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Technology and factors influencing Greek-style yogurt – a Review

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Abstract

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Introduction. Analytical studies of Greek-style yoghurt manufacturing methods, formation and physicochemical characteristics of acid milk gels and factors that define yogurt quality are presented.

Material and methods. The review is based on all the most important scientific papers ever published on the subject.

Results and discussion. Different yoghurt production methods: traditional methods based on mechanical separators, methods based on membrane processes and methods based on direct recombination, give product with differences in properties. Two of the most important parameters: the textural attributes and the water holding capacity define yogurt quality and determine consumer acceptance. Numerous manufacturing parameters, such as severe heat treatments, excessive whey protein to casein ratios, high incubation temperatures, certain types of starter cultures and the use of excessive amounts of starter culture, are associated with textural defects of stirred yogurt like graininess (particles) or surface roughness (irregularities in the yogurt matrix). This method of direct recombination has advantages over others, because it is more environmentally friendly and increases the nutritional value of the finished product. Still, rheological properties of recombined concentrated yogurt are different from those of strained yogurt. Usually they form weaker gels than those made by traditional or UF methods. Different dry dairy ingredients (especially with elevated content of whey protein) should be used for production of concentrated yogurt.

Conclusions. Future research should be focused on the production of concentrated yogurt by direct recombination.

Review structure

1. Introduction
 2. General technology of yogurt manufacturing
 - 2.1. Milk standardization
 - 2.2. Homogenization
 - 2.3. Heat treatment
 - 2.4. Incubation/fermentation
 - 2.5. Cooling and storage
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 - 3.1. Traditional method
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 - 3.3. Methods based on membrane processes
 - 3.4. Methods based on direct recombination
 4. Formation and physicochemical characteristics of acid milk gels
 - 4.1. Casein micelle structure
 - 4.2. Formation of acid milk gels
 - 4.3. Effects of heat treatment on the formation of acid milk gels
 5. Important factors that define yogurt quality
 - 5.1. Rheology
 - 5.2. Whey separation
 - 5.3. Clusters formation
- Conclusions

1. Introduction

Yogurt is defined as the “food produced by culturing one or more of the optional dairy ingredients [cream, milk, partially skimmed milk or skimmed milk] with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*” [1]. Yogurts differ according to their chemical composition, method of production, flavour used and the nature of post-incubation processing [2]. There are substantial differences in composition of Greek-style yoghurts measured in products in different countries. The carbohydrate, fat and protein contents ranged between 1–12, 0–20 and 3.3–11 g/100 g, respectively [3].

Greek-style yogurt, also known as strained yogurt, concentrated yogurt or thick yogurt, is a semisolid fermented milk product derived from yogurt by draining away part of its whey. As a result of this draining action, the final product has higher total solids and lower lactose contents than regular yogurt (Table 1).

Table 1

Typical chemical compositions (g 100g⁻¹) of industrial full and low-fat strained yogurt

Composition	Full-fat	Low-fat
Total solids	22.0	14.3
Protein	4.9	9.9
Fat	10.1	0.2
Carbohydrate	6.0	3.5
Ash	1.0	0.6

Source: Tamime (2003) [61].

The product has a cream/white color, a soft and smooth body, good spreadability with little syneresis and a flavor that is clean and slightly acidic [4]. Concentrated yogurt is widely consumed in the Middle East and Balkan regions [5, 6]. Evidence of its production can be found in many countries in Turkestan, the Balkans, the eastern Mediterranean, and the Indian subcontinent [7]. Table 2 shows the variety of names by which this product is known in different countries.

Table 2

Synonyms for concentrated yogurt in different countries

Traditional names	Countries/Regions
Labneh, labaneh, lebneh, labna	Eastern Mediterranean
Ta, than	Armenia
Laban zeer	Egypt, Sudan
Stragisto, sakoulas, tzatziki	Greece
Torba, suzme	Turkey
Syuzma	Russia
Mastou, mast	Iraq, Iran
Basa, zimne, kiselo, mleko-slano	Yugoslavia, Bulgaria
Ititu	Ethiopia
Greek-style	United Kingdom
Chakka, shrikhand	India
Ymer	Denmark
Skyr	Iceland

Source: Tamime & Robinson (2007a) [7].

Strained yogurt has a higher lactic acid concentration than normal yogurt (1.8–2.0% as lactic acid). As a result, it presents a better keeping quality than the latter form [8-10]. High lactic acid concentrations can be expected to curtail the growth of bacterial pathogens, but yeasts, moulds and some lactic acid bacteria can still contribute to spoilage problems. At 7 °C, concentrated yogurt can be kept for two weeks [4]. Any sharp taste resulting from the high lactic acid concentration will be masked by diacetyl produced during fermentation; and by the high fat content, which is typically around 10%, and [5, 9]. Furthermore, concentrated yogurt has superior nutritional properties to those of regular yogurt: it has higher protein [2.5x] and mineral [1.5x] concentrations; a higher number of viable lactic acid bacteria [there is a tendency for these bacteria to be retained in the crud during the concentration process]; a very low lactose concentration, which makes strained yogurt even more suitable for lactose intolerant individuals than regular yogurt; and a fat content which can be varied according to consumer demand [4, 11–14]. The perceived nutritional benefits and storage characteristics of Greek-style yogurt led to its increasing popularity and economic importance during the last decade of the past century [15–17]. Nowadays, concentrated yogurt is establishing as a popular nutritious product possessing a healthy image equal to or greater than that of regular yogurt [4, 6]. The CODEX ALIMENTARIUS classifies strained yogurt as a type of concentrated fermented milk and its composition and quality standards are described in: CODEX STAN 243-2003 [18]. Yogurt, essentially from the Eastern hemisphere, has gained considerable popularity as a wholesome and nutritious food in America. Indeed, its health properties, which extend beyond nutrition, are now being recognized [19, 20]. Reported health benefits associated with yogurt and probiotic cultures include growth promotion, enhancement of mineral absorption, lactose digestion (the ability to reduce symptoms of

lactose intolerance), antimicrobial function (the ability to enhance resistance to colonization by pathogenic organisms), anticholesterol effect (the ability to reduce the risk of cardiovascular disease by lowering serum cholesterol), anticarcinogenic factor (the ability to reduce risk factors for colon cancer initiation), stimulation of the host immunological system, restoration of normal balance of gastrointestinal microflora, and positive contribution to longevity [19, 21–26]. Consumption of yoghurt, despite the relatively high content of saturated fat, has been lately linked with lower blood pressure, but the mechanism is unclear [27]. Added to this, yogurt is commonly supplemented with various functional ingredients, such as probiotics, prebiotics, fiber, plant sterol esters, omega-3 fatty acids, minerals and vitamins to impart an even healthier image to the final product [19]. The developments of new products, along with increased consumer awareness of the health benefits associated with yogurt cultures and probiotics, had led to a sharp increase in the per capita consumption of yogurt in Canada and the U.S. during the last decades. According to the Canadian Dairy Information Centre, Canadians consumed 9.87 liters (per capita) of yogurt in their diet in 2018, almost twice as much as they had two decades ago [28]. The introduction of Greek yogurt changed American tastes in yogurt, which helped increase sales of the overall yogurt category. As of 2016, the Americas held a share of over 50 percent of the global Greek yogurt market. In contrast to conventional yogurt, the Greek type is bought equally by men and women and consumers are drawn to the creamy texture and its high protein content. The Greek yogurt category experienced tremendous growth in the United States over the past few years. Americans consumed 13.7 liters (per capita) of yogurt in their diet in 2017 with 37.8% of Greek-type share [29].

2. General technology of yogurt manufacturing

At present, there is a wide variety of yogurt types on the market [24, 30]. Yogurts are usually classified based on their fat content (full-fat, reduced-fat, and low-fat) and on the method of production and the physical structure of the coagulum (set or stirred yogurts). Set yogurt is the product formed when the fermentation of milk is carried out in a retail container, and the yogurt produced is in a continuous semisolid mass. In contrast, stirred yogurt results when the coagulum is produced from milk, and the gel structure is broken before cooling and packaging. Fluid yogurt can be considered as stirred yogurt of low viscosity [2]. The main processing steps involved in these two types of yogurt manufacturing (Figure 1) include the standardization of milk (fat and protein content), homogenization, milk heat treatment, incubation/fermentation, cooling, and storage [31].

2.1. Milk standardization

Nowadays, three systems are available to standardize the fat and protein content of the milk base: (1) the addition of milk powders to liquid milk, (2) the evaporation of water from liquid milk under vacuum, (3) the removal of water from liquid milk by membrane processes [9]. Milk bases should be formulated to comply with regulations and meet consumer expectations [32]. Stabilizers (gelatin, starch, pectin) and sweeteners can also be added to further impact the physical properties of the final product [33]. Increasing the total solids increases the firmness, complex viscosity, storage modulus, fracture stress, apparent viscosity, oral viscosity, consistency index, and water holding capacity – WHC of the resultant gel [31, 34–46]. Thus, it improves the textural attributes of the gel, giving a higher sensory acceptability to the final product [12, 47–49].

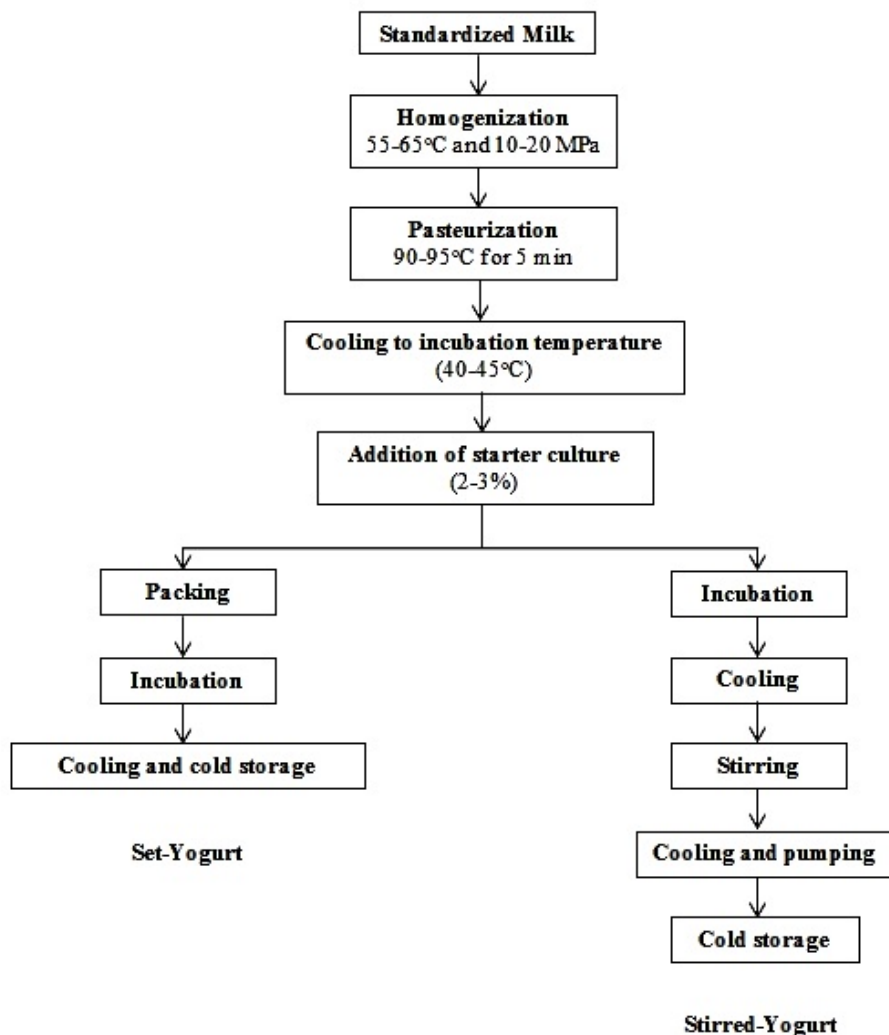


Figure 1. Main processing steps in the manufacture of set and stirred yogurt
Source: Adapted from Lee & Lucey (2010) [31].

2.2. Homogenization

Homogenization is the typical industrial process used to effect stabilization of the lipid phase against separation by gravity. During this process the average diameter of fat globules (3–4 μm) is reduced to 1 or 2 μm . As a result, the fat globules do not cream during the incubation of the yogurt. Because of the size reduction, there is usually a four-to-six-fold increase in the surface area [2]. Upon homogenization, the fat-globule membrane is destroyed, and caseins and whey proteins form the new surface layer of fat globules, which increases the number of possible structure-building components in yogurt made from homogenized milk. Homogenized milk fat globules act like protein particles due to the

presence of protein on the fat surface [31]. Therefore, homogenization also improves gel strength upon fermentation due to greater protein–protein interaction [24]. As the fat globule membrane is destroyed during homogenization, lipids are vulnerable to attack by lipase. To prevent lipolysis, milk must be pasteurized immediately after homogenization [2]. Homogenization pressures used are usually between 10 and 20 MPa and, since the efficiency of homogenization is much better when the fat phase is in a liquid state, the process is usually carried out at high temperatures (55°C to 80°C) [33].

