes only as it will be tied to special story development with unique relevant background stories, feelings, or experiences that have been brought into being by mascots. Almost all independent variables have emotional connection as mediator: That is, there will be incremental increases resulting from other causes affecting purchase intention.

Lastly, the unexpected inverse relationship whereby a person might form some attitude toward a mascot tends to be an ominous sign needing caution and in-depth scrutiny; to identify and counteract any negative perceptions that might arise; businesses need to appraise customer attitudes on a regular basis in relation to their mascots. Most probably, this should include market research and an analysis of social media conversation to prepare for the adaptation or refinement of mascot strategies if they attract unfavorable reactions. Hence, it could be said that the very perspective with which a mascot can create positive feelings may sometimes, at a clumsy attempt, pull an apathetic audience away from the consumer.

Conclusions

This study demonstrates that brand mascots play a significant strategic role in shaping purchase intentions in the food industry by fostering emotional connections with consumers. Mascot characteristics, subjective norms, and perceived behavioral control positively influenced purchase intentions, while emotional connection strongly mediated these effects. However, attitude toward mascots showed an unexpected negative relationship with purchase intention, highlighting the complexity of mascot-driven consumer responses. Overall, the findings emphasize the importance of managing mascots as dynamic branding tools and suggest the need for further research into non-rational drivers of consumer behavior, including authenticity, trust, and mascot fatigue.

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Why Uzbekistan pays premium prices for sugar: Institutional and market analysis

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Abstract

Introduction. Among the fifteen largest global sugar importers by value, only three countries pay average prices exceeding $700 per ton: the United States ($761), Italy ($709), and Uzbekistan ($706). The first two countries implement extensive programs to protect domestic production. Uzbekistan produces virtually no sugar. This study examines how a country with nothing to protect pays prices comparable to protectionist economies.

Materials and methods. This study analyses data on sugar imports classified under the Harmonised System code HS1701 for the period 2017–2024, obtained from two main sources: the United Nations Commodity Trade Statistics Database (UN Comtrade) and the International Trade Centre Trade Map (ITC Trade Map). The study employs comparative price analysis across three dimensions – time, cross-country, and supplier-specific – in combination with natural experiment designs and a counterfactual welfare estimator.

Results and discussion. Using HS1701 import data from 2017 to 2024 for Central Asian and Commonwealth of Independent States (CIS) countries, we identify systemic procurement inefficiencies. Uzbekistan pays 17.4% more than Kazakhstan, 8.8% more than Tajikistan, and 7.9% more than Kyrgyzstan, despite importing more sugar than all regional competitors combined. Natural experiment comparisons separate institutional from geographic factors: Kyrgyzstan (EAEU member, similar geography) and Tajikistan (non-member, worse geography) both achieve better prices, ruling out location as the cause. For Brazilian sugar – where no preferential agreements apply – Uzbekistan pays a 39.6% premium over Kazakhstan, confirming procurement inefficiency independent of EAEU effects. Estimated welfare losses reach $67–117 million annually (0.06–0.10% of GDP), totalling $362 million in 2021–2024.

Conclusions. The study makes three contributions. First, it shows that institutions can outweigh geography: Tajikistan's lower prices despite worse location challenge the idea that landlockedness alone drives trade costs. Second, it quantifies the EAEU exclusion penalty (12.1%) while proving domestic factors matter – non-members like Tajikistan and Azerbaijan match EAEU prices. Third, it overturns bargaining theory: despite importing more sugar than all neighbours combined, Uzbekistan pays the highest prices. This inverse volume-price relationship reveals that institutional weaknesses can erase the benefits of scale.

Introduction

In 2024, Uzbekistan ranked fifteenth among the world's largest sugar importers by value and third by average import price, behind only the United States and Italy (Workman, 2025). This ranking requires explanation. The United States maintains a complex regime of tariff quotas and price supports to protect domestic sugar beet and cane producers. Prices in Italy reflect the protection of its sugar industry under the European Union's Common Agricultural Policy. Both countries accept higher import costs as the price of maintaining domestic production.

Uzbekistan has no such justification. Its continental climate, summer temperatures exceeding 40°C and annual precipitation of 100–400 mm, make commercial sugar production unprofitable (FAO, 2025). The country produces virtually no sugar, has no domestic industry requiring protection, and does not support an agricultural lobby demanding import barriers. Yet, it pays prices comparable to the world's most protectionist markets. Why?

The literature on landlocked developing countries documents significant trade cost penalties. Raballand (2003) found that transport costs are approximately 50% higher than those of coastal countries. UN-OHRLLS (2024) reports that landlocked countries spend almost twice as much of their export earnings on transport and insurance. For Central Asia, Carrere and Grigoriou (2011) estimate that improving infrastructure could increase exports by almost 50%, while Akbarov (2025) characterises the region as facing "some of the highest trade costs in the world" – 1.4 times higher than those of coastal developing economies.

A parallel literature examines the effects of regional integration. Tarr (2016) estimates that EAEU membership reduces trade costs by 15–30% due to harmonised standards and simplified customs procedures. Vinokurov (2017) documents preferential pricing of goods for EAEU members beyond formal tariff structures. Recent studies by Cieślik and Gurshev (2022) apply gravity models to Central Asian trade integration.

However, the aforementioned literature remains disconnected from product-level procurement analysis. Studies of landlocked countries' trade costs focus on aggregate indicators; studies of the EAEU examine trade flows rather than price efficiency; and the procurement literature focuses on government contracts in advanced economies. No existing research examines whether Central Asian countries facing similar geographic constraints achieve comparable import prices for specific goods. This gap is significant: if landlocked countries with identical locations pay systematically different prices for the same products from the same suppliers, geography cannot explain the difference. Something else must be at play.

This paper fills a gap by analysing commodity-level prices for sugar imports in Central Asia. Sugar provides an ideal test case: a standardised commodity (HS1701) with internationally defined purity requirements, traded globally in sufficient volumes to obtain reliable price data, and important enough to imply welfare effects.

The analysis utilises two natural experiments. First, the comparison of Uzbekistan and Kyrgyzstan holds geography roughly constant while varying EAEU membership. Both countries do not border dominant regional exporter, are located at similar latitudes, face comparable distances to suppliers, and must transit imports through Kazakhstan. Kyrgyzstan joined the EAEU in 2015; Uzbekistan remains outside it. If membership provides price advantages, Kyrgyzstan should outperform Uzbekistan despite its smaller import volumes, which, according to standard theory, should provide worse terms.

Second, the Brazilian sugar market isolates the efficiency of domestic procurement from the effects of regional trade agreements. Brazil does not have preferential agreements with any Central Asian country and exports a standardised commodity. Price differentials among Central

Asian importers of Brazilian sugar cannot reflect membership in the Eurasian Economic Union or bilateral political relations, but only differences in procurement institutions.

To test the robustness of our findings, we compare Uzbekistan not only with Kazakhstan (the region's largest economy with excellent infrastructure and access to Caspian ports), but also with Tajikistan – Central Asia's smallest economy with the region's most complex geography, weakest infrastructure, and longest transit routes. Even if Tajikistan achieves better prices than Uzbekistan, explanations based on scale, infrastructure, or geography become untenable.

Literature review

Three distinct strands of literature inform this study: trade costs in landlocked countries, the effects of regional trade agreements, and procurement efficiency in developing countries. While each offers important insights, none examines comparisons of product-level price efficiencies among countries with similar geographic constraints – a gap that this paper addresses. This part synthesises the relevant findings and develops the theoretical framework motivating our empirical analysis.

Trade costs and institutional quality. Research on landlocked developing countries has evolved from documenting geographic penalties to emphasising the institutional determinants of trade costs. Limão and Venables (2001) provided fundamental evidence that infrastructure quality explains significant variation in trade costs: poor infrastructure explains 40% of the predicted transport costs for coastal countries and 60% for landlocked countries. Improving infrastructure from the 75th to the 50th percentile increases trade by 50%.

However, subsequent research has found that geography alone cannot explain variation among landlocked countries. Anderson and Marcouiller (2002) demonstrated that institutional quality, levels of corruption, contract enforcement, and regulatory transparency affect trade costs regardless of distance. Their analysis finds that inadequate institutions restrict trade in the same way tariffs do, acting as a "hidden tax" on international exchange. Arvis et al. (2016) extended this framework to 178 countries, finding that logistical efficiency explains 20–60% of the variation in delivered prices, significantly exceeding pure transport differentials.

The Central Asian context has received particular attention. Carrere and Grigoriou (2011) estimate that improving transit country infrastructure to median levels among landlocked countries would increase Central Asian exports by 49%, emphasising that the region's trade costs reflect removable institutional failures rather than unchangeable geography. Shepherd and Wilson (2009) identify customs modernisation and regulatory harmonisation as the main sources of potential trade cost reductions of 25–40% in the region.

Regional trade agreements and preferential pricing. The literature on the EAEU documents the benefits of membership, but rarely quantifies the costs for non-members. Tarr (2016) provides basic estimates: membership reduces trade costs by 15–30% due to harmonised standards, simplified customs procedures, and tariff elimination. Vinokurov (2017) extends this beyond formal mechanisms, documenting preferential pricing of regional energy and goods for EAEU members, reflecting informal bargaining advantages and integrated supply chains.

The distribution of benefits remains controversial. Knobel (2015) finds significant trade creation among members, while Isakova et al. (2016) document uneven benefits, with smaller economies facing adjustment costs. A recent paper by Cieślik and Gurshev (2022) applies gravity models to Central Asian trade integration, finding that EAEU membership significantly influences trade patterns.

Volume-price relationship and procurement efficiency. Standard trade theory predicts that larger import volumes yield lower unit prices through economies of scale and increased

bargaining power. Harrigan (1993) formalises this relationship for product markets, while Goldberg and Knetter (1997) document volume discounts of 10–25% for large buyers of homogeneous products. If these mechanisms are effective, Uzbekistan, the largest sugar importer in Central Asia, should achieve the lowest prices in the region.

The purchasing literature identifies conditions under which this relationship breaks down. Bandiera et al. (2009) demonstrate that Italian state-owned organisations paid at least 22% more than semi-autonomous agencies for standardised goods, with the variation explained by management quality rather than procurement scale. Their distinction between "active waste" (corruption) and "passive waste" (inefficiency) suggests that institutional design determines whether scale advantages are realised.

For the post-Soviet context, Evenett and Hoekman (2005) document that state-owned trading firms often fail to utilise bargaining power due to capture by intermediaries, fragmented powers, and weak competitive pressures. Murrell (2002) attributes persistent inefficiencies to institutional path dependence, which isolates decision makers from market signals. In commodity markets, Pirrong (2014) shows that intermediaries can extract significant margins when buyers lack market information or face limited sourcing options, while Gilbert (2010) documents import price premiums of 15–35% in countries with concentrated import channels and limited procurement transparency.

Theoretical framework and value added. The preceding literature converges on a central finding: trade costs for landlocked countries reflect institutional quality as much as geography, and procurement efficiency depends on governance structures, not just on purchasing scale. Three mechanisms potentially explain why a large importer may pay premium prices despite the volume that should warrant discounts:

First, exclusion from regional trade arrangements may impose costs beyond formal tariff disadvantages. While EAEU membership provides informal price advantages through integrated supply chains and negotiating networks, non-members face a systematic disadvantage regardless of import volumes.

Second, inefficient domestic procurement may hinder the transformation of purchasing power into bargaining leverage. Capture by intermediaries, fragmented power, or weak competitive pressures may explain why larger buyers pay higher prices.

Third, supply-side factors can create price differentials through geopolitical preferences or bilateral relationship effects independent of buyer characteristics.

The Brazilian sugar market provides a critical test for distinguishing this third mechanism from the first two. Brazil has no preferential arrangements with any Central Asian country and exports a standardised commodity with identical commercial terms for all buyers. Price differentials among Central Asian importers of Brazilian sugar cannot reflect EAEU membership or supplier preferences – only differences in the efficiency of domestic procurement.

Materials and methods

Materials

Source Identification. This study analyses data on sugar imports classified under the Harmonised System code HS1701 for the period 2017–2024, obtained from two main sources: the United Nations Commodity Trade Statistics Database (UN Comtrade) and the International Trade Centre Trade Map (ITC Trade Map).

Description of Sources. UN Comtrade compiles official international trade statistics reported by national statistical offices, covering bilateral trade flows for over 170 countries. ITC Trade Map provides processed trade statistics with user-friendly interfaces for analysing trade patterns. Both databases report import values in US dollars and quantities in metric tons, allowing for the calculation of unit prices. All prices are quoted on a CIF (Cost, Insurance, and Freight) basis, including transportation and insurance costs to the destination border.

Data Limitations. Three limitations should be noted. First, CIF prices exclude domestic distribution costs, customs processing delays, and domestic distribution markups. Observed price differentials thus represent conservative lower estimates of actual price differentials. Second, confidential terms of bilateral contracts – payment terms, credit arrangements, volume commitments, and quality specifications – remain unobservable in aggregate trade data. Third, Turkmenistan's trade data are subject to partial reporting limitations, limiting certain comparisons.

Hypotheses

Based on the theoretical framework developed, this study tests four hypotheses:

H1: Uzbekistan faces systematic price differentials in sugar imports that persist across suppliers and cannot be explained by economies of scale or geography.

H2: Institutional factors dominate geographic constraints in determining import price differentials among Central Asian countries.

H3: Membership in the EAEU provides systematic price advantages for sugar supplied by the dominant regional exporter imports that exceed volume-based bargaining power.

H4: Uzbekistan's price disadvantages impose significant and persistent welfare costs that reflect structural institutional failures rather than temporary market conditions.

Analytical methods

The study employs comparative price analysis across three dimensions – time, cross-country, and supplier-specific – in combination with natural experiment designs and a counterfactual welfare estimator.

The analytical framework proceeds in three stages:

Stage 1: Documenting price patterns. The time analysis tracks Uzbekistan's import prices by supplier for 2017–2024 to identify structural breaks and shifts in market composition. The cross-country analysis compares average CIF prices for Central Asian importers, establishing regional benchmarks. The supplier-specific analysis compares prices paid for imports from identical suppliers (dominant regional exporter, Brazil) by different importing countries.

Stage 2: Institutional effect isolation. Two natural experiments separate institutional and geographic determinants:

The Uzbekistan-Kyrgyzstan comparison exploits variation in EAEU membership while holding geography roughly constant. Neither country borders dominant regional exporter, are located at similar latitudes (Tashkent 41.3° N, Bishkek 42.9° N), are at comparable distances from regional suppliers, and are forced to transit imports through Kazakhstan. Kyrgyzstan joined the EAEU in 2015; Uzbekistan remains outside. The price differential between these generally similar countries isolates the impact of EAEU membership from geographic factors.

A test of the Brazilian sugar market allows us to isolate the effectiveness of domestic procurement from the impact of regional trade agreements. Brazil does not have preferential

agreements with any Central Asian country and exports a standardised commodity with identical commercial terms for all buyers. The difference in Brazilian sugar import prices cannot reflect EAEU membership or supplier preferences, but rather reflects differences in domestic procurement institutions.

To ensure that the observed differences reflect institutional factors rather than quality differences within the 4-digit HS1701 classification, the comparison of Uzbekistan and Tajikistan examines pricing at the more detailed 6-digit level (HS170112 raw sugar; HS170199 refined sugar).

Step 3: Welfare quantification. The economic losses from unfavourable prices are estimated using counterfactual analysis:

Aggregate welfare loss:

$$WL_{total} = Q_{UZ} \times (P_{UZ} - P_{benchmark})$$

Supplier-specific welfare loss:

$$WL_{supplier} = Q_{UZ,supplier} \times (P_{UZ,supplier} - P_{benchmark,supplier})$$

where WL denotes the welfare loss in US dollars, Q represents the import volume in tons, P denotes the average CIF price per ton, and the subscripts indicate the respective country (UZ for Uzbekistan) and the origin of the supplier.