2.3. Heat treatment

Heat treatments, which are much more severe than fluid milk pasteurization, are necessary to:

1. Generate a yogurt with the desired textural properties. Thus, the heating/holding regime both alters the physicochemical properties of the caseins and denatures the whey proteins, so that β -lactoglobulin, in particular, may become attached to the casein micelles; this linkage improves the texture (set yogurt) or viscosity (stirred yogurt) of the final product.
2. Cause some breakdown of the whey proteins to liberate free amino acids that stimulate the activity of the starter culture.
3. Expel oxygen from the processed milk because, as the starter bacteria are microaerophilic, deaeration provides the correct environment for rapid growth.
4. Kill any non-spore-forming pathogens that may be present, helping to ensure that yogurt retains its image as a “safe” product [9].

To meet these requirements, milk is generally heated, using a continuous plate heat exchanger, at 85 to 95 °C for 10 to 30 minutes [30]. According to Chandan and O’Rell [33], optimum results are obtained by using a heat treatment of 90–95 °C and a holding time of 5–10 minutes.

2.4. Incubation/fermentation

After heat treatment, the milk base is cooled to the incubation temperature used for growth of the starter culture. An optimum temperature of the thermophilic lactic acid bacteria, i.e., *Streptococcus* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, is around 40–45 °C. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of milk. During the acidification of milk, the pH decreases from 6.7 to ≤ 4.6 . In unheated milk gels, gelation occurs at around pH 4.9, while in heated milks gelation occurs at pH 5.2–5.4 (because denatured β -lactoglobulin has a higher isoelectric point than casein) [31, 50, 51].

The essential flora of yogurt (*Sc. thermophilus* and *Lb. delbrueckii*ssp. *bulgaricus*) displays an obligate symbiotic relationship during their growth in a milk medium. The rates of acid and flavor production by mixed yogurt cultures are considerably higher than by either of the two organisms grown separately [33, 41]. *Lb. delbrueckii*ssp. *bulgaricus* hydrolyzes milk proteins, the caseins, thus releasing essential amino acids, including valine, which stimulate the growth of *Sc. thermophilus*. Initially, *Sc. thermophilus* grows rapidly, reducing the pH to around 5.4, which stimulates the growth of *Lb. delbrueckii* ssp. *bulgaricus*, which is acid-tolerant and produces large amounts of lactic acid, which reduces the pH. *Sc. thermophilus* uses oxygen during its growth, which makes oxidation–reduction potential more favorable for *Lb. delbrueckii* ssp. *bulgaricus*; it also produces purine, pyrimidine, CO₂, formic acid, oxaloacetic acid, and fumaric acid that stimulate the growth of the lactobacillus [2, 33, 41]. During the growth in milk, *L. delbrueckii*ssp. *bulgaricus* apparently exhibits a

preference for utilizing β -casein over other proteins as a nitrogen source, indicating that the type of protein is also an important factor influencing the growth of this culture [41].

Starter bacteria can continue to produce acid until a very low pH [e.g. ~ 4.0] is attained when bacteria become inhibited by the low pH; in practice bacterial gels are cooled when sufficient acidity has been attained [pH ~ 4.6]. The rate of pH change during fermentation or addition of acid is controlled by the acid-base buffering properties of milk [38].

2.5. Cooling and storage

Since the yogurt organisms show limited growth activity around 10 °C, the primary objective of cooling is to drop the temperature of the coagulum from 30–45 °C to <10 °C as quickly as possible so as to control the final acidity of the product. The process of cooling yogurt may be carried out using one-phase or two-phase cooling [52]. In single-phase cooling, the temperature of fermenting milk is directly reduced from 43 °C to <10 °C. This model is more appropriate for plain set-type yogurt production. Two-phase cooling is widely employed for stirred-type yogurt production. In the first phase, fermenting milk is stirred gently in a tank to obtain a homogeneous body, and cooled to 20–24 °C. At this stage, fruit is added and the yogurt cups are filled. The filled cups are then cooled to <10 °C over a period of 10–12 hours [41]. To improve yogurt quality, the second stage of cooling should be carried out as slowly as possible over a 12-hour period [2]. The rate of cooling is of critical importance in obtaining a product with the desired textural quality. Cooling too quickly can cause a weak body and stimulate whey separation during cold storage [41]. Storing yogurt for 1–2 days improves the viscosity. During the first 24–48 hours of cold storage, an improvement in the physical characteristics of the coagulum is observed, mainly because of hydration and/or stabilization of casein micelles. Proper hydration is required to avoid syneresis. It is therefore important to delay the sale or distribution of yogurt for 24–48 hours [2].

3. Greek-style yogurt manufacturing methods

Much of the concentrated yogurt consumer acceptability is dependent on its sensory properties, which in turn, seem to be heavily dependent on the method of processing of the material [14, 16, 53, 54]. Depending on the used process, Greek-style yoghurt can be 10 times better than traditional yoghurt to deliver probiotic bacteria. Fresh ultrafiltrated or centrifugated Greek-style yoghurts had between 3 and 7 times higher counts of *Lb. helveticus* and *S. thermophilus* than the regular stirred yoghurt [55]. Concentrated yogurt is traditionally manufactured by straining the natural set yogurt in cloth bags [56]. However, nowadays there are other methods available to manufacture this product in large volumes. The current methods available for manufacturing concentrated yogurt have been widely reviewed by Tamime and Robinson [7, 8, 57], Robinson and Tamime [58], Özer [59], Salji [21], Nsabimana et al. [4], Tamime [60, 61], Tamime and Marshall [62], Tamime et al. [63] and can be classified as follows:

1. Traditional method (cloth bag) [10, 53, 64–73].
2. Methods based on mechanical separators [74–76].
3. Methods based on membrane processes [10, 53, 64–71, 77–81].
4. Methods based on direct recombination [53, 67–70, 82, 83].

3.1. Traditional method

The basic principle of using the traditional cloth bag method is to extract water from plain yogurt until the desired total solids level has been reached. The duration of drainage for yogurt in cloth bags takes about 15-20 hours at $<10^{\circ}\text{C}$. The whey separation can be achieved either by gravity drainage (small scale production) or by pressing (large scale production, i.e., by piling 25-kg bags on top of each other); however, the drainage time can be shortened by up to 6 hours by applying pressure of 2 kg kg^{-1} on the yogurt [59].

The sensory properties of the product made with this traditional system are excellent [9]. However, this method could be described as slow, labour intensive and unhygienic by the nature of the process, and the yield obtain is rather low due to residues left in the bag [9, 10, 64, 82, 84]. Consequently, this system is not suitable for large-scale processing [21, 53]. Despite this, the traditional production method is still preferred in some countries in the Middle East, as the investment in mechanised systems of production is rather high [59].

3.2. Methods based on mechanical separators

Mechanical separators have been used successfully for the industrial-scale production of strained yogurt [62]. Salji et al. [11, 85] reported the use of this method for factory-scale production in Saudi Arabia. This method requires the use of a nozzle or Quarg separator. Only, skimmed milk should be used when manufacturing yogurt in this way; if whole milk is used, the fat globules will clog the separator nozzles. However, recent developments in the design of centrifugal separators have made it feasible to use fermented whole milk to produce strained yogurt [61]. Producing concentrated yogurt by centrifugation is a two-step procedure. First, milk is fermented until it achieves the desired level of acidification (pH 4.6-4.8). After acidification, fermented skimmed milk is stirred vigorously, heated up to $55\text{--}60^{\circ}\text{C}$ to inactivate the culture and control the level of acidity, and cooled to 40°C . Next, any large clots or clumps are removed by passing the fermentate through a metal sieve before it enters the separator. The fermented milk is also de-aerated for 15–20 minutes before entering the centrifuge to assist the separation of whey in the separator. Once in the separator, the fermented milk is concentrated to the desired total solids level. The concentrated product leaving the separator is blended with any source of fat or cream, to provide the desired fat level in the final product. Then it is cooled and packaged [4, 7, 8, 59–61]. Capacities of such separators are up to 6.5 tonnes h^{-1} , depending on the composition of the milk used and the acidity of the fermented milk prior to concentration [61, 62]. According to Dagher and Ali-Ghariebeh [74], strained yogurt, produced from heated yogurt by centrifugation for 5 minutes at different speeds between 4, 000 and 11, 700g, had organoleptic characteristics similar to those of control samples made by the traditional method.

3.3. Methods based on membrane processes

Membrane techniques, especially ultrafiltration (UF), have been successfully used in the yogurt industry for the last 20-25 years [59, 81]. Production of strained yogurt by reverse osmosis (RO) has also been studied. However, previous scientific works revealed that using RO to produce concentrated yogurt created weaker structures which did not give gel properties close to those of concentrated yogurt made by the traditional method [53, 67–70, 78]. Two different systems of UF have been used to produce concentrated yogurt: (a) the fermentation of UF retentate that has the solids content desired in the final product [79, 80, 71], and (b) UF of yogurt at $40\text{--}50^{\circ}\text{C}$ [10, 66, 77] to produce a concentrated product with the desired total solids content [7]. Several scientific works studied the microstructures and rheological properties of concentrated yogurt obtained by these two UF methods [10, 64, 67–

70, 78]. Greek yoghurts prepared by concentrating a milk base through UF to 13.8% exhibited a hard structure, low syneresis and a high protein and fat content [86]. Applying both, UF and straining, resulted yoghurts with required structural attributes while substantially reducing the generation of AW. Researchers concluded that the concentrated yogurt made from UF milk retentate had much greater firmness than the products manufactured using the traditional method or UF of yogurt [4, 7, 10, 61, 64]. The concentration of milk by UF before yogurt-making carries a risk of bitterness in the final product since the calcium content will be higher [59]. On the other hand, the quality of strained yogurt made by UF of warm yogurt closely resembles the traditional product in terms of elasticity, firmness, and structure [10, 61]. Unfortunately, UF of yogurt affects process efficiency due to permanent membrane fouling [81]. The manufacturing process is as follows: after the fermentation period, the warm yogurt is heated to 58-60 °C for 3 minutes in the plate heater exchanger, to inactivate the culture and control the level of acidity, cooled to 40 °C, concentrated in a two-to-four stage UF plant (depending on the desired degree of concentration), cooled in a plate cooler to about 20 °C and finally packaged [4]. According to Özer et al. [53] and Tamime et al. [10, 64], UF applications can be used as an industrial alternative to the traditional strained yogurt-making process. Several studies which have investigated the rheology of concentrated yogurt produced by a range of techniques for increasing total solids have concluded that compared to other techniques (such as RO and direct recombination), UF of yogurt gives the gel properties that are closest to those of the traditional product [53, 67, 78]. Other advantages of UF as compared with other conventional methods are: higher yield (10% increase), shortening of processing time (e.g., by 25%), reduced wheying-off, and easy automation and process control [4, 59]. In addition, when using UF instead of the traditional method, the volumes of milk and starter cultures are reduced by around 10% and 80%, respectively [59]. Due to all these advantages, a wide range of UF plants are now available on the market for the production of strained yogurt on a large scale [58, 60, 61].

3.4. Methods based on direct recombination

According to the Food and Agriculture Organization of the United Nations, a recombined milk product is a product resulting from the combining of milk-fat and milk-solids-non-fat in their preserved forms with or without the addition of water to achieve the appropriate milk product composition [87]. In order to eliminate the drainage stage during the manufacture of concentrated yogurt, it is feasible to manufacture this product from recombined dairy ingredients [59–61]. The process involves reconstituting powders in water, up to the total solids level required in the final product, and blending the reconstituted milk with anhydrous milk fat and stabilisers [61]. After the recombination is complete, the recombined milk is handled and processed in a similar way to the production of traditional yogurt [8]. The quality of recombined dairy products is directly related to the composition, properties, and microbiological standards of the ingredients used [83]. According to Gilles and Lawrence [82], good quality yogurt can be obtained from milk powders as long as the powders are free of off-flavours. Odet [88] stated that there were no organoleptic differences between yogurt produced from recombined and fresh milks. The introduction of membrane techniques to the dairy industry has enabled the production of different types of milk powders containing diverse protein to lactose ratios and altered whey protein to casein ratios (e.g., milk retentate, milk permeate, whey retentate, and whey permeate powders) [89, 90]. The use of these latter powders has enabled the production of recombined dairy products containing high protein and low lactose contents, such as concentrated yogurt [14]. Several authors recommended using these types of powders to fortify the milk base during yogurt

production and/or to produce concentrated yogurt using recombination technology [36, 61, 82, 91–93]. In order to obtain a recombined strained yogurt with good textural and physicochemical properties, experts recommend using heat-treated high protein dairy powders (with reduced lactose content) free of inhibitory substances that can slow or restrain the growth of lactic bacteria [82, 94, 95]. However, if processing steps include a high heat treatment, low-heated milk powders can also be used effectively to produce a good quality product [95]. Since recombined products generally contain high amounts of water, it is important to have a high quality water source. Excessively hard water can lead to problems with powder solubility and stability [95]. According to the recommendations from the International Dairy Federation (IDF), water used to recombine dairy products should not exceed the following maximum salt concentrations: total hardness, 100 $\mu\text{g g}^{-1}$ of calcium carbonate g^{-1} ; chloride, 100 $\mu\text{g g}^{-1}$; sulfate, 100 $\mu\text{g g}^{-1}$; nitrate, 45 $\mu\text{g g}^{-1}$ [96]. Nichols and Kozak [97] discussed in depth the importance of water used for recombining milk and milk products. Milk powders used for recombination are very stable and have a shelf-life of 12 months at ambient temperatures without refrigeration, although storage at 20°C or below is recommended [98]. The long durability and good thermal stability of ingredients makes direct recombination a suitable option to provide a nutritious and high-quality source of dairy products in areas where a fresh raw milk supply is not readily available or is in short supply [17]. Because refrigeration and transportation may not be readily available in some regions, utilization of preserved milk ingredients may be the only viable means of producing dairy products [95]. Several authors [99–103] reported the use of recombination technology to produce milk and dairy products in developing countries where, as a result of geographic/climatic/economic conditions, setting up a conventional dairy industry base using local milk production is impractical [104]. On the other hand, in industrialized countries where there is a milk surplus, milk recombination offers the opportunity to transfer raw materials (milk powders, anhydrous milk fat, etc.) from surplus production areas to deficiency areas, in order to compensate for the abovementioned problems and to open up new markets [14, 96]. Therefore, it is believed that a widespread use of this technique to produce concentrated yogurt will potentially increase the international trade of powders high in protein and low in lactose [14, 89]. However, it is important to point out that indiscriminate distribution of dairy ingredients for recombining purposes can, under certain circumstances, be detrimental to local milk producers [104]. The production of concentrated yogurt by direct recombination offers important advantages over other industrial production methods. Direct recombination does not involve whey disposal problems (there is less environmental damage, and the yogurt produced is more nutritious because all whey proteins are retained in the final product) and requires low investment and production costs (depending on the local market) [89, 98]. However, several scientific publications stated that the rheological properties of recombined concentrated yogurt were different from those of strained yogurt produced by the traditional method or by UF [7, 8]. Özer et al. [53, 67, 69, 70] concluded that strained yogurt made by directly recombining full-cream milk powder to 23% (w/v) total solids formed weaker gels than those made by traditional or UF methods. Although numerous scientists have studied or reviewed the production of recombined concentrated yogurt [7, 8, 53, 59–61, 67–70, 82, 83, 95], there is little evidence of the manufacture of recombined non-fat strained yogurt. In the future it is crucial to find an effective formulation for producing a recombined non-fat, additive-free type of Greek-style yogurt.

4. Formation and physicochemical characteristics of acid milk gels

Acid-induced milk gels are formed by aggregation of casein particles as the pH of milk decreases and the isoelectric point (pH 4.6) of casein is approached [105]. Acid casein gels have a particulate, heterogeneous structure, consisting of fairly large conglomerates and holes (void spaces where the aqueous phase is confined). These conglomerates are thought to be built of smaller ones, which, in turn, consist of casein particles aggregated in strands and nodes. This heterogeneity, which depends, e.g., on the temperature during gel formation, largely determines the mechanical properties of the gel [38, 106]. Casein gels are very dynamic and rearrangements of the clusters and particles forming the network may occur before or during gel formation [105]. The physical characteristics of these particulate gels are determined by both strong permanent bonds (covalent bonds: SH/S-S exchange) formed during the aggregation, and subsequent rearrangements of protein particles (noncovalent bonds: electrostatic, hydrophobic interactions and, probably, the ever-present Van der Waals attraction, as well as steric and entropic effects related to protein conformation). The balance between these strong and weak bonds controls the rheology of yogurt gels [53, 70, 106]. Milk protein gels are irreversible, in contrast to many other food gels. Although milk gels are usually classified as particle gels, it is now recognized that they are not simple particle gels because the internal structure of the casein particle plays an important role in the rheological properties of milk gels [37].

4.1. Casein micelle structure

At least three types of models for the structure of casein micelles have been proposed. Schmidt [107] and Walstra [108] suggested a model that proposes that the micelle core is divided into discrete sub-units [sub-micelles] with distinctly different properties [37]. In this model, the individual caseins come together in their appropriate portions to form internal sub-micelles, if depleted in κ -casein, or external sub-units rich in κ -casein, colloidal calcium phosphate (CCP) is regarded as the cement which links these discrete sub-units together. Another model, proposed by Holt [109], regards the micelle as a mineralized, cross-linked protein gel in which the CCP nanoclusters are the agents responsible for cross-linking the proteins and holding the network together [110]. A major failing of these two models is their lack of a plausible mechanism for assembly, growth and, more importantly, termination of growth of the casein micelles. All such elements are in place in a recent model, proposed by Horne [110], which suggests a dual-binding (polycondensation-type) mechanism for gel assembly [37, 111]. In the dual-binding model, micellar assembly and growth take place by a polymerization process involving, as the name suggests, two distinct forms of bonding: crosslinking through hydrophobic regions of the caseins or bridging across CCP nanoclusters. Central to the model is the concept that micellar integrity and hence stability is maintained by a localized excess of hydrophobic attraction over electrostatic repulsion [111]. The energy of interaction between molecules present inside the micelle is calculated as the sum of electrostatic repulsion and hydrophobic attraction as

$$IE = ER + HI \quad (1)$$

where, IE: interaction energy; ER: electrostatic repulsion; HI: hydrophobic interaction [110]. This model sees the micellar CCP not just as cross-links but also as neutralizing agents which, being positively charged, bind to negatively charged phosphoserine clusters to reduce the protein charge to the level where the attractive interactions between the hydrophobic regions of the caseins can be allowed to dominate [110]. Figure 2 illustrates the structure of the casein micelle according to the dual-binding model and can be used to explain the two types of linkage postulated between protein molecules. The first linkage is hydrophobic, where two or more hydrophobic regions from different molecules form a bonded cluster.

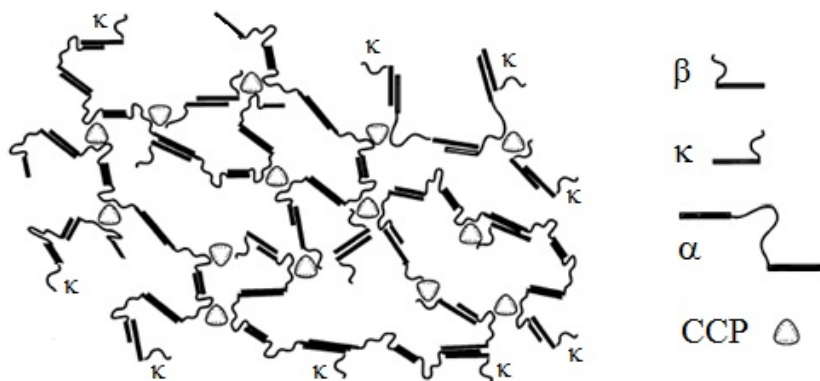


Figure 2. Dual-binding model of structure of casein micelle

Bonding occurs between the hydrophobic regions, shown as rectangular bars, and by linkage of hydrophilic regions containing phosphoserine clusters to CCP clusters. Molecules of κ -casein limit further growth and are labeled with the letter ' κ '. *Source:* Adapted from Horne (1998) [110].

The growth of these polymers is inhibited by the protein-charged residues whose repulsion pushes up the interaction free energy. Neutralization of the phosphoserine clusters by incorporation into the CCP diminishes that free energy as well as producing the second type of cross-linking bridge, since it is considered that up to four or more phosphoserine clusters from different casein molecules can be accommodated at each CCP nanocluster [Horne, 1998] [110]. Although the κ -casein molecules can interact via their hydrophobic domains with the hydrophobic regions of the other caseins, further growth beyond the κ -casein is not possible because it possesses neither a phosphoserine cluster for linkage via CCP [the only phosphoserine residue in κ -casein lies in the macropeptide which forms the putative hairy layer deemed essential for micellar stability in all accepted models, thus, this residue cannot be involved in any cross-linking via CCP], nor another hydrophobic anchor point to extend the chain via this route. κ -Casein acts as a terminator for both types of growth. Unless circumvented by the growing network, it will become part of the surface structure of the micelle. Hence its surface location, a prime requirement for any structural model, arises naturally in this model [110]. This concept of a localized excess of hydrophobic attraction over electrostatic repulsion allows the visualization of micellar growth and successfully accommodates the response of the micelles to changes in pH, temperature, urea addition or removal of CCP by sequestrants, all in accordance with experimental observations [112]. Urea does not rupture the CCP linkages but disrupts the hydrophobic bonds, bringing about micellar disintegration. Further, micellar integrity is largely maintained when the CCP is dissolved out by acidification because the phosphoserine negative charges are neutralized by the acid medium. If the milk is dialyzed and the pH is then restored to that of the original milk, dissociation of the micelle complex is observed as the negative charges of the phosphoserine residues are not neutralized and the electrostatic repulsion effect predominates over the hydrophobic attraction. The same dissociation is observed at natural pH when the CCP is removed by sequestration with EDTA. Increasing pH from the natural value in milk leads to dissociation of the micelles. Whether this is due to conversion of the phosphoserine residues from singly to doubly negatively charged units which are no longer capable of linking to the CCP nanoclusters, or whether the increase in charge itself is sufficient to upset the balance of electrostatic repulsion and hydrophobic attraction in favour of electrostatic

repulsion and the micelles dissociate. Decreasing the temperature decreases the level of hydrophobic attraction and any β -casein not linked through its phosphoserine cluster could then be released into the serum phase [110-112]. These facts suggest that CCP does not cement the micelle together, as described by the earlier models, but rather it helps to control and modulate the effects of calcium and charged groups on caseins. It is also clear that hydrophobic interactions and hydrogen bonding are important for micelle integrity [37].

4.2. Formation of acid milk gels

As the pH of milk is reduced, CCP is dissolved, the micelle structure is altered (the charge on individual caseins is altered and the ionic strength of the solution increased) and caseins are liberated into the serum phase [38, 113]. The extent of liberation of caseins depends on the temperature at acidification (low temperatures result in a decrease in the level of hydrophobic attractions inside the casein micelle); at fermentation temperatures commonly used for yogurt manufacture ($>30^{\circ}\text{C}$), no dissociation of casein likely occurs [113]. When the isoelectric point of caseins ($\text{pH} \approx 4.6$) is approached, aggregation occurs and low-energy bonds, mainly hydrophobic, are progressively established between proteins [114]. Three pH regions in the acidification of milk from pH 6.7 to 4.6 can be distinguished:

- a. *pH from 6.7 to 6.* The decrease in pH causes a decrease in the net negative charge on the casein micelles, thereby reducing electrostatic repulsion. Only a relatively small amount of CCP is dissolved above pH 6.0, so the structural features of the micelles are relatively unchanged (e.g., size) [115].
- b. *pH from 6 to 5.* As the pH of milk decreases further from pH 6.0 to 5.0, the net negative charge on casein micelles greatly decreases and the charged “hairs” of κ -casein may shrink (or curl up). This results in a decrease in electrostatic repulsion and steric stabilization, which are both responsible for the stability of casein micelles in the original milk. At $\text{pH} \leq 6.0$ the rate of solubilization of CCP increases, which weakens the internal structure of casein micelles and increases the electrostatic repulsion between the exposed phosphoserine residues. In milk, CCP is completely solubilized in casein micelles by $\text{pH} \sim 5.0$ [31].
- c. *pH ≤ 5 .* When the pH of milk becomes close to the isoelectric point of casein ($\text{pH} 4.6$), there is a decrease in the net negative charge on casein, which leads to a decrease in electrostatic repulsion between casein molecules. On the other hand, casein-casein attractions increase due to increased hydrophobic and electrostatic charge interactions (and van der Waals’ forces) [31, 50]. In unheated milk, gelation occurs at around pH 4.9, while in heated milks, gelation occurs at pH 5.2–5.4 (because denatured β -lactoglobulin has a higher isoelectric point than casein) [31, 50, 51]. Casein particles aggregate as a result of (mainly) charge neutralization [50]. The acidification process results in the formation of a three-dimensional network consisting of clusters and chains of caseins [31].