Rationale for the choice of method. Comparative price analysis is appropriate because sugar is a standardised commodity with minimal quality differences, and price differences primarily reflect procurement efficiency and trade costs. The natural experiment design exploits institutional differences (EAEU membership) and supplier neutrality (Brazil), which provide identification unavailable in studies of total trade costs. The counterfactual welfare valuation follows standard methods in trade economics for quantifying efficiency losses.

Benchmark selection

Three baseline scenarios provide estimates under different performance assumptions:

Benchmark	Description	Rationale
Kazakhstan	Region's lowest average import price	Optimistic scenario: Uzbekistan achieves best regional performance
Regional average (excl. Uzbekistan)	Mean price across Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan	Moderate scenario: Uzbekistan achieves typical regional performance
Supplier-specific	For Brazilian sugar: Kazakhstan-Tajikistan average; For sugar supplied by the dominant regional exporter: post-Soviet regional average excl. Uzbekistan	Isolates inefficiency within dominant supply relationships

The average price for Brazilian sugar between Kazakhstan and Tajikistan combines excellent logistics (Kazakhstan's connection to the Caspian Sea) with unfavourable conditions (Tajikistan's disadvantageous geographic location), providing a balanced benchmark for efficiency. The post-Soviet regional average for sugar supplied by the dominant regional exporter accounts for different conditions for regional importers, EAEU members with institutional advantages, non-EAEU countries with different geographic locations, and countries of varying sizes.

Multi-year welfare tracking (2021–2024) allows us to distinguish temporary market failures from chronic institutional failures. Losses are scaled relative to GDP to assess macroeconomic significance.

Methodological limitations. Several caveats should be considered. First, the welfare assessment assumes that benchmark prices represent achievable alternatives for Uzbekistan, which may overstate potential savings if Uzbekistan faces unique constraints not observed in the data. Second, comparisons within a natural experiment suggest that unobserved country characteristics are not systematically correlated with either EAEU membership or import prices. Third, the analysis fails to distinguish alternative institutional mechanisms (intermediary capture, power fragmentation, weak competition) that could explain the observed inefficiencies.

Results and discussion

This section addresses the central question underlying this paper: why does Uzbekistan, a country without its own sugar industry to protect, pay import prices comparable to those of the world's most protectionist economies? Two caveats special attention: CIF prices do not include domestic distribution costs, so the observed differences represent conservative lower-bound estimates; and confidential contract terms remain unobserved in aggregate trade data. Given these limitations, the analysis tests four hypotheses: Uzbekistan faces a systematic pricing disadvantage vis-à-vis suppliers (H1); institutional factors dominate geographic constraints (H2); membership in the Eurasian Economic Union (EAEU) provides price advantages (H3); and, consequently, welfare losses reflect persistent institutional failures (H4).

Market structure and price dynamics

We begin by analysing the dynamics of Uzbekistan's import prices over the study period. Table 1 presents the average prices at which Uzbekistan imported sugar from major suppliers between 2017 and 2024.

Table 1

Uzbekistan import prices ($/t) of HS1701 from selected major suppliers

Countries	2017	2018	2019	2020	2021	2022	2023	2024
World	615	421	422	425	1369	633	756	705
Primary regional supplier (EAEU)	475	482	397	411	598	1335	706	702
Brazil	631	389.3	415	431	1450	628	780	784
Ukraine	502	471	488	516	591	470	n/a	n/a
Pakistan	400	302	326	520	591	444	556	n/a
Kazakhstan	467	473	456	489	591	n/a	622	411
Mexico	n/a	n/a	423	n/a	n/a	614	n/a	n/a
secondary EAEU producer	427	467	460	439	n/a	n/a	755	713
India	n/a	383	23352	381	n/a	667	483	836

Source: Own elaboration based on ITC & UN Comtrade 2025 Database, n/a – not available/not applicable

Table 1 reveals two distinct periods. Between 2017 and 2020, prices remained relatively stable, falling from US$615.7 per ton in 2017 to approximately US$422–426 per ton in 2018–2020. 2021 saw a sharp price spike, with average import prices jumping to US$1,369.6 per ton, more than triple the 2020 level. This jump likely reflects pandemic-related supply chain disruptions and global commodity inflation. The price of Brazilian sugar reached US$1,450.6 per ton in 2021, while the price of sugar supplied by the dominant regional exporter jumped to US$1,335.9 per ton in 2022. However, what happened after the crisis is noteworthy. Although prices declined, they did not return to pre-crisis levels. By 2024, Uzbekistan's average import price stabilised at $705.8/t, still 65.7% higher than the 2020 level ($425.9/t). This steady increase raises several questions: did price changes reflect changes in Uzbekistan's sugar supply sources? Perhaps the 2021 crisis forced a switch to more expensive suppliers. Or did geopolitical events, particularly the post-2022 geopolitical shock and dominant regional exporter's subsequent reorientation toward Central Asian markets, alter regional commodity flows, affecting pricing?

For analysis, Table 2 presents the value structure of sugar imports to Uzbekistan by supplier for the same period.

Table 2

Uzbekistan's import values (millions $) of HS1701 from selected suppliers

Countries	2017	2018	2019	2020	2021	2022	2023	2024
World	330.2	336.6	265.7	240.0	333.4	520.9	471.9	790.8
Primary regional supplier (EAEU)	14.3	93.3	29.1	154.7	3.0	0.3	68.2	342.0
Brazil	304.2	30.7	91.7	42.7	291.9	460.1	376.2	336.2
Ukraine	1.2	106.3	33.6	6.6	2.3	0.0	n/a	n/a
Pakistan	0.0	44.8	0.1	0.4	0.1	5.4	12.4	n/a
Kazakhstan	10.2	27.3	2.0	7.6	0.8	n/a	5.2	57.0
Mexico	n/a	n/a	89.5	n/a	n/a	4.3	n/a	n/a
secondary EAEU producer	0.2	11.1	4.6	18.1	n/a	n/a	0.6	7.5
India	n/a	0.1	0.0	0.0	n/a	34.6	3.9	3.9

Source: Own elaboration based on ITC & UN Comtrade 2025 Database | n/a – not available/not applicable

Table 2 demonstrates the radical transformation of suppliers. In 2017, Brazil dominated sugar imports to Uzbekistan with a volume of US$304.2 million (92.1% of the total of US$330.2 million). dominant regional exporter was a minor player, supplying only US$14.3 million (4.3%). By 2024, the picture had completely reversed: dominant regional exporter supplied US$342.0 million (43.2% of imports), while Brazil supplied US$336.2 million (42.5%), virtually equaling parity between the two.

The growth in regional imports is particularly striking. In 2022, sugar imports from dominant regional exporter fell to just US$0.3 million (0.06% of imports). By 2024, they reached US$342.0 million, a 1,140-fold increase in just two years. This radical shift coincided with the conflict in Ukraine (note Ukraine's complete disappearance from Table 2 after 2022) and dominant regional exporter's intensified economic engagement with Central Asian countries during this period. Whether this supplier reorientation reflects Uzbekistan's strategic diversification, the aggressive entry of regional exporters seeking new markets, or a combination of both is impossible to determine from trade data alone.

Returning to the question of price: did this supplier shift explain the price increase? Analysing Tables 1 and 2 together, the answer appears to be no. sugar supplied by the dominant regional exporter costs $702.8/t in 2024, while Brazilian sugar costs $784.6/t; both suppliers are setting higher prices. Moreover, Brazilian sugar prices actually increased from $628.0/t (2022) to $784.6/t (2024), despite Uzbekistan remaining Brazil's largest regional consumer (Table 1). Standard bargaining theory predicts that large buyers should negotiate better, not worse, terms over time. Comparing the two tables reveals another pattern: total import volumes rose sharply from 243,400 tons in 2021 to 1,120,424 tons in 2024. However, average prices remained high rather than declining with increasing scale. The increase in volumes did not translate into price improvements.

These patterns raise a central question for subsequent sections: are Uzbekistan's high prices typical of landlocked Central Asian countries, or is Uzbekistan worse off than its neighbours, facing similar geographic constraints?