Solubilization of CCP during the acidification process undoubtedly changes the structural integrity of the casein micelles [48]. When CCP is depleted from the micelle, the casein molecules will have more dispersed structures with a higher number of interaction sites [112]. Therefore, the loss of CCP from casein micelles dramatically influences the properties of casein gels [115]. If the acidification is proceeding slowly, then this may allow equilibration and rearrangement into localized denser structures with few linkages between, giving rise to weaker gels. More rapid drops in pH may lock the protein into a more dispersed structure with greater density of possibly stronger strands [112]. These statements are verified by the experimental work done by Lee and Lucey [116]. These authors reported that higher

inoculation rates resulted in lower fermentation times and stiffer gel networks. They support their results by stating that the solubilization of CCP in milk during acidification is a slow process, and may require a slightly lower pH to completely dissolve CCP under conditions of fast acidification. When CCP dissolves at a lower pH, caseins at this lower pH value may be less sensitive to excessive rearrangements (due to the fact that at lower pH values there will be lower electrostatic repulsion and higher hydrophobic interactions between casein particles); thus, stiffer gel networks are obtained. Consequently, the solubilization of CCP appears to alter the balance between viscous and elastic components in the gel network [37]. Hydrophobic interactions are unlikely to play a direct role in the strength of acid gels as the stiffness of acid gels increases as the measurement temperature decreases. Cooling results in an increase in the stiffness of the gel, probably as a result of the swelling of casein particles (caused by the weaker hydrophobic interactions) and an increase in the contact area between particles. A similar trend occurs when lower incubation temperatures are used. The use of lower incubation temperatures leads to longer incubation times, but firmer and more viscous gels that are less prone to whey syneresis are formed. At a lower incubation temperature, there is an increase in the size of the casein particles because of a reduction in hydrophobic interactions which, in turn, leads to an increased contact area between the casein particles [50]. Higher incubation temperatures (i.e., higher gelation pH) also make the gel network more prone to rearrangements during gelation, and these changes can lead to greater whey separation [50, 116].

4.3. Effects of heat treatment on the formation of acid milk gels

With the exception of proteose-peptone, whey proteins are very sensitive to heat treatment. Unlike caseins, whey proteins have three-dimensional structures or configurations. Each configuration is stabilized by hydrogen and hydrophobic bonds, and other forces. Secondary and tertiary structures of whey proteins tend to be broken down by heat treatment because heating weakens hydrogen and hydrophobic bonds [41]. Denaturation of whey proteins occur above 60 °C. At temperatures up to 90 °C, unfolding of the protein is rate-limiting but further increases in the heating temperature result in only small increases in the rate of denaturation as aggregation of the proteins becomes rate-limiting [117]. Below 65 °C, at least in theory, denaturation or functional changes of whey proteins (mainly β -lactoglobulin) are reversible, but above 70 °C irreversible functional changes in whey proteins occur [41]. The most abundant whey protein is β -lactoglobulin in which a heat-induced conformational change results in the exposure of a reactive thiol group (Figure 3).



Figure 3. Schematic representation of β -lactoglobulin denaturation: breakage of its tertiary structure and exposure of thiol groups

Source: Adapted from Bylund (1995) [136].

This thiol group can form disulfide bonds with other cysteine-containing proteins, such as β -lactoglobulin or bovine serum albumin, or with proteins having disulfide bridges, such as α -lactalbumin, κ - and α_{S2} -casein. The latter process occurs through thiol group-disulfide bridge exchange reactions, resembling a polymerization process in which heat-denatured β -lactoglobulin is the initiator. Interaction of β -lactoglobulin with κ -casein, present at the exterior of the casein micelle, leads to coating of the casein micelles with β -lactoglobulin. Interactions of β -lactoglobulin with cysteine-containing serum caseins might lead to casein-whey protein aggregates. Additionally, interactions of β -lactoglobulin with cysteine-containing whey proteins, such as α -lactalbumin and β -lactoglobulin molecules, result in the formation of whey protein aggregates [118]. Hydrogen bonding and electrostatic and hydrophobic interactions have also been suggested as major forces in whey protein aggregation [119]. To summarize, heat treatment of milk results in a complex mixture of native whey proteins and denatured whey proteins present as whey protein aggregates, casein-whey protein aggregates and whey protein coated casein micelles [118]. The association of denatured whey proteins to casein micelles significantly increases the casein micelle size [114, 119]. According to Pesic et al. [120], after exposing bovine milk to a severe heat treatment (90 °C; 10 minutes) at natural pH (6.71), about 30% of denatured whey proteins were involved in soluble complexes. Figure 4 shows a schematic representation of the effects of heat treatment and subsequent acidification on casein micelles and whey proteins present in skim milk.

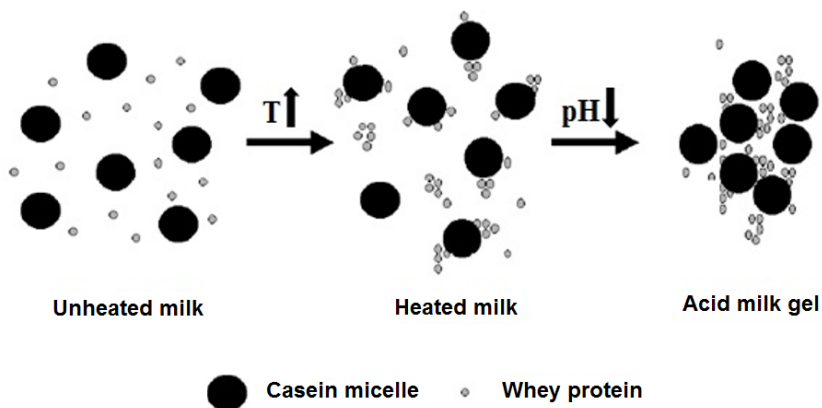


Figure 4. Schematic representation of the heating of skim milk and the subsequent acidification resulting in the formation of a protein network
 Source: Adapted from Vasbinder et al. (2003) [118].

The extent and rate of denaturation of whey proteins are determined by a number of factors. Amongst these are the pH value, the ionic strength and the ionic composition, the protein concentration and casein to whey protein ratio of the heat treated whey protein solution, and the duration and temperature of the heat treatment [121]. Increasing the pH above the natural pH of milk markedly accelerates the rate of denaturation of β -lactoglobulin. Generally a decrease in the pH of milk systems prior to heating results in an increased association between the denatured whey proteins and the casein micelle. Even small changes in pH can shift the distribution of the association of the denatured whey proteins with the casein micelle. For example, at a level of 95% whey protein denaturation, approximately

70% of denatured whey proteins are associated with the casein micelle at pH 6.55. This decrease to approximately 30% when the pH of milk prior to heating is 6.7. The difference in association level is reflected in the increase in the casein micelle size when milk is heated at the lower pH [117]. According to Vasbinder et al. [118], the denatured whey protein aggregates that form contain a ratio of α -lactalbumin to β -lactoglobulin which is representative of the ratio of total denatured whey proteins in milk. α -lactalbumin is more easily incorporated in aggregates than it is involved in coating of micelles, while the whey protein coating of the casein micelles clearly contains more β -lactoglobulin. Vasbinder and de Kruif [122] stated that at high pH, β -lactoglobulin- β -lactoglobulin interactions causing whey protein aggregates are favoured over κ -casein- β -lactoglobulin interactions, while κ -casein- β -lactoglobulin- β -lactoglobulin reactions hardly take place. At lower pH, formation of separate whey protein aggregates hardly occurs, but clusters of whey proteins are formed on the surface of the casein micelle. Apparently, at these conditions κ -casein-(β -lactoglobulin)_n interactions are favoured over κ -casein- β -lactoglobulin interactions. Figure 5 summarizes the different interactions that take place between casein micelles and denatured whey proteins when different pH mediums are considered prior to heating.

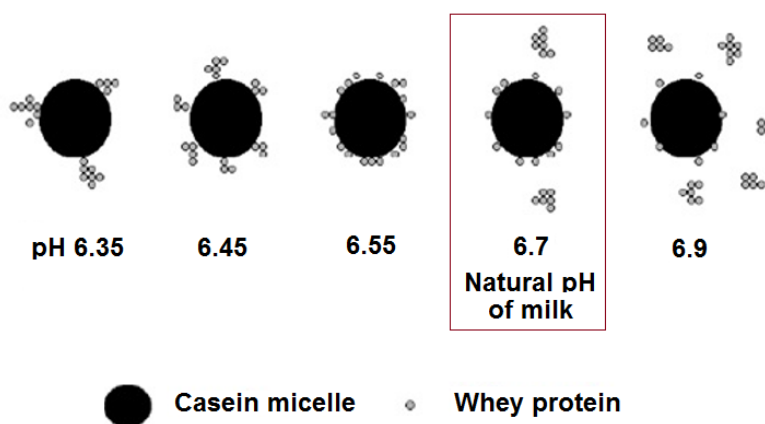


Figure 5. Schematic representation of the interactions between casein micelles and whey proteins occurring in milk during heat treatment for 10 min at 80 °C at pH values ranging from 6.35 to 6.9.

Native whey proteins are not included in the figure.

Source: Adapted from Vasbinder et al. (2003) [118].

Anema [40] explains this phenomenon by stating that as the pH of the milk is increased from about pH 6.5 to pH 7.1 before heating, κ -casein progressively dissociates from the casein micelles so that, at pH 6.5, the majority of the κ -casein is associated with the casein micelles, whereas at pH 7.1, about 60–70% of the κ -casein is found in the milk serum. As the denatured whey proteins interact with the casein micelles via disulfide bonding with the κ -casein, this dissociation of κ -casein probably explains why the association of the whey proteins with the casein micelles is pH-dependent. It is important to note that a more severe heat treatment at a constant pH will cause more denaturation of whey proteins, but the ratio of denatured whey proteins associated with the casein micelle and present in aggregates will remain constant [118]. Heat-induced interactions of casein micelles and whey proteins are also affected by the casein to whey protein ratio of the milk base. It is believed that κ -casein

presents limited number of available binding sites for β -lactoglobulin association. Thus, when these sites are saturated, denatured whey proteins will interact with each other, increasing the amounts of whey protein aggregates in the system. According to Cho et al. [123], after a heat treatment at pH 6.7, a maximum number of disulfide bonds between κ -casein and whey proteins is formed when using a casein to whey protein ratio of 4:1 [124]. Calcium ions promote the association of β -lactoglobulin with casein micelles, perhaps due to the ability of ions to influence the degree of electrostatic attraction or repulsion between β -lactoglobulin and κ -casein by providing an ionic environment around the interacting molecules. Additionally, salts could be affecting the reactivity of thiol groups. Furthermore, lactose concentration is a limiting factor for the whey protein denaturation. The glucosyl residues are bound to β -lactoglobulin via gluconic acid or melibiononic acid, making this whey protein fraction stable against heat treatment. Lactose concentrations of milk with normal chemical composition do not have any negative effect on the rate of whey protein denaturation. However, if the lactose level of yogurt milk is increased during standardization, the rate of whey protein denaturation is likely reduced. In order to overcome this handicap, milk should be exposed to a higher heat treatment (at >90 °C for 10–15 min) [41]. The association of denatured whey proteins with micellar caseins on heating gives improved yogurt texture and gel strength [125]. Yogurt prepared from heated skim milk and 2% protein from whey protein concentrate had higher storage modulus, firmness, water holding capacity and a denser microstructure than those prepared only from skim milk [126]. On the other hand, it is thought that native whey proteins do not interact with casein micelles during the acidification of unheated milk and act as a destructive filler, or a structure breaker, in acid milk gels [51]. Lee and Lucey [127] reported that yogurt gels made from milk heated at high temperatures [>80 °C] presented a higher cross-linked and branched protein structure with smaller pores than gels made from milk heated at low temperatures [31]. This branched microstructure increases the elasticity, gel strength and water binding capacity of the final gel [38, 121, 127–129]. Therefore, yogurts produced from heated milks will have greater firmness and lower susceptibility to syneresis. According to Sodini et al. [51], a heating that ensures 60 to 90% of β -lactoglobulin denaturation generally optimizes both the WHC and the rheological properties of the final gel. On the other hand, a too severe heating generally (above 90% β -lactoglobulin denaturation) has a slightly detrimental effect on yogurt's physical properties.

5. Important factors that define yogurt quality

Two of the most important parameters that define yogurt quality and determine consumer acceptance are, unquestionably, the textural attributes and the WHC of the gel network [31, 37, 38, 51, 54, 130–132].

5.1. Rheology

Textural attributes, including the desired oral viscosity, are very important criteria that determine the identity, quality and consumer acceptance of yogurt [130, 131, 133]. Although texture is related to the sensory perception of a food product, rheology and structure of a product evaluated by instrumental methods also provide relevant information on its textural properties [51]. Skriver et al. [47], Richardson et al. [134], and Stanley and Taylor [135] reported that sensory texture analyses are highly correlated with the rheological properties of stirred yogurt and other semi-solid foods. Due to this fact, rheological properties of milk gels

are important physical attributes which contribute to the overall sensory perception and functionality of these products [37].

Yogurt is defined as a weak viscoelastic gel system which is unable to keep its structural integrity during high shear [31, 53, 67]. Several authors reported the advantages of using oscillatory dynamic tests over other destructive rheological techniques (e.g., penetrometer, rotational viscometers) to evaluate the rheological characteristics of viscoelastic semisolid foods [31, 53, 67, 69, 136, 137]. The principal advantage of dynamic tests is that they enable measurements to be made without incurring structural damage to the samples. Therefore, this type of tests can be used to relate dynamic rheological parameters to molecular structures [137]. On the other hand, each penetration into or rotation in a gel network causes a breakdown in the elastically effective bonds, and the procedure thus fails to measure the actual physical characteristics of the gel. Once the gel structure is disturbed, it is rarely possible to re-form the gel structure in the same way, because yogurt is a metastable gel and any change in its enthalpic/entropic nature creates irreversible deformation. Thus, any kind of destructive effect may lead to atypical physical properties in the yogurt, and provide erroneous results. Due to this fact, dynamic studies are much more reliable than destructive rheological techniques for studying the physical properties of concentrate yogurt [67]. Consequently, during the last decades, dynamic tests have been widely used to investigate the rheological aspects of acid milk gels [43, 44, 48, 49, 113, 114, 116, 127–129, 138–148]. Small amplitude oscillatory tests are used to compare the rheological aspects of experimental and commercial samples of concentrate yogurt. Small deformation is defined as a small relative deformation which, when applied, does not disrupt the gel network structure, i.e., within the linear viscoelastic region. This type of test involves applying an oscillatory (sinusoidal) stress or strain to the material and measuring the strain or stress responses [31]. The magnitude and phase shift of the transmission depend on the material's viscoelastic nature. Much of the stress is transmitted in highly elastic materials while it is dissipated in frictional losses in highly viscous ones. The phase shift is large for highly viscous materials but small for highly elastic materials [149]. Several rheological parameters are determined in a small amplitude oscillatory rheology test. The storage modulus (G') expresses the magnitude of the energy that is stored in the material or recoverable per cycle of deformation (indicates the solid-like properties). The loss modulus (G'') is a measure of the energy which is lost as viscous dissipation per cycle of deformation (reflects the liquid-like properties). Therefore, for a perfectly elastic solid, all the energy is stored, that is, G'' is zero and the stress and the strain will be in phase. In contrast, for a liquid with no elastic properties, all the energy is dissipated as heat, that is, G' is zero and the stress and the strain will be out of phase by 90° . For a specific food, magnitudes of G' and G'' are influenced by frequency, temperature, and strain. For strain values within the linear range of deformation, G' and G'' are independent of strain. The loss tangent ($\tan \delta$) is the ratio of the energy dissipated to that stored per cycle of deformation and indicates the type of viscoelastic properties in a material. A high $\tan \delta$ value (i.e., $G'' > G'$) means that the material has liquid-like behavior [31, 150]. These parameters are defined as follows:

$$G' = \left[\frac{\sigma_0}{\gamma_0} \right] \cos \delta \quad (2)$$

$$G'' = \left[\frac{\sigma_0}{\gamma_0} \right] \sin \delta \quad (3)$$

$$\tan \delta = \left[\frac{G''}{G'} \right] \quad (4)$$

where σ_0 is the amplitude of the share stress, γ_0 is the amplitude of the strain and δ is the phase angle difference between the stress and the strain [38, 150, 151]. In acid milk gels, the G' is determined by the number and/or strength of non-relaxing protein bonds (covalent bonds), whereas the G'' is determined by rapidly relaxing bonds (non-covalent bonds) [53]. The G' and G'' are similarly related to the spatial distribution and the number of protein-protein bonds, which, therefore, suggests that $\tan \delta$ is related to the nature of the protein bonds [69].

5.2. Whey separation

Whey separation, i.e., the appearance of whey on the surface of a milk gel, is a common defect in fermented milk products such as yogurt [38, 132]. Whey separation negatively affects consumer perceptions of yogurt, as consumers think there is something microbiologically wrong with the product [31]. Due to this fact, manufacturers try to prevent whey separation by increasing the total solids content of milk, subjecting the milk to a severe heat treatment (to increase whey protein denaturation) or by adding stabilizers such as gelatin, pectin, starches, or gums [37]. Spontaneous syneresis is the usual cause of whey separation [31]. Syneresis is defined as shrinkage of a gel and this occurs concomitantly with expulsion of liquid or whey separation. Spontaneous syneresis is contraction of a gel without the application of any external forces (e.g., centrifugation) and is related to instability of the gel network (i.e., large scale rearrangements) resulting in the loss of the ability to entrap all the serum phase [132]. Hence, excessive rearrangements of particles in the gel network are responsible for high levels of whey separation [105]. Previous studies showed that several manufacturing conditions, such as low total solids content (protein content) of the mix, very low acid production ($\text{pH} \geq 4.8$), excessive heat treatment of the mix, and very high incubation temperatures, promote whey separation [38, 105]. Increasing content of protein by whey protein concentrate addition decreases the syneresis [152]. Whey separation is intimately related to the gel network's microstructure. Extensive rearrangements of protein particles in the gel network may be associated with increased local breakage of weak protein strands that make up the junctions in the network. This may result in the formation of weak spots and a less stable gel network [131]. Several authors reported that a high number of relaxing (non-covalent) protein bonds present in the gel favor rearrangements in the network and results in greater whey separation [105, 116, 127, 153]. As the number of non-relaxing (covalent) protein bonds increases, the level of rearrangements in the gel network decreases and a lower level of whey separation is obtained [105, 116, 127, 154]. Hence, high $\tan \delta$ values together with low G' values can be correlated with high levels of whey separation [105]. On the other hand, whey separation is also related to the permeability of the gel network. Finer networks with a higher level of cross-links and smaller pores will have less of a tendency to exhibit whey drainage under the force of gravity than coarser, more open structures [145].

5.3. Clusters formation

Undesired clusters can have a negative effect on a yogurt's texture. Numerous manufacturing parameters, such as severe heat treatments, excessive whey protein to casein ratios, high incubation temperatures, certain types of starter cultures and the use of excessive amounts of starter culture, are associated with textural defects of stirred yogurt like graininess (particles) or surface roughness (irregularities in the yogurt matrix) [51, 155]. Remeuf et al. [114] reported that graininess can be related to an increase in the casein micelles size caused by the interaction of micelles with denatured whey proteins. Puvanenthiran et al. [145] associated the observed grainy texture with the formation of big whey protein aggregates.

Although manufacturing parameters have a direct influence on the formation of clusters, according to Lee and Lucey [31], stirred yogurts are likely to have clusters of protein aggregates which are presumably created by the collisions and shearing during the mixing process involved in their production.

Conclusions

Future research should be focused on the production of concentrated yogurt by direct recombination. It offers important advantages over other industrial production methods as it is more environmentally friendly and the product can be more nutritious. Still, rheological properties of recombined concentrated yogurt are different from those of strained yogurt. Usually they form weaker gels than those made by traditional or UF methods. Different dry dairy ingredients (especially with elevated content of whey protein) should be used for production of concentrated yogurt. Such products could be used as supplements of diet for sportsmen and physically active people. In the future it is also crucial to find an effective formulation for producing a recombined non-fat, additive-free type of Greek-style yogurt. Increased whey protein content (in form of whey protein isolate with low content of fat) could solve the problem of weaker texture of non-fat product.

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Biological active compounds from native food sources for fermented dairy products

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Abstract

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Introduction. The present research aims to identify and quantify valuable compounds from native products such as honey, walnut and sea buckthorn in order to produce fermented product from cow's milk.

Materials and methods. Honey bees of native production polyphora from the Drăgășani area (Romania) were used, and for the identification and quantification of the volatile compounds a GC-MS system was used. Qualitative and quantitative analyzes of the polyphenols were performed using the Agilent 1200 HPLC system consisting of a photodiode array (PDA) detector and an electrospray ionization mass detector.

Results and discussion. In poliflora honey bees, phenolic acids reach 79.284 mg/100 g sample, isoprenoids reach 127.449 mg/100 g sample, and flavonoids at 168.475 mg/100 g sample, and the results obtained by chromatographic analysis of honey. Bees have been found in this product a wide range of aroma compounds, namely: terpenic compounds, higher alcohols, aldehydes and ketones, esters, so that the aldehydes reach values of 7.889 mg/100g, ketones at 2.337 mg/100g, alcohols higher ones accumulate at amounts of 3.212 mg/100g, and the esters reach values of 8.993 mg/100g. Following the chromatograms obtained, the content of polyphenols in the walnut kernel was established at an amount of 786.553 mgGAE/100 g, and for the berries the content in polyphenols was 343.229 mgGAE/100 g. Also the highest concentration significant of tocopherols is found in the form of alpha tocopherol, with 33.245 mg/100g, followed by beta tocopherol with an amount of 12.723 mg/100 g of oil. Values lower than 4.553 mg/ 100g and 1.286 mg/100g respectively are visible in the case of the tocopherol gamma and the tocopherol delta.

Conclusions. The compounds identified and quantified from the three indigenous products such as polyflora honey, walnut and sea buckthorn will lead to the achievement of a harmonious aromatic profile and with certain taste qualities of the new fermented dairy product.

1. Introduction

The actual task is to analyze new perspectives and key areas for future research in the development of high-quality innovative dairy products as a function of valuable compounds from native food products and some new processing techniques. Previous studies [16–18] have shown that bee honey, walnuts and sea buckthorn have a number of positive effects in human nutrition and health, with beneficial action especially in the area of the nervous and vascular system, infectious diseases exerting a pronounced antibacterial action.

The current research has been done is to identify and quantify valuable compounds from honey, walnuts and sea buckthorn in order to produce an innovative fermented product from cow's milk.

2. Analysis of valuable compounds from native products used in obtaining innovative dairy products

2.1. Bee honey: characteristics and valuable components

Bee honey is a food naturally derived from the processing of floral nectar by bees [32]. It is sweet, so it contains many sugars, aromatic, of different consistencies, with a variable color palette, specific to its composition [8, 10]. The color palette starts from colorless, yellow, reddish, orange and can reach black [1, 2].

Honey is the result of the activity of the bees that collect the nectar with the help of the horn with which they are endowed, keep it in the gose, mix it with saliva and then a transfer to the bees left in the hive in a sugary form, which continues the processing until it reaches the known form [16, 33].

2.1.1. Bee honey and its sensory characterization. The appearance of honey and the color can be appreciated according to the degree of transparency examined in the direct sunlight [3, 7].

The taste and smell are appreciated by noting the intensity of the aroma and the sweet taste, or of the possible secondary shades: sour, bitter, fad, etc. conferred including polyphenols (Table 1) [4, 5].

Table 1

Sensory characteristics

No	Characteristics	Conditions of admissibility
1	Color	From colorless, bright yellow, to orange
2	Taste	Sweet, pleasant,
3	Appearance	Semifluid, viscous or crystallized
4	Flavor	Floral, characteristic of the type of honey

2.1.2. Beehoneyandits chemical composition. The chemical composition of honey bees depends to a large extent on the climatic factors and the vegetal nature of the collection area, the mode of exploitation of the product by the beekeepers, its properties being in direct correlation with them [6–10].

Bee honey contains protein substances, a number of microelements and vitamins (C, B1, B6, B2), small quantity organic acids (citric, lactic, malic, oxalic, succinic), dextrans, odorants and dyes, with clinical effects. Relevant, but also presents a microbiological load worth studying [11–15].

All honey is acidic and has a pH value generally between 3.5 and 5.5, due to the presence of organic acids that contribute to honey aroma and stability to microorganisms [11]. In honey, the main acid is gluconic acid, which is found together with the respective glucono-lactone in a variable equilibrium (Table 2) [9].

Table 2

Physico-chemical and microscopic characteristics

No	Parameter	Acacia honey	Forest honey	Other assortments
1	Water,% max	20	20	20
2	Acidity, ml NaOH 1N/ 100g, max	4	5	4
3	Reducing sugar, expressed in invert sugar,% min	70	60	70
4	Amylase index, min	6,5	13,9	10,9
5	Ash,% max	0,5	1,0	0,5
6	Hydroxymethylfurfural, mg/100g max	1,5	1,5	1,5
7	Colorimetric index, mm, max (on the Pfund scale)	12	65	-
8	Water insoluble substances, max	0,1	0,2	0,1
9	Lightly hydrolyzable sugar, expressed as sucrose,% max	5	10	5

2.1.3. Microbiological indicators of honey. Microbiological indicators of honey are presented in Table 3[12].

Table 3

Microbiological indicators of honey

No	Specification	NTM/g	Number of yeasts / g	Molds	Pathogenic microflora
1	Normal honey	<300	2-3	absent	Absence
2	Honey with limited conservabilitate	<300	10-10 ²	absent	Absence
3	Honey coming out of consumption	>300	10 ⁴	present	Present

In order to detect forgeries, the physico-chemical control of honey is done by several methods: determination of acidity index, determination of sucrose (based on the identification of sucrose from honey forged with sugar syrup by reaction with silver nitrate), determination of the addition of flour or starch (based on the color reaction of starch with iodine), identification of honey nitrites [12, 13].