Systematic price disadvantage in Central Asian countries (H1)

To determine whether Uzbekistan's prices are competitive in the region, we compare them with those of other Central Asian importing countries. If all landlocked countries in the region pay similar prices, Uzbekistan's higher costs may simply reflect unavoidable geographic realities. If its neighbours achieve higher prices, this difference must be explained by something specific to Uzbekistan.

H1 predicted that Uzbekistan faces a systematic price disadvantage that persists across suppliers and cannot be explained by economies of scale or geography. Table 3 presents import data for 2024 for all Central Asian countries.

Table 3

Central Asian Countries' HS1701 import patterns (2024)

Country	Total imports (million $)	Avg. price ($/t)	Main supplier	Import value from main supplier (million $ (%))	Price from main supplier ($/t)
Uzbekistan	790.8	705.8	DRE	341.6 (43.2)	702.8
Kazakhstan	345.8	601.4	DRE	277.0 (80.1)	609.1
Kyrgyzstan	28.9	646.3	DRE	27.6 (95.4)	651.5
Tajikistan	130.2	648.7	DRE	59.7 (46.4)	639.8
Turkmenistan	21.3	688.7	Azerbaijan	21.0 (98.6)	691.7

* DRE - Dominant Regional Exporter

Source: Own elaboration based on ITC & UN Comtrade 2025 Database

The comparison reveals a striking anomaly: Uzbekistan, the region's largest importer, pays the highest prices. Uzbekistan imported $790.8 million worth of sugar, more than double the value of the next-largest importer (Kazakhstan, at $345.8 million) and all other Central Asian countries combined. However, Uzbekistan's average price of $705.8 per ton exceeds every regional competitor.

Kazakhstan, which imports less than half of Uzbekistan's value, purchased sugar at $601.4 per ton, a $104.4 per ton advantage (17.4%). Even the region's smallest importers

achieved higher prices: Kyrgyzstan at $646.3 per ton (8.4% less than Uzbekistan), Tajikistan at $648.7 per ton (8.8% less), and Turkmenistan at $688.7 per ton (2.4% less). The expected relationship between volume and price is not only absent, but inverse. The largest buyer pays the most. Table 3 also shows that dominant regional exporter is the primary supplier for four of the five countries (Turkmenistan relies primarily on Azerbaijan). This allows for a direct comparison of prices for the same product from the same supplier. Uzbekistan paid $702.8/t for sugar supplied by the dominant regional exporter, the highest price among all buyers. Kazakhstan paid $609.1/t (15.4% less), Tajikistan $639.8/t (9.8% less), and Kyrgyzstan $651.5/t (7.9% less).

Could Uzbekistan's disadvantage be limited to sugar supplied by the dominant regional exporter, reflecting bilateral political factors? To determine whether this trend applies to all suppliers, Table 4 provides detailed information on each country's supplier portfolio.

Table 4

Central Asian countries' HS1701 import patterns (2024) extended

Uzbekistan				**Kyrgyzstan**			
Exporters	Share (%)	Quantity (1000t)	Price $/t	Exporters	Share (%)	Quantity (1000t)	Price $/t
DRE	43.2	486.5	702.8	DRE	95.4	42.3	651.5
Brazil	42.5	428.6	784.6	SP	3.9	2.2	525.1
Kazakhstan	7.2	138.5	411.3	Iran, Islamic Republic of	0.3	0.1	776.8
Azerbaijan	1.8	16.7	833.2	Kazakhstan	0.2	0.1	865.7
Morocco	1.4	12.2	933.3	**Turkmenistan**			
Thailand	1.4	12.0	893.9	Azerbaijan	98.6	30.4	691.7
SP	0.9	10.5	714.0	Pakistan	1.3	0.6	520.0
Kyrgyzstan	0.9	9.8	756.2	**Tajikistan**			
India	0.5	4.7	836.1	DRE	46.4	93.2	639.8
Kazakhstan				SP	16.6	33.3	847.1
DRE	80.1	454.9	609.1	India	15.1	30.4	557.0
Brazil	14.5	89.0	561.9	Pakistan	8.8	17.7	538.4
SP	5.3	30.8	593.0	Kazakhstan	4.9	9.8	580.7
Central Asia excl. Uzbekistan		852.0	646.0	Brazil	0.2	0.5	551.1

* DRE - Dominant Regional Exporter; SP – secondary EAEU producer

Source: Own elaboration based on ITC & UN Comtrade 2025 Database

These results support hypothesis H1: Uzbekistan faces a systematic price disadvantage that persists across all major suppliers and cannot be explained by economies of scale or geography. Uzbekistan pays 17.4% more than Kazakhstan and 8.8% more than Tajikistan, despite the latter facing greater geographic barriers. This trend holds for both sugar supplied by the dominant regional exporter (+15.4% compared to Kazakhstan) and Brazilian sugar (+39.6% compared to Kazakhstan), confirming a systemic disadvantage rather than a supplier-specific one.

Institutional and geographical determinants (H2, H3)

The price disadvantages described above could theoretically be due to geographic location. Uzbekistan's unique landlocked location may impose costs that its neighbours avoid. H2 predicts that institutional factors, not geography, determine price differences. H3 predicts that membership in the EAEU, in particular, provides price advantages.

Central Asia provides an ideal setting for testing these hypotheses, as all five countries are landlocked. If geography determines prices, countries with similar locations should pay similar prices. If institutions matter, countries with different institutional arrangements should pay different prices regardless of geographic similarity.

Comparison with Kyrgyzstan. The Uzbekistan-Kyrgyzstan pair represents a natural experiment, particularly for sugar supplied by the dominant regional exporter imports. Both countries have remarkably similar geographic characteristics: neither directly borders dominant regional exporter (their main supplier), both are located at similar latitudes (Tashkent 41.3° N, Bishkek 42.9° N), both are comparable distances from regional suppliers, and both must transit imports through Kazakhstan.

This comparison is motivated by an important institutional context. All post-Soviet regional member states, including Uzbekistan and Kyrgyzstan, participate in the Commonwealth of Independent States Free Trade Area, established by the 2011 post-Soviet regional Free Trade Area Agreement, which eliminates import duties on most goods, including sugar, between signatory states (WTO, n.d.-a). Uzbekistan joined this agreement in 2013. Thus, both countries benefit from duty-free sugar imports from dominant regional exporter under the post-Soviet regional agreements. The key institutional difference lies elsewhere: Kyrgyzstan joined the EAEU in 2015, gaining access to deeper integration benefits, including harmonised customs procedures, simplified border controls, and preferential supply chain agreements that go beyond tariff waivers. Uzbekistan remains outside the EAEU.

If prices were determined by geography, both countries should pay roughly the same amount for sugar supplied by the dominant regional exporter, given their comparable positions. If EAEU membership provides additional benefits beyond the post-Soviet regional FTA, Kyrgyzstan should pay less, despite its smaller import volumes.

Returning to Table 3, the data support the institutional explanation. Kyrgyzstan pays $651.5 per ton for sugar supplied by the dominant regional exporter, while Uzbekistan pays $702.8 per ton, giving Kyrgyzstan an advantage of $51.3 per ton (7.9%). This advantage is achieved despite the fact that Kyrgyzstan imports only 42,300 tons compared to Uzbekistan's 486,500 tons (Table 4). Standard bargaining theory predicts that larger volumes should yield more favourable terms. Instead, the smaller EAEU member outperforms the larger non-member. A similar geographic factor leads to different results depending on institutional membership.

Tajikistan's stress test. Kyrgyzstan's advantage may be due precisely to its EAEU membership. But what about countries that, like Uzbekistan, are not members? Tajikistan presents a complex challenge. Among the Central Asian countries, Tajikistan faces the most challenging circumstances: the smallest economy, the weakest infrastructure, and the most isolated geography, requiring transit through two countries to reach regional suppliers. Many believe that if any country should pay higher prices due to its geographic disadvantage, it is Tajikistan. However, as shown in Table 3, Tajikistan pays $639.8 per ton for sugar supplied by the dominant regional exporter, which is $63.0 per ton (9.8%) less than Uzbek sugar at $702.8 per ton. Neither country is a member of the EAEU and operates under the same post-Soviet regional FTA system, so this difference cannot reflect the benefits of membership.

The country in the region, which is most geographically disadvantaged, achieves better prices than Uzbekistan, which is located in the centre of the country. Geographic factors cannot explain this pattern.

Could the price difference in Tajikistan reflect differences in quality? Perhaps Tajikistan imports lower-quality sugar, which is sold at lower prices. To test this hypothesis, Table 5 compares prices at a more detailed six-digit code level.

Table 5

Uzbekistan-Tajikistan HS6-level sugar price comparison (2024)

HS code	Tajikistan import price $/t	Uzbekistan import price $/t	Tajikistan quantity (1000t)	Uzbekistan quantity (1000t)
170112	616.4	720.8	12.5	264.6
170199	643.4	680.9	80.7	221.9

Source: Own elaboration based on UN Comtrade 2025 Database

Table 5 rules out the quality explanation. For both raw sugar (HS170112) and refined sugar (HS170199), Tajikistan achieves better pricing despite smaller volumes. The differential is particularly large for raw sugar: Tajikistan pays $616.4/t while Uzbekistan pays $720.8/t, a 16.9% premium for Uzbekistan. For refined sugar, Tajikistan pays $643.4/t versus Uzbekistan's $680.9/t, a 5.8% premium. Quality variation within the HS1701 classification does not explain Uzbekistan's disadvantage.

Post-Soviet regional-wide patterns. Having established that both EAEU membership (Kyrgyzstan comparison) and domestic factors (Tajikistan comparison) matter, we now examine the full pattern across all post-Soviet regional sugar supplied by the dominant regional exporter importers. Table 6 presents the complete picture.

Table 6

Post-Soviet regional Countries' regional HS1701 import prices (2024)

Rank	Country	Import value (millions $)	Quantity (1000 t)	Price $/t	EAEU member
1	Uzbekistan	342.0	486.5	702.8	No
2	Kazakhstan	277.1	454.9	609.1	Yes (founding member)
3	Tajikistan	59.6	93.2	639.8	No
4	Azerbaijan	31.4	49.2	638.0	No
5	Kyrgyzstan	27.6	42.3	651.5	Yes (since 2015)
6	Armenia	24.4	38.6	631.6	Yes (since 2015)
7	Turkmenistan	0.0	0.0	n/a	No
8	secondary EAEU producer	0.0	0.0	n/a	Yes (founding member)
	post-Soviet regional aggregate excl. Uzbekistan	420.1	678.2	634.0	
	EAEU countries	329.0	535.8	614.1	
	Non-EAEU countries	433.0	628.9	688.4	

Source: Own elaboration based on UN Comtrade 2025 Database; Eurasian Economic Commission (2024); Ministry of Foreign Affairs of Armenia (2024)

Table 6 includes data for all post-Soviet regional countries for a complete picture. Turkmenistan and secondary EAEU producer did not import sugar supplied by the dominant regional exporter in 2024. Turkmenistan purchases it almost exclusively from neighbouring Azerbaijan (Table 4), while secondary EAEU producer, a founding member of the EAEU and with its own significant sugar production capacity, does not import sugar supplied by the dominant regional exporter.

Aggregate data for active importers demonstrates the systematic effect of membership. In EAEU countries (Kazakhstan, Kyrgyzstan, Armenia), the average price of sugar supplied by the dominant regional exporter is $614.1/t. In non-EAEU countries (Uzbekistan, Tajikistan, Azerbaijan), the average price is $688.4/t. This gap of $74.3/t represents a membership premium of 12.1%, consistent with Tarr's (2016) estimate that EAEU membership reduces trade costs by 15–30%. In support of H3, membership status provides systematic price advantages that appear to extend beyond the duty-free access already available under the post-Soviet regional FTA.

The table also demonstrates that membership trumps volume. Armenia imports only 38,600 tons, but achieves a price of $631.6 per ton, higher than Uzbekistan ($702.8 per ton), despite Uzbekistan importing 12.6 times more. Kazakhstan imports slightly less than Uzbekistan (454,900 versus 486,500 tons), but pays 15.4% less. Volume advantages are not realised for non-EAEU countries.

However, one important trend complicates the situation for the EAEU: among non-EAEU countries, Uzbekistan performs the worst. Tajikistan ($639.8 per ton) and Azerbaijan ($638.0 per ton) achieve prices closer to those of EAEU member countries than to those of Uzbekistan ($702.8 per ton). Uzbekistan pays $63.0 per ton (9.8%) more than Tajikistan and $64.8 per ton (10.2%) more than Azerbaijan, which are also non-EAEU members but operate under the same post-Soviet regional FTA structure. If exclusion from the EAEU explained Uzbekistan's premium, all non-EAEU countries would have to pay similar prices. However, this is not the case. Something specific to Uzbekistan, beyond its geographic location and trade agreement status, is driving additional costs.

These results support hypotheses H2 and H3. A comparison with Tajikistan shows that geography cannot explain the difference: the country in the most disadvantaged position achieves more favourable prices. A comparison with Kyrgyzstan and an analysis of the post-Soviet regional confirm that EAEU membership provides systematic benefits (on average 12.1%) compared to those available solely through the post-Soviet regional Free Trade Agreement. However, Uzbekistan also performs worse than other non-EAEU countries, suggesting internal institutional factors that exacerbate the penalty for non-membership.

Procurement inefficiency in neutral markets

The previous analysis established that EAEU membership matters, but it cannot fully explain Uzbekistan's premium, despite other non-EAEU countries offering higher prices. To isolate domestic procurement efficiency from the impact of trade agreements, we need a market in which neither EAEU membership nor post-Soviet regional free trade agreements provides any advantage. Brazilian sugar provides such a criterion.

Brazil does not have preferential agreements with any Central Asian country. It exports a standardised commodity traded commercially worldwide. Brazil has no geopolitical interest in offering differential pricing to Central Asian buyers. Therefore, price differences between Central Asian importers of Brazilian sugar reflect only buyer-side factors, primarily procurement efficiency, and not supplier preferences or the impact of trade agreements.

Table 7 presents the import prices of Brazilian sugar for three Central Asian countries importing from Brazil in 2024.

Table 7

Brazilian sugar HS1701 import prices (2024)

Country	Import value (million $)	Quantity (1,000t)	Price ($/t)
Uzbekistan	336.2	428.6	784.6
Kazakhstan	50.0	89.0	561.9
Tajikistan	0.2	0.5	551.1

Source: Own elaboration based on UN Comtrade 2025 Database

The results are striking. Uzbekistan pays $784.6/t, while Kazakhstan pays $561.9/t, representing a premium of $222.7/t (39.6%) for Uzbekistan. Tajikistan, despite importing only 450 tons (952 times less than Uzbekistan), pays $551.1/t, the lowest price in the region and $233.5/t (42.4%) less than Uzbekistan.

A comparison of Brazilian and regional premiums demonstrates the relative importance of the EAEU's influence compared to domestic purchases. For sugar supplied by the dominant regional exporter (Table 6), Uzbekistan pays 15.4% more than Kazakhstan. For Brazilian sugar, Uzbekistan pays 39.6% more than Kazakhstan. The Brazilian premium is 2.6 times higher than the regional one.

This comparison has important implications. sugar supplied by the dominant regional exporter prices partially reflect the impact of EAEU membership; Kazakhstan enjoys preferential access that Uzbekistan does not. However, Brazilian sugar prices cannot reflect the influence of the EAEU, as Brazil does not offer preferential treatment to anyone. The Brazilian premium of 39.6% reflects the pure inefficiency of domestic procurement. Since this premium exceeds the regional premium of 15.4%, domestic factors lead to higher costs than non-membership in the EAEU.

The results for Tajikistan confirm this conclusion. Tajikistan, with the weakest infrastructure and the longest transit times, achieves the best price in the region for Brazilian sugar – $551.1/t. Geographical disadvantage does not lead to higher prices; rather, the relationship is inverse. The only explanation consistent with all the observed patterns is differences in domestic procurement efficiency. The specific mechanisms underlying this inefficiency cannot be determined from trade data alone: intermediary markups, internal rents, fragmented procurement authority, weak competitive bidding, or information asymmetries remain unobservable. However, this pattern strongly suggests that achieving competitive prices will require institutional reform of procurement processes, not just EAEU membership. This finding further supports hypothesis H2: institutional factors, particularly domestic procurement institutions, dominate over geographic constraints.