2.2. Walnuts: characteristics and valuable components

Walnuts (*Juglans regia* L.) belong to the family Juglandaceae. The native region of which they come is Central Asia, the western Himalayan chain, the region of Kyrgyzstan [19]. They arrived in Europe before the Roman era, and later spread to America and North Africa [21]. Walnuts and hazelnuts (*Corylus avellana* L.) make up about 60% of walnut production in Europe [20].

The results of clinical and epidemiological studies clearly indicate that regular consumption of nuts reduces the risk of cardiovascular disease, diabetes, cancer and inflammatory diseases [21]. According to many authors, these properties result from the chemical composition of walnuts. First, they are a rich source of fat (up to 70% dry mass), including unsaturated fatty acids, as well as phytochemicals such as phenols, tocopherols and sterols [19, 20]. In addition, walnuts contain polyunsaturated fatty acids (PUFAs) that lower LDL cholesterol and increase HDL cholesterol [22]. Moreover, nuts contain dietary fiber and essential micronutrients [23].

Nuts are also a rich source of complete proteins, which are abundant in exogenous amino acids, as well as minerals, including potassium and magnesium [24]. In walnut oil, there are a number of active phytochemicals that consist mainly of polyphenols [26]. In terms of polyphenol content, nuts dominate among all nuts; their average polyphenol content is about 1591.5 mg/100 g, and in peanuts it varies widely up to 900 mg/100 g [21–23]. Of all the polyphenols identified in walnuts, elagitannins are the most numerous group, followed by phenolic acids, flavanols and dihydroclacones [24–26].

2.3. Sea buckthorn (*Hippophae rhamnoides* L.): characteristics and valuable components

The sea buckthorn (*Hippophae rhamnoides* L.) presents a series of properties beneficial to the human body, through its rich content in minerals and vitamins. It presents a series of bactericidal and bacteriostatic effects, being rich in antioxidants that have the effect of neutralizing free radicals, is implicated by its composition in the elimination of toxins from the body [27].

Sea buckthorn contains over 200 bioactive components and volatile compounds, many vitamins (including A, P, C, B1, F, B2, E, K), carotenoids, tocopherols, sterols, flavonoids, phenolic compounds, lipids, ascorbic acid, citric acid and more than 15 microelements (including Fe, Mn, B, Al, K, F, Ti) [27].

Catenin reduces liver injury by antioxidant activity [28]. The sea buckthorn pulp is rich in carotenoids, tocopherols, sterols, lipids, ascorbic acid, flavonoids, triterpenes, etc. These compounds have biological activities and therapeutic properties such as antioxidants, antitumor and immunomodulators [29, 30]. In addition to medicinal use, the pulp is processed into different products, such as juice and jam [28].

Studies have shown the efficiency of dog consumption in the recovery of cancer patients, those with epidermal problems, being an optimal raw material including in the cosmetic industry [31].

3. Materials and methods

3.1. Experimental samples

Polyflora honey, walnut kernel and sea buckthorn berries are valued as a source of natural compounds with antioxidant properties and pleasant sensory profile. In this study honey of polyflorous bees of local production in the Drăgășani area (Romania), walnut kernel arising from Station for Horticulture Research and Development (SCDP) (Romania), and sea buckthorn from Agricultural Cooperative from Biocătina (CAB) (Romania) were used.

3.2. Determination of phenolic compounds in honey

For quantification and determination of phenolic compounds, 100 g of honey were dissolved in 500 ml of acidified water (pH = 2, HCl) [4]. The solution was then filtered with cotton to remove the solid particles. The obtained filtrate was passed through an Amberlite XAD-2 column, which has the ability to selectively retain phenolic compounds. To remove sugars and other polar compounds, a wash was carried out with the passage of acidified water to pH=2. Subsequently, the phenolic fraction was eluted with methanol and the extract was concentrated under reduced pressure at 40 °C. The final residue was then redissolved in 5 ml of distilled water and subjected to liquid/liquid extraction, using diethyl ether as the extraction solvent. The extracts were concentrated under reduced pressure, and the residue was dissolved in methanol.

The chromatographic system consisted of a quaternary pump, an automatic sampler maintained at 5 °C, a degasser, a photodiode detector and an automatic compartment for thermostatic columns. Chromatographic separation was performed with a C18 column of particles having a size of 1.8 μm and maintained at 30 °C. The mobile phase was composed of 0.1% formic acid diluted in water and 0.1% formic acid dissolved in acetonitrile, previously degassed and filtered using a nylon membrane filter with a porosity of 0.22 μm. The injection volume was 3 μl. Spectral data for all peaks were detected in the range 190-600 nm.

Each sample was filtered through a 0.2 μm nylon membrane (Whatman). The mass analysis was performed on a Varian 240 mass spectrometer in negative mode, equipped with an ESI electro-spray ionization source: spray voltage, 5 kV; capillary voltage, -20V; capillary tube voltage, -65V; capillary temperature, 325 °C; gas flow and auxiliary gases (N₂), respectively 50 and 10 (arbitrary units). The mass spectra were obtained in the mass range 100-1000 m/z.

The collision energy used in the MS_n experiments was 35 (arbitrary units). Data acquisition was performed using Xcalibur ® software (Thermo Scientific, CA, USA). The quantification was performed with calibration curves of the standard substances for caffeic acid, quercetin, caempferol, chrysin and pinocembrine. In the absence of standards the compounds were expressed by equivalents of the more structurally similar phenolic compound (method adopted and adapted after Silva Caveiro [31]).

3.3. Determination of volatile compounds in honey

A GC-MS system was used to identify and quantify volatile compounds: GC-MS Varian 240 with column C18, helium carrier gas, the temperature being set upwards by 3 °C/min up to 170 °C, later with 25 °C/min up to 290 °C/min. The volatile compounds were identified by comparing the spectra obtained with those from the laboratory library [6, 25].

3.4. Determination of phenolic compounds in walnuts and sea buckthorn fruit

Qualitative and quantitative analyzes of the polyphenols were performed using the HPLC Agilent 1200 system consisting of a photodiode array (PDA) detector and an electrospray ionization mass detector. The polyphenolic compounds were separated on a C18 column using a linear gradient of 8 minutes. Solvents were used: solvent B containing 40% acetonitrile and 0.1% formic acid and solvent A: ultrapure water containing 0.1% formic acid at a flow rate of 0.30 mL / min. The injection volume of the samples was 5 μ L. The readings of the polyphenolic compounds were performed at the following wavelengths: hydroxycamates at 320 nm and the ellagic tannins at 240 nm. Gallic acid was used as an equivalent [20, 23].

3.5. Determination of tocopherol compounds in walnuts and sea buckthorn fruit

To analyze the content of tocopherols, the oil extracted from nuts and sea buckthorn was used using the Soxhlet method. The tocopherol content (α , β , γ , δ) was measured by high performance liquid chromatography (HPLC) according to the method adapted by Pycia et al. The chromatography system consisted of an Agilent 1200 HPLC chromatograph, a C18 column. As a mobile phase, methanol with a flow rate of 0.8 mL/min and a Shimadzu fluorescence detector with the extinction wavelength set at 290 nm and the emission wavelength at 330 nm were used. The injection volume of a measured sample was 20 μ L. Tocopherols were quantified using standard curves calculated by linear regression analysis [19].

4. Results and discussions

4.1. Identification and quantification of phenolic compounds in honey

In order to approach as accurately as possible, the phenolic compounds in the honey of the bees subjected to the study, they were summed according to their chemical classification, namely: phenolic acids, flavonoids, isoprenoids (Figure 1).

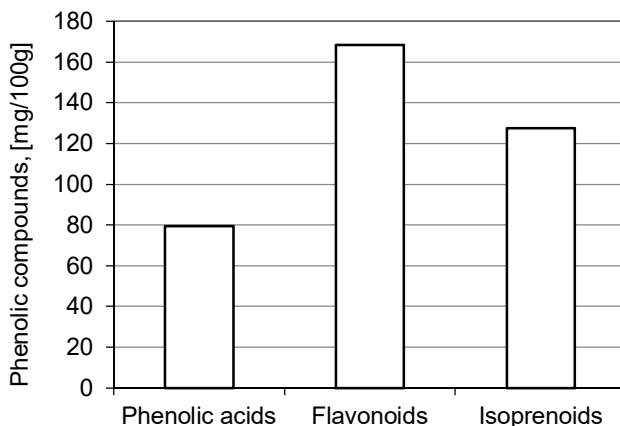


Figure 1. Sum of the phenolic compounds identified and quantified in the honey of poliflorine bees from Drăgășani

The amount of these compounds varies in the sample taken from 79.284 mg/100 g to 168.475 mg/100 g (Figure 1). Phenolic acids reach 79.284 mg/100 g sample, isoprenoids reach 127.449 mg/100 g, and flavonoids to 168.475 mg/100 g sample.

4.2. Identification and quantification of volatile compounds in honey

The presence of volatile compounds in honey can provide information on its botanical origin, allowing it to verify whether it was produced by bees from the nectar of flowers or exudates secreted by plants or insects [3, 6]. Over 500 volatile compounds have been identified in honey as complex mixtures of different classes of compounds such as terpenes, isoprenoids, phenolic compounds, benzene derivatives, alcohols, ketones, aldehydes, esters, fatty acids, linear hydrocarbons and cyclical [33]. As a result of the results obtained by the chromatographic analysis of honey bee, it was found that in this product there is a wide range of aroma compounds, namely: terpenic compounds, higher alcohols, aldehydes and ketones, esters (Figure 2).

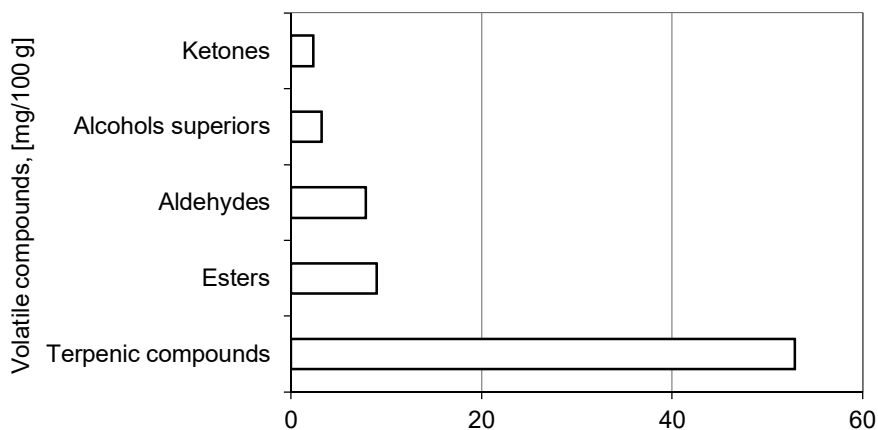


Figure 2. Sum of the volatile compounds identified and quantified in the honey of polyflorous bees from Drăgășani

We observe the determined quantities, noting especially the terpenic ones (Figure 2). From the results obtained the hotrienol is identified in remarkable amounts, the identified values being 47.335 mg/100g, and the sum of the terpenic compounds reaches 52.867 mg/100g. Aldehydes reach values of 7.889 mg/100g and ketones reach 2.337 mg/100g. Higher alcohols accumulate at 3.212 mg/100g, and esters reach 8.993 mg/100g.

4.3. Identification and quantification of phenolic compounds in walnuts and fruits of sea buckthorn

Polyphenols are chemical compounds with multiple beneficial effects on the human body, generally acting as powerful antioxidant elements [20]. They are found in many fruits and vegetables, offering protection against free radicals [29, 30]. During the study it was determined the total polyphenol content in walnuts and sea buckthorn samples (Figure 3).