Welfare implications

Having established that Uzbekistan faces systematic price declines driven primarily by domestic institutional factors, we now quantify the resulting welfare losses. H4 predicts that these losses will lead to substantial and persistent losses, reflecting structural failures rather than temporary conditions.

Table 8 presents welfare loss estimates for 2024 under alternative benchmark scenarios.

Table 8

Welfare loss estimates under alternative benchmark scenarios (2024)

Benchmark	Benchmark price ($/t)	Price premium ($/t)	Total welfare loss (million $)	% of total sugar import value	% of Uzbekistan's GDP*	Scenario type
Kazakhstan	601.4	104.4	117.0	14.8%	0.10%	Optimistic
Regional average**	646.0	59.8	67.0	8.5%	0.06%	Moderate
Welfare loss on Brazilian sugar only: Uzbekistan imports of 428 552 tons						
Kazakhstan-Tajikistan average***	556.5	149.3	64.0	14.9%	0.06%	Moderate
Welfare loss on sugar supplied by the dominant regional exporter only: Uzbekistan imports of 486 544 tons						
Post-Soviet regional average excl. Uzbekistan	634.0	71.8	34.9	7.2%	0.03%	Moderate

Source: Own elaboration based on ITC & UN Comtrade 2025 Database. * Uzbekistan GPD (2024) estimated at $114.97 billion (World Bank, 2025). **Regional average – average import prices of Central Asian countries excluding Uzbekistan. *** Average import price for the Brazilian sugar of Kazakhstan and Tajikistan combined

Annual welfare losses range from US$67.0 million (moderate scenario) to US$117.0 million (optimistic scenario), representing 0.06–0.10% of GDP. For comparison, the US$67.0 million loss under the moderate scenario exceeds the combined sugar import costs of Kyrgyzstan ($28.9 million) and Turkmenistan ($21.3 million), as shown in Table 3. Purchasing inefficiencies in Uzbekistan are more costly than the combined sugar import costs of the two neighbouring countries.

A breakdown by supplier reveals where the inefficiencies are concentrated and confirms previous findings. Losses from Brazilian sugar reach US$64.0 million, almost double the US$34.9 million lost from sugar supplied by the dominant regional exporter. Total supplier-related losses of $98.9 million exceed those under the moderate scenario ($67.0 million), reflecting the concentration of inefficiencies in dominant supply relationships. Brazilian sugar prices reflect pure procurement inefficiencies (excluding the EAEU effect), while sugar supplied by the dominant regional exporter prices partially reflect the disadvantages of membership. The welfare distribution confirms the same pattern: disruptions in domestic procurement lead to greater costs than non-membership in the EAEU. Even if Uzbekistan were to join the EAEU tomorrow, significant losses from inefficient procurement of Brazilian sugar would persist.

Temporal Sustainability. The magnitude of the losses matters, but so does their sustainability. Temporary disruptions can resolve themselves; structural disruptions require policy intervention. Table 9 tracks welfare losses over 2021–2024 to distinguish between these options.

Table 9

Cumulative welfare losses (Regional average benchmark)

Year	Uzb. import value (millions $)	Uzb. price ($/t)	Regional average* price ($/t)	Premium ($/t)	Quantity (1,000t)	Welfare loss (millions $)
2021	333.4	1369.6	517.8	851.7	243.4	207.3
2022	520.9	633.2	598.3	34.9	822.6	28.7
2023	471.9	756.8	662.9	93.9	623.5	58.5
2024	790.8	705.8	646.0	59.8	1120.4	67.0
Total	2117.0	n/a	n/a	n/a	n/a	**361.6**

Source: Own elaboration based on ITC & UN Comtrade 2025 Database | n/a – not available/not applicable

*Regional average – average import prices of Central Asian countries excluding Uzbekistan

The sharp price spike in 2021 (losses of US$207.3 million) reflects the effects of the pandemic and can reasonably be considered temporary. However, subsequent events cannot be considered temporary. Losses persisted even after market normalisation: US$28.7 million (2022), US$58.5 million (2023), and US$67.0 million (2024).

Two trends point to structural rather than temporary causes. First, losses more than doubled from 2022 to 2024 (from US$28.7 million to US$67.0 million), despite the stabilisation of global sugar markets. If Uzbekistan's disadvantage were due to temporary supply chain disruptions, losses should have declined as conditions returned to normal. Instead, they continued to grow.

Secondly, losses increased despite a 4.6-fold increase in import volumes – from 243,400 tons (2021) to 1,120,424 tons (2024). Increasing volumes should promote economies of scale and strengthen bargaining power. Instead, Uzbekistan's losses increased as its market share expanded. The procurement system is unable to translate increased purchasing power into better prices.

Cumulative losses for 2021–2024 amounted to US$361.6 million, accounting for 17.1% of total import expenditures for this period. This amount represents a significant transfer of resources from Uzbek consumers and businesses to foreign suppliers and intermediaries, which could otherwise support domestic consumption or investment.

These results support hypothesis H4. The persistence of premiums through 2022–2024, the increase in losses despite the growth in volumes, and the concentration of losses in the politically neutral Brazilian market all point to structural institutional failures that require political intervention rather than patience.

Conclusions

This study examines a striking anomaly: why does Uzbekistan, a country without its own sugar industry requiring protection, pay import prices comparable to those of the world's most protectionist economies? Among the fifteen largest global sugar importers, only the United States ($761/t), Italy ($709/t), and Uzbekistan ($706/t) pay average prices exceeding $700 per ton. The first two countries employ extensive domestic protection regimes. Uzbekistan has no such justification, as it produces virtually no sugar and has no industries requiring protection.

The analysis tested four hypotheses using HS1701 sugar import data (2017–2024) to Central Asian and post-Soviet regional countries. All were confirmed (Table 10).

Table 10

Summary of hypothesis tests

Hypothesis	Prediction	Key Evidence	Verdict
H1: Systematic price disadvantages	Uzbekistan pays more to suppliers regardless of size	17.4% of the total premium compared to Kazakhstan; 15.4% (dominant regional exporter), 39.6% (Brazil), 25–40% (minor suppliers); the largest regional importer pays the highest prices (Tables 3, 4, 7)	Supported
H2: Institutional factors dominate geography	Prices vary between countries with similar geographic locations; a poorer geographic location does not mean the highest prices	Tajikistan (the worst geography) pays 9.8% less than Uzbekistan; the Brazilian premium (39.6%) in the neutral market exceeds the regional premium (15.4%) (Tables 3, 5, 6, 7)	Supported
H3: EAEU membership confers price advantages	Member countries pay less than non-member countries	The difference between members and non-members is 12.1%; Armenia (38,648 t) is ahead of Uzbekistan (486,544 t) (Table 6)	Supported
H4: Persistent welfare costs from institutional failures	Losses continue after market normalisation; losses increase despite volume growth	Cumulative losses amounted to US$361.6 million (2021–2024); losses increased from US$28.7 million (2022) to US$67.0 million (2024), and volumes increased by 4.6 times (Tables 8, 9)	Supported

H1 (Systematic price disadvantages): Uzbekistan pays 17.4% more than Kazakhstan, 8.8% more than Tajikistan, and 7.9% more than Kyrgyzstan, despite importing more than all regional competitors combined. The disadvantage persists for all suppliers: +15.4% for sugar supplied by the dominant regional exporter, +39.6% for Brazilian sugar, and 25-40% for small suppliers. The expected volume-price relationship is inverse; the largest buyer pays more.

H2 (Institutional factors dominate geography): Tajikistan, the smallest economy in the region with the worst geographic location and weakest infrastructure, achieves prices 9.8% more favorable than geographically central Uzbekistan. The premium on Brazilian sugar (39.6%) significantly exceeds the regional one (15.4%), confirming that domestic procurement inefficiencies entail higher costs than non-membership in the EAEU.