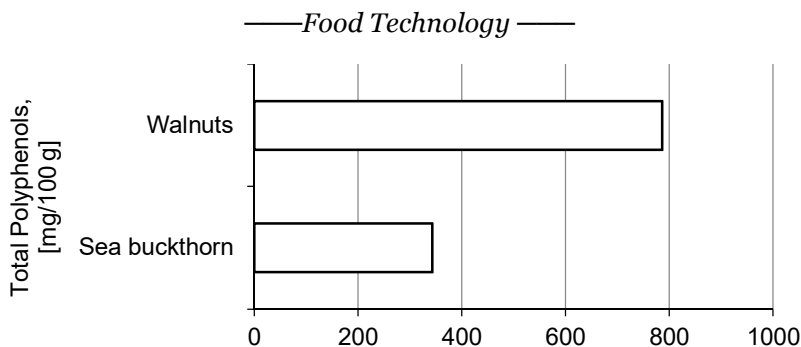


Figure 3. Total polyphenol concentration identified in walnut kernel and sea buckthorn expressed in gallic acid equivalent

Following the chromatograms obtained, the content of polyphenols in the walnut kernel was established, which is at an amount of 786.553 mgGAE/100 g according to Figure 3. For the berries the content in polyphenols is 343.229 mgGAE/100 g.

4.4. Identification and quantification of tocopherols in walnuts and fruits of sea buckthorn

Tocopherols or vitamin E is beneficial to the human body by its pronounced antioxidant character which leads to neutralization and elimination of free radicals [19]. The most active tocopherol is alpha, but it is found in walnut and buckwheat oil and as tocopherol beta, gamma or delta, or tocotrienol [24, 29]. During this study there were identified and determined several tocopherols: alpha, beta-, gamma and delta in the oil extracted from walnut (Figure 4).

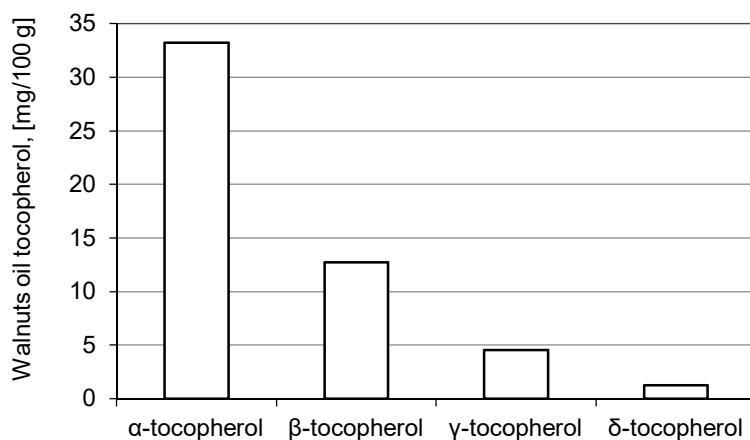


Figure 4. The concentration of tocopherols identified and quantified in the oil extracted from walnut

The most significant concentration of tocopherols (Figure 4) is found as alpha tocopherol, with 33.245 mg/100g, followed by beta tocopherol with an amount of 12.723 mg/100 g oil. Values lower than 4.553 mg/100g and 1.286 mg/100g respectively are visible in the case of the tocopherol gamma and the tocopherol delta.

Experimental results obtained regarding the tocopherols concentration in the oil extracted from the sea buckthorn are presented in Figure 5.

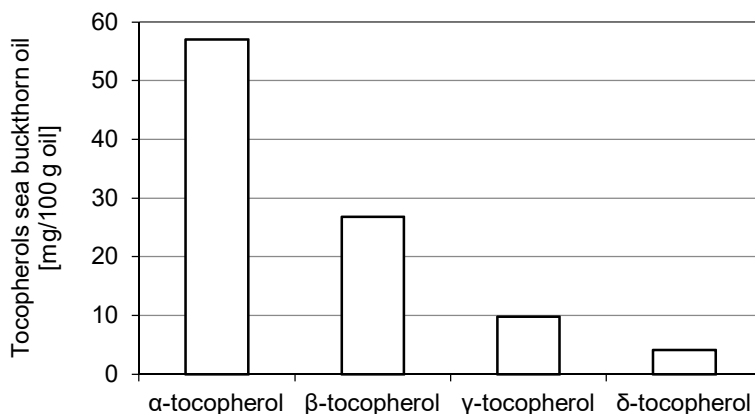


Figure 5. The concentration of tocopherols identified and quantified in the oil extracted from the fruit of sea buckthorn

A maximum concentration of alpha tocopherols in the amount of 56.973 mg/100g is observed, followed by 26.771 mg/100g beta tocopherols (Figure 5). Lower values of 9.774 mg/100g were identified in the case of tocopherol gamma and of 4.123 mg/100g in the case of the tocopherol delta. Compared with the values identified in walnut oil, the values found in sea buckthorn oil are more significant.

Conclusions

Bee honey contains significant values of phenolic compounds, compounds that confer antioxidant qualities associated with nutritional ones.

The volatile compounds identified and quantified in honey bees lead to the achievement of a harmonious aromatic profile and with certain gustatory qualities, the share having terpenic compounds and esters.

The tocopherols identified and quantified in the oil extracted from the walnut core have significant values especially in the form of alpha tocopherols. Compared with the values identified in sea buckthorn oil, they are lower on average by 45%.

The ratio of alpha tocopherols to beta, gamma and delta tocopherols is on average 7:3, slight oscillations being visible in those identified and quantified in sea buckthorn oil.

The results of the present study suggest that bee honey, walnuts and sea buckthorn berries can be used as a valuable source of natural antioxidants and volatile compounds for obtaining innovative dairy milk products.

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Investigation of the ferric ions complexes with bioligands of probiotic origin

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Abstract

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Introduction. The peculiarities of obtaining stable, easily digestible and safe ferric ion complexes with bioligands of the probiotic origin and their characteristics have been investigated.

Materials and research methods. As bioligands, the products of metabolism and processing of biomass *Lactobacillus delbrueckii subsp. Bulgaricus B-3964*, namely, lactic acid, amino acids, low molecular weight peptides and muropeptides, were used. The destruction of biomass peptidoglycans was performed by alternating treatment of sonication and papain. The progress of complexation was monitored using the turbidimetry method.

Results and discussion. The destruction of biomass peptidoglycans, performed by alternating sonication and the papain enzyme preparation, resulted in a mixture of amino acids, low molecular weight peptides and muropeptides, the concentration of which was 10.24 mg/cm³, 6.45 mg/cm³ and 2.25 mg/m³ respectively. Lactic acid was isolated from the culture fluid by crystallization of calcium lactate. Three polydentate systems were used to form Fe³⁺ complexes: peptidoglycan destruction products; lactic acid; a mixture of degradation products and lactic acid. It was found that the bioligand systems under study bind Fe³⁺ ions in the amounts of 32, 40 and 46 mol/dm³·10⁻² respectively. Electrostatic and coordination interactions are involved in the formation of the complex of iron (III) and probiotic bioligands. The behavior of complexes at different pH values and temperatures was studied. Most stable is the complex formed with the participation of the bioligands system containing the products of peptidoglycans degradation and lactic acid. Such system of bioligands provides the formation of ferric ions chelate complexes, stable in the pH range of 1–10 units. Obtained complex is a promising ingredient of dietary supplements and dietetic products whose technology involves high-temperature processing. That was proven that, the complex is stable in the temperature range of 20–122 °C. When the temperature reaches 122–125 °C, the mass loss is 3% for the complex and 14% for the mechanical mixture. In the temperature range of 122–178 °C, an endothermic reaction is observed during the thermal treatment of the complex, and no thermal effects are observed during the mechanical mixture treatment. The weight loss of the complex in this temperature range is 22%, mechanical mixture – 16%. The presence of an endothermic peak on the differential thermal analysis curve of the complex may indicate the presence in its structure of chelate bonds, and during their destruct the enthalpy changes are occurred.

Conclusion. The research results indicate the effectiveness of the use of polydentant mixed ligand systems of probiotic origin for complexation with ferric ions.

Introduction

Iron is one of the most important trace elements that is part of more than 100 enzymes of the human body, participates in respiration, hematopoiesis, immunobiological processes, redox reactions, etc. [1]. Insufficient iron supply to the body can provoke iron deficiency anemia (IDA). It is a disease of the blood system caused by iron deficiency in the body [1, 2]. IDA is accompanied by changes in metabolism parameters, a decrease in hemoglobin concentration, which can lead to disorders of the cardiovascular, nervous system, decrease in women's reproductive function, changes in intelligence and behavioral moods, chronicity of various diseases, etc. [3, 4].

Analysis of recent research and publications

As is known [1], food contains 2 types of iron – heme (red meat) and non-heme, or ionized (vegetables, fruits, cereals). Moreover, non-heme iron covers 70% of the human body's need for iron.

The entry of iron into the body occurs through the intestinal mucosa, where it is adsorbed by food enterocytes (the common name of epithelial cells lining the intestinal mucosa, which are highly specialized cells that coordinate the absorption and transport of iron). The absorption of iron in the intestine occurs in three main ways: with the transporter of divalent cations, as part of the mobilferrin integrated complex, as well as a special way for the absorption of heme iron from food (Figure 1) [2, 3].

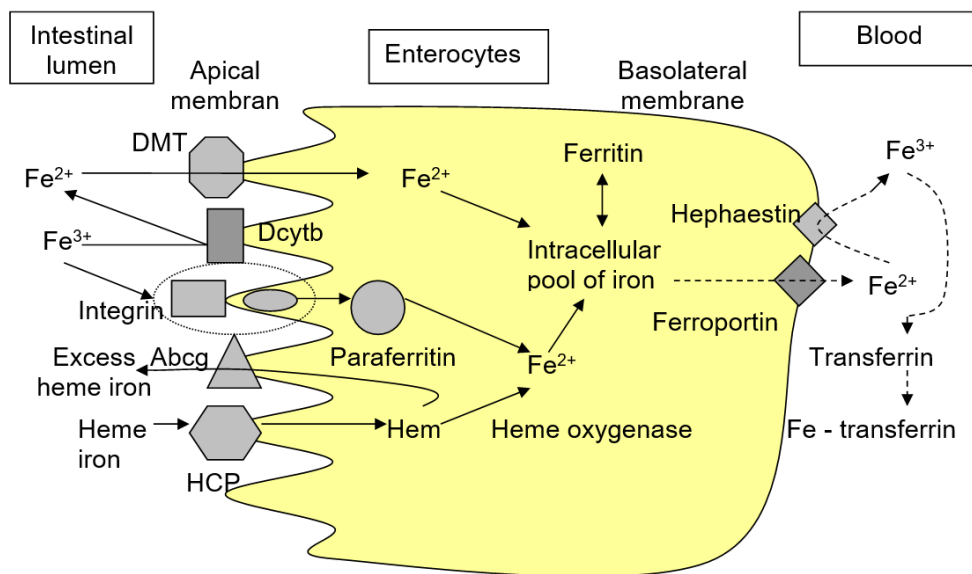


Figure 1. Scheme of iron transport in the enterocyte [2]

Inorganic, organic and multivitamin preparations in oral form are usually used to replenish iron deficiency (Figure 2) [4].

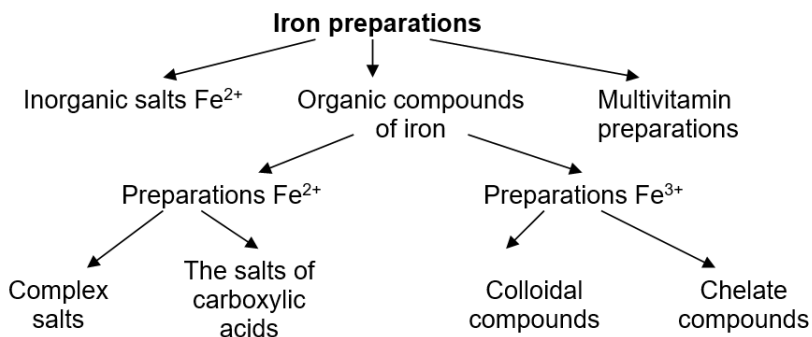


Figure 2. Classification of iron preparations [4]

The use of inorganic preparations in the ferrous form (Fe^{2+}) can provoke a number of complications. In the gastrointestinal tract, the ferrous ion is oxidized to the ferric-ion, inducing the appearance of a free electron, resulting in the generation of free radicals that disrupt DNA synthesis, affect the activity of several enzymes, cause the peroxidation of polyunsaturated lipids in cell membranes [4]. Moreover, increased concentrations of ferrous iron in the intestine contribute to its passive transport of into enterocytes, which leads to the entry of toxic ions into the cells of the intestinal mucosa and can cause their death. Direct cytotoxic effect of ferrous ions on cells of different organs, especially brain and liver, was also noted [3]. To counteract these disadvantages, to ferrous drugs additionally include antioxidants (such as ascorbic acid), or fix Fe^{2+} ions on special matrices that cause their prolonged release [4].

Ferric ion salts (Fe^{3+}) have better solubility and higher elemental iron content than ferrous salts, but Fe^{3+} has a higher charge/radius ratio than Fe^{2+} ion, which causes a significant degree of hydrolysis of iron (III) salts [2, 5]. During the hydrolysis of salts of ferric ions, the formation of insoluble $\text{Fe}(\text{OH})_3$ hydroxide occurs. Thus, inorganic agents containing Fe^{3+} are more likely to be converted to ferric hydroxide in an alkaline medium of the intestine, which makes it impossible for them to absorb. Such the ferric properties complicate its use as an oral agent for the correction of IDA [2, 4].