H3 (EAEU membership provides price advantages): EAEU countries pay an average of $614.1/t for sugar supplied by the dominant regional exporter; for non-EU countries, the

average price is $688.4/t – a 12.1% premium for membership. Even Armenia (38,600 tons) achieves better prices than Uzbekistan (486,500 tons).

H4 (Persistent welfare costs): Annual losses range from $67 million to $117 million (0.06–0.10% of GDP). Cumulative losses for 2021–2024 amount to $361.6 million. Losses increased from $28.7 million to $117 million. US (2022) to US$67.0 million (2024), despite a 4.6-fold increase in volumes, which confirms structural rather than temporary reasons.

Contributions. The study advances three lines of research. First, it demonstrates that institutional factors can dominate geography among landlocked countries: higher prices in Tajikistan, despite its poorer geographic location, challenge the assumption that landlockedness alone determines trade costs. Second, it quantifies the costs of excluding the EAEU (a 12.1% premium), while simultaneously showing that domestic factors matter independently: non-EAEU members such as Tajikistan and Azerbaijan achieve prices comparable to those of EAEU members. Third, it challenges conventional bargaining theory: economics textbooks predict that large buyers obtain higher prices through economies of scale and bargaining power, yet Uzbekistan, which imports more sugar than all its neighbors combined, pays the highest prices in the region. This inverse relationship between volume and price suggests that institutional weaknesses can completely offset the benefits that market power should provide.

Policy implications

The "Tajikistan question". Before introducing reforms, Uzbek policymakers should study what Tajikistan is doing differently. Tajikistan – smaller, poorer, and more remote – has achieved more favorable prices within existing regional structures, without membership in the EAEU. Understanding the specific mechanisms (the structure of import channels, intermediaries, and contractual practices) will allow for targeted replication of this experience.

Information transparency. Intermediaries profit when buyers lack market information. A practical option would be to require quarterly publication of Uzbekistan's import price data, comparing them with regional benchmark prices. Ensuring accountability, explaining why Uzbekistan paid 17.4% more than Kazakhstan, would help reduce information asymmetry.

Competitive discipline. Uzbekistan pays premiums to nine suppliers, suggesting systemic problems rather than supplier-specific ones. The introduction of competitive pressure, mandatory tenders for large contracts, direct relationships with suppliers, and transparent evaluation criteria could undermine arrangements in which relationships prevail over price competition. Several specific measures should be considered:

- **Granting the State Competition Committee** authority to monitor import prices of essential goods, with the authority to investigate cases of persistently exceeding regional premiums.
- **Establishing a commodity exchange** in Tashkent, ensuring transparent pricing and reducing the opacity of intermediaries, based on Uzbekistan's existing commodity exchange infrastructure.
- **Requiring import license** holders to demonstrate competitive sourcing through documented quotations from multiple suppliers for contracts exceeding thresholds.
- **Leveraging bilateral relations** between Uzbekistan and Kazakhstan to explore joint procurement mechanisms that combine Kazakhstan's access to the EAEU with Uzbekistan's advantages in procurement volume.

Regional integration: beyond EAEU. While the 12.1% EAEU premium represents real costs, joining the customs union may not serve Uzbekistan's interests. As the world's only doubly-landlocked country, Uzbekistan's prosperity depends on connectivity with all neighbors, not alignment with a single bloc. EAEU membership would surrender tariff autonomy to dominant regional exporter's industrial priorities precisely when flexibility matters most.

A more promising vision: Uzbekistan leading deeper Central Asian integration, 80 million consumers with complementary economies, that preserves sovereignty over external tariffs while reducing regional barriers. The procurement inefficiencies documented here are domestic problems requiring domestic solutions; solving them independently strengthens Uzbekistan's position for shaping future regional arrangements.

Limitations. CIF prices do not include internal distribution costs, so the observed differences represent conservative estimates. The analysis does not allow us to identify the specific mechanisms underlying Uzbekistan's disadvantage, such as intermediary markups, internal rents, fragmented government, or information asymmetries. Importantly, trade statistics do not reveal the composition of buyers: whether sugar is imported by state-owned organizations, private traders, or producers. This distinction is important because reforms aimed at public procurement are fundamentally different from competition policy for private channels. Natural experiment comparisons suggest that unobserved characteristics are not correlated with EAEU membership, and the data for Turkmenistan are based on mirror statistics due to reporting limitations.

Future research. Supply chain analysis that identifies intermediaries and institutional bottlenecks can help develop targeted interventions. A comparative analysis of commodities (wheat, vegetable oil, fuel) will allow us to determine whether sugar reflects broader inefficiencies. Most importantly, customs-level data, which distinguishes between public and private procurement, can reveal efficiency gaps between the public and private sectors in Central Asian markets, directly determining whether public procurement, private competition, or intermediary structures require reform.

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Mirzavaliev B., Rovny P., Smutka L., Cabelkova I., Prochazka D.A. (2025), Why Uzbekistan pays premium prices for sugar: Institutional and market analysis, *Ukrainian Food Journal*, 14(4), pp. 792–812, https://doi.org/10.24263/2304-974X-2025-14-4-14

APA Style

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Instructions for authors

Dear colleagues!

The Editorial Board of scientific periodical
Ukrainian Food Journal
invites you to publish your research findings in our journal

Manuscripts should present original research that has not been previously published and is not under consideration for publication elsewhere. Submission of the manuscript implies that its publication has been approved by all co-authors and by the appropriate institutional authorities at the organization where the research was conducted.

A cover letter to the editor is mandatory and should include a brief description of the research topic, its novelty and significance. The letter must also confirm that all authors agree to submit the manuscript to the Ukrainian Food Journal and that the work is original and authored solely by the listed contributors.

Manuscript requirements

Authors must prepare the manuscript according to the guide for authors. Editors reserve the right to adjust the style to certain standards of uniformity.

Language – English

Manuscripts should be submitted in Word.

Use 1.0 spacing and 2 cm margins.

Use a normal font 14-point Times New Roman for text, tables, and in the figure captions.

Present tables and figures in the text of manuscript.

Consult a recent issue of the journal for a style check.

Number all pages consecutively.

Abbreviations should be defined on first appearance in text and used consistently thereafter. No abbreviation should be used in title and section headings.

Please submit math equations as editable text and not as images (It is recommend software application MathType or Microsoft Equation Editor).

Minimal size of the article (without the Abstract and References) is 10 pages. For review article is 25 pages (without the Abstract and References).

Manuscript should include:

Title (should be concise and informative). Avoid abbreviations in it.

Authors' information: the name(s) of the author(s); the affiliation(s) of the author(s), city, country. One author has been designated as the corresponding author with e-mail address. If available, the 16-digit ORCID of the author(s) information on the title page.

Declaration of interest

Author Contribution Statement

Abstract. The **abstract** should contain the following mandatory parts:

Introduction provides a rationale for the study (2–3 lines).

Materials and methods briefly describe the materials and methods used in the study (3–5 lines).

Results and discussion describe the main findings (20–26 lines).

Conclusions provides the main conclusions (2–3 lines).

The abstract should not contain any undefined abbreviations or references to the articles.

Keywords. Immediately after the abstract provide 4 to 6 keywords.

Text of manuscript

References

Manuscripts should be divided into the following sections:

Introduction
Materials and methods
Results and discussion
Conclusions
References

Introduction. Provide a background avoiding a detailed review of literature and declare the aim of the present research. Identify unexplored questions, prove the relevance of the topic. The Introduction section should not exceed 1.5 pages in length.

Materials and methods. Provide sufficient detail to enable an independent researcher to replicate the study. For methods that are already published, cite the original source and summarize the procedure briefly. Detailed descriptions are required only for new techniques or significant modifications of existing methods.

Results and discussion. Results should be presented clearly and concisely, supported by appropriate tables and/or figures. The significance of the findings should be discussed in the context of existing literature, highlighting similarities, differences, and potential implications.

Conclusions. Conclusions should be clearly derived from the study's findings and summarized briefly in a separate Conclusion section.

Acknowledgments (if necessary). Acknowledgments of individuals, grants, or funding sources should be included in a separate section. List all persons who contributed assistance during the research. Names of funding organizations must be written in full.

Organize your article into clearly defined sections and, if necessary, subsections. Each subsection should have a concise and descriptive heading.

References

Please, check references carefully.

The list of references should include works that are cited in the text and that have been published or accepted for publication.

All references mentioned in the reference list are cited in the text, and vice versa.

Cite references in the text by name and year in parentheses. Some examples:

(Drobot, 2008); (Qi and Zhou, 2012); (Bolarinwa et al., 2019; Rabie et al., 2020; Sengev et al., 2013).

Reference list should be alphabetized by the last name of the first author of each work.

If available, please always include DOIs links in the reference list.

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Journal article

Please follow this style and order: author's surname, initial(s), year of publication (in brackets), paper title, *Journal title (in Italic)*, volume number (issue), first and last page numbers. If available, please always include DOIs as full DOI links in your reference list (e.g. "https://doi.org/abc").

Journal names should not be abbreviated.

Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science*, *Technique and Technologies*, 2(2), pp. 104–108, https://doi.org/11.1016/22-33-85

Book

Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

Book chapter in an edited book

Kochubei-Lytvynenko O., Kuzmyk U., Yushchenko N. (2022), Spices for dairy products. In: O. Paredes-López, O. Shevchenko, V. Stabnikov, V. Ivanov (Eds.), *Bioenhancement and Fortification of Foods for a Healthy Diet*, pp. 157–178, CRC Press, Boca Raton, https://doi.org/10.1201/9781003225287-11

Online document

Mendeley J.A., Thomson, M., Coyne R.P. (2017), *How and When to Reference*, Available at: https://www.howandwhentoreference.com

Conference paper

Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018,* NUFT, Kyiv, pp. 52–57, https://doi.org/11.1016/22-33-85

Figures

All figures must be created using a graphic editor with Arial font.
The font size on the figures and the text of the article should be the same.
Black and white graphic with no shading should be used.
The figure elements (lines, grid, and text) should be presented in black (not gray) colour.
Figure parts should be denoted by lowercase letters (a, b, etc.).
All figures are to be numbered using Arabic numerals.
Figures should be cited in text in consecutive numerical order.
Place figure after its first mentioned in the text.
Figure captions begin with the term Figure in bold type, followed by the figure number, also in bold type.

Each figure should have a caption describing what the figure depicts in bold type.

Submit all figures along with the corresponding Excel files containing the graph data as separate attachments.

If your manuscript includes figures previously published elsewhere, you must obtain permission from the copyright owner(s) prior to submission.

Tables

Number tables consecutively in accordance with their appearance in the text.

Place footnotes to tables below the table body and indicate them with superscript lowercase letters.

Place table after its first mentioned in the text.

Ensure that the data presented in tables do not duplicate results described elsewhere in the article.

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Submission

Email for all submissions and other inquiries:

ufj_nuft@meta.ua

Шановні колеги!

Редакційна колегія наукового періодичного видання «**Ukrainian Food Journal**» запрошує Вас до публікації результатів наукових досліджень.

Вимоги до оформлення статей

Мова статей – англійська.

Мінімальний обсяг статті – **10 сторінок** формату А4 (без врахування анотацій і списку літератури)**.**

Для всіх елементів статті шрифт – **Times New Roman**, кегль – **14**, інтервал – 1.

Всі поля сторінки – по 2 см.

Структура статті:

1. **Назва статті.**
2. Автори статті (ім'я та прізвище повністю, приклад: Денис Озерянко).
3. *Установа, в якій виконана робота.*
4. Анотація. **Обов'язкова** структура анотації:
   - Вступ (2–3 рядки).
   - Матеріали та методи (до 5 рядків)
   - Результати та обговорення (пів сторінки).
   - Висновки (2–3 рядки).
6. Ключові слова (3–5 слів, але не словосполучень).

Пункти 2–6 виконати англійською і українською мовами.

7. Основний текст статті. Має включати такі обов'язкові розділи:
   - Вступ
   - Матеріали та методи
   - Результати та обговорення
   - Висновки
   - Література.

За необхідності можна додавати інші розділи та розбивати їх на підрозділи.

8. Авторська довідка (Прізвище, ім'я та по батькові, вчений ступінь та звання, місце роботи, електронна адреса або телефон).
9. Контактні дані автора, до якого за необхідності буде звертатись редакція журналу.

Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути **співрозмірним** (!) тексту статті. **Фотографії можна використовувати лише за їх значної наукової цінності.**

Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

Рисунки та графіки EXCEL з графіками додатково подаються в окремих файлах.

Скорочені назви фізичних величин в тексті та на графіках позначаються латинськими літерами відповідно до системи СІ.

У списку літератури повинні переважати англомовні статті та монографії, які опубліковані після 2010 року.

Оформлення цитат у тексті статті:

Кількість авторів статті	Приклад цитування у тексті
1 автор	(Arych, 2019)
2 автора	(Kuievda and Bront, 2020)
3 і більше авторів	(Bazopol et al., 2022)

Приклад тексту із цитуванням: It is known (Arych, 2019; Bazopol et al., 2022), the product yield depends on temperature, but, there are some exceptions (Kuievda and Bront, 2020).
У цитуваннях необхідно вказувати джерело, звідки взято інформацію.
Список літератури сортується за алфавітом, літературні джерела не нумеруються.

Правила оформлення списку літератури

В Ukrainian Food Journal взято за основу загальноприйняте в світі спрощене оформлення списку літератури згідно стандарту Garvard. Всі елементи посилання розділяються **лише комами**.

1. Посилання на статтю:
Автори А.А. (рік видання), Назва статті, *Назва журналу (курсивом)*, Том (номер), сторінки, DOI.
Ініціали пишуться після прізвища.
Всі елементи посилання розділяються комами.
Вказуються всі автори.
Назву журналу скорочувати не можна.

Приклад:
Popovici C., Gitin L., Alexe P. (2013), Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction, *Journal of Food and Packaging Science, Technique and Technologies,* 2(2), pp. 104–108, https://doi.org/5533.935-3.

2. Посилання на книгу:
Автори (рік), *Назва книги (курсивом)*, Видавництво, Місто.
Ініціали пишуться після прізвища.
Всі елементи посилання розділяються комами.
Приклад:
Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

3. Посилання на розділ у редагованій книзі:
Автори (рік), Назва глави, In: Редактори, *Назва книги (курсивом)*, Сторінки, Видавництво, Місто.
Приклад:
Kochubei-Lytvynenko O., Kuzmyk U., Yushchenko N. (2022), Spices for dairy products, In: O. Paredes-López, O. Shevchenko, V. Stabnikov, V. Ivanov (Eds.), *Bioenhancement and Fortification of Foods for a Healthy Diet*, pp. 157–178, CRC Press, Boca Raton, https://doi.org/10.1201/9781003225287-11

4. Тези доповідей конференції:

Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018,* NUFT, Kyiv, pp. 52–57, https://doi.org/5533.935-3.

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Приклад:

Cheung T. (2011), *World's 50 most delicious drinks*, Available at: http://travel.cnn.com/explorations/drink/worlds–50-most-delicious-drinks–883542

Список літератури оформлюється лише латиницею. Елементи списку українською та російською мовою потрібно транслітерувати. Для транслітерації з українською мови використовується паспортний стандарт.

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УДК 663/664

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

Тематика публікацій в Ukrainian Food Journal:

Харчова інженерія	Процеси та обладнання
Харчова хімія	Нанотехнології
Мікробіологія	Економіка та управління
Фізичні властивості харчових продуктів	Автоматизація процесів
Якість та безпека харчових продуктів	Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

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- Index Copernicus (2012)
- EBSCO (2013)
- Google Scholar (2013)
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- CABI full text (2014)
- Online Library of University of Southern Denmark (2014)
- Directory of Open Access scholarly Resources (ROAD) (2014)
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- InfoBase Index (2015)
- Chemical Abstracts Service Source Index (CASSI) (2016)
- FSTA (Food Science and Technology Abstracts) (2018)
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Рецензія рукопису статті. Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний науковець.

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