Organic iron compounds are more widely used as a means of correcting iron deficiency [4, 5]. Thus, the use of an organic Fe^{2+} complex with two nicotinamide molecules is known [4]. Ferum (II) salts and carboxylic acids, namely fumaric, lactic, gluconic, have also been used as they have been shown to have protective properties similar to those of ascorbic acid. The problem of introduction of ferric ion into the body was solved in the group of chelating agents. There is an agent based on the chelate complex of ferum protein succinylate, in which the ligands provide the stability of the ferric ion, and the stability constants are not less than in the ferric hydroxide. This provides preservation of Fe^{3+} in a soluble state in the intestinal lumen and increases the likelihood of its absorption [4, 6].

There are agents to combat IDA based on insoluble iron oxide in colloidal solution. In the micelles of such a colloidal solution, insoluble $\text{Fe}(\text{OH})_3$ was used as the nucleus of the micelles, and the dense layer was created by carbohydrate compounds, such as maltose, sucrose, sorbitol, isomaltose [4].

Despite some advances in the development of effective anti anemia agents, the search for new, more perfect forms of iron for oral use is still ongoing [7, 8]. Since iron in the living body is always in the composition of bioorganic complexes with amino acids, proteins and other bioligands [2, 4], the use of mixed ligand systems to obtain safe soluble easily digestible forms of non-toxic ferric ions is worthy of attention [9]. The study of mixed ligand complexes of biometals has become widespread, the methods of obtaining and characteristics of some of them are described in the literature [10–12]. Both natural and synthetic compounds are used as bioligands, but there is no information in the literature on the possibility of using metabolism products and processing of probiotic bacteria to produce mixed ligand systems containing iron [13–15]. Despite the extensive experience and production of probiotic cultures cultivation, this idea is very relevant [16–17]. The culture fluid contains a large number of metabolites, in particular organic acids, capable of chelation with biometals [18, 19]. With the disintegration of probiotic cells, degradation products of the peptidoglycans of their cell walls can be obtained, namely, amino acids, low molecular weight peptides, muropeptides, which also contain functional groups capable to form ionic and coordination bonds with metal ions. In addition, the substances of the muropeptide series have their own physiological activity – they are powerful immunotropic compounds [20–21].

The purpose of this work is obtaining and characteristic of the ferric ions complexes with metabolites and low molecular weight degradation products of peptidoglycans of *Lactobacillus delbrueckii subsp. Bulgaricus B-3964*.

Materials and methods

Materials

Biomass (BM) of lactic acid bacteria *Lactobacillus delbrueckii subsp Bulgaricus B-3964* with a concentration of $4.8 \cdot 10^9$ CFU/cm³ from the collection of the Scientific and Production enterprise Ariadna (Odesa), papain with proteolytic activity of 10 Un/mg (Swanson Health Products, USA), FeCl₃·6H₂O h (China) were used for the research.

Obtaining degradation products of peptidoglycans *Lactobacillus delbrueckii subsp. Bulgaricus B-3964*

The isolation of cells from the culture fluid was carried out by centrifugation for 15 min at 8000 min⁻¹. The precipitated cells were washed with distilled water, resuspended and carried out the ultrason treatment using ultrasonic baths “PSB-1335-05” with a working frequency of 40 kHz, duration of the treatment was 300 s. The enzymatic destruction of BM cell walls was carried out by papain treatment at 37 °C and pH = 7.4. The enzyme: substrate ratio (BM solids content) was 1:200, the incubation time of the reaction mixture was 300 min. The enzymatic hydrolysis was stopped by emergency heating to a temperature of 100 °C, the mixture was cooled, the liquid phase was separated from the solid by centrifugation for 10 min at 8000 min⁻¹. In the liquid phase, the content of free amino acids was controlled by formol titration method [22]. The content of low molecular weight peptides (LMWP) was determined by the Benedict method [22] after precipitation of high molecular weight proteins by 10% trichloroacetic acid. The content of muropeptides was determined after purification of the hydrolysate on an ion exchange column with cation exchanger [23] and the subsequent determination of the carbohydrate component in the composition of muropeptides by the Antron method [24].

Lactic acid isolation

The isolation of lactic acid from the culture fluid was performed by a classical method, which included crystallization of calcium lactate with subsequent processing of crystals with sulfuric acid and removal of insoluble calcium sulfate precipitate [25].

Ferric complexes preparation

The complexes were obtained by combining solutions of bioligands and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ with vigorous stirring for 180 s, the temperature of complexation was 40 °C. Iron-containing complexes were obtained by combining $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solutions with: hydrolysate of *Lactobacillus delbrueckii* subsp. *Bulgaricus* B-3964 (Complex I), lactic acid at a concentration of 10 mg/cm³ (Complex II); hydrolysate and lactic acid with concentration 5 mg/cm³ in the mixture (Complex III). The complex formation ability of Fe^{3+} ions with the bioligands was determined by a turbidimetric method in the presence of Na_2CO_3 on a spectrophotometer SF-2000 at 450 nm [26]. Different volumes of 0.5n $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were added to aliquots of mixtures containing bioligands, mixed and left for 180 s for complete chelation. 1N Na_2CO_3 was then added to the solutions to achieve a pH of 7 units. Under these conditions, Fe^{3+} ions, which did not participate in the complexation, interact with sodium carbonate to form insoluble $\text{Fe}(\text{OH})_3$ particles, which provoke turbidity of the system [27].

Complexes pH-stability study

The behavior of the complexes was investigated in the range of pH values of the medium 1–10 units. The concentration of Fe^{3+} was determined by the thiocyanate method [28]. The required pH was achieved using standard NaOH and H_2SO_4 solutions. The stability of the chelate complexes was calculated by the formula (1):

$$C = \frac{a-b}{a} 100, \% \quad (1)$$

where a is the total amount of Fe^{3+} ions in the complex, mg/cm³; b is the amount of Fe^{3+} ions released into the reaction medium, mg/cm³.

Complexes thermal stability study

The studies were performed using the differential scanning calorimetry (DSC) method. DSC thermograms were obtained in the temperature range of 25–250 °C at a constant heating rate of 5 °C/min on a Derivatograph Q1500-D calorimeter. In order to determine under what conditions the complete decomposition of the samples would take place, the heating was continued to a maximum temperature of 450 °C. A 500 mg portion was placed in a ceramic crucible. The accuracy of determining the temperature was ± 1 °C, the thermal effect – $\pm 3\%$.

IR spectra of samples were recorded in the range of wavelengths from 4000 to 400 cm⁻¹ in a spectrometer with a Fourier transformer FTIR IR Affinity-1, Shimadzu (Japan) [29].

Results and discussion

Preparation and characterization of mixed-ligand complexes of ferric ions

Probiotic bioligands, namely, peptidoglycan degradation products and metabolism of *Lactobacillus delbrueckii subsp. Bulgaricus* B-3964, were used to form Fe^{3+} chelate complexes. Destruction of peptidoglycans of cell walls of BM was performed by the procedure above. As a result, a mixture of amino acids, LMWPs and mucopeptides was obtained, the concentration of which was 10.24 mg/cm^3 , 6.45 mg/cm^3 and 2.25 mg/cm^3 , respectively. An effective chelating agent, namely lactic acid, that is a metabolism product, was also isolated from the BM culture fluid. As a result of the manipulations above, a solution of lactic acid at a concentration of 10% was obtained. A mixture of amino acids, LMWPs, mucopeptides and lactic acid is a mixed ligand system that can cause the formation of stable chelate complexes of biometals, but it is difficult enough to determine the exact denticity of such system, that complicates the calculation of the required amount of metal in inorganic form for its complete chelation at complex formation [9, 27].

As a rule, the synthesis of biometals chelate complexes is carried out with a known composition of ligands of definite denticity. This allows to take on to the complexation reaction required amount of metal, which provides the effect of complete chelation [7, 27]. In our case, it was necessary to determine in what form the metal is in the system of biological agents: organic or inorganic. The classical methods for determining the concentration of a metal ion in a reaction medium are unjustified because the reagents used are sufficiently aggressive. This may cause the destruction of the ionic, coordination bonds of the complexes, which will not provide reliable results and will not allow to determine what form the metal was in the system. Therefore, the determination of free Fe^{3+} ions in the reaction medium was carried out using nephelometry, namely, by determining the turbidity of the system, the presence of which was provided by the reaction products of free ferric ions and sodium carbonate. Three probiotic bioligand systems were used for the studies: low molecular weight hydrolysis products of peptidoglycans (Complex I), lactic acid (Complex II) and their mixture (Complex III). The concentration of additional ligands for complexation was chosen based on theoretical quantum-chemical ideas about possible configurations of Fe^{3+} chelate complexes. The results of the studies are presented in Figure 3.

As can be seen from Figure 3, the turbidity of mixed ligand systems in the presence of Fe^{3+} and Na_2CO_3 ions is minimally stable (0.08 opt. Units) until a certain concentration of metal is reached in the mixture. For the systems under study, this value has a significant difference. Thus, the rapid turbidity growth of Complex I system occurs at concentrations of Fe^{3+} ions in a mixture of $32 \text{ mol/dm}^3 \cdot 10^{-2}$, Complex II – $40 \text{ mol/dm}^3 \cdot 10^{-2}$, Complex III – $40 \text{ mol/dm}^3 \cdot 10^{-2}$. Such behavior of the investigated systems indicates that, before reaching these concentrations, the metal is in bound state in the composition of mixed ligand complexes, that makes impossible its interact with the sodium carbonate present in the system and the appearance of insoluble ferum hydroxide, which causes turbidity. From figure 3 implies that Complex III contains the largest amount of bound metal. This is because the denticity of the mixed ligand system of this complex is the highest, based on the total concentration of all its components [7, 30].

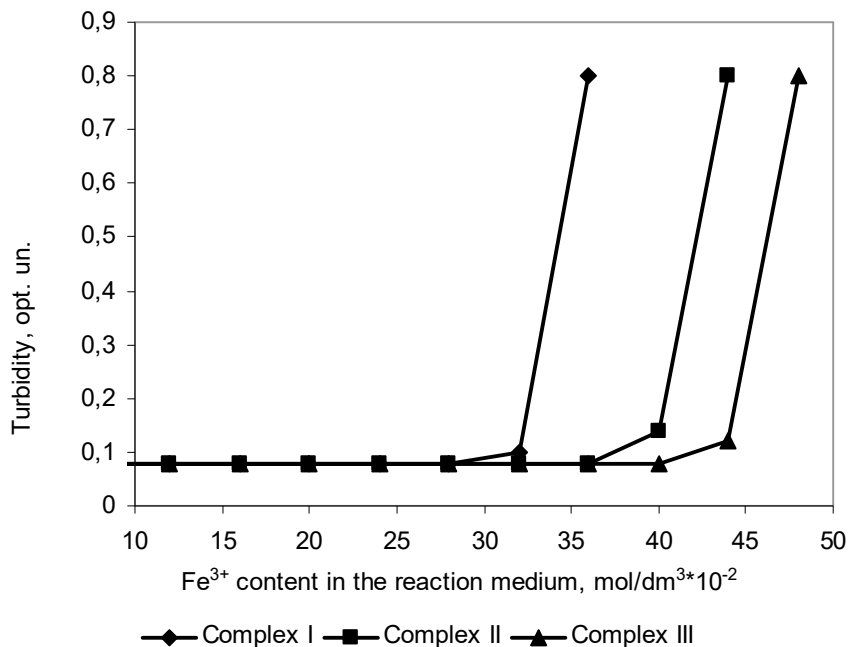


Figure 3. Dependence of the turbidity of mixed ligand system of probiotic origin on the content of Fe³⁺ ions in the presence of Na₂CO₃

IR spectroscopy was used for a deeper study of the chemical structure of the complex. The following samples were studied by IR spectroscopy: 1 – bioligands (a mixture of peptidoglycic hydrolysis products and lactic acid); 2 – a complex of ferric ions with bioligands (Figure 4). Ferric ions and the mechanical mixture of the complex components were not investigated by IR spectroscopy, since the FeCl₃·6H₂O compound is very hygroscopic, that complicates the analysis and may affects on the correct interpretation of the results.

When comparing the IR spectra of bioligands and the resulting complex, there is a difference between the absorption bands. In particular, a significant band broadening in the region of 3000–3500 cm⁻¹ was observed, which corresponds to the valence vibrations of primary amino groups of amino acids, protonated amino groups, free hydroxyl groups [29], metal complexes of amino acids (3200–3400 cm⁻¹) [31]. In addition, a band at frequencies of 2933 cm⁻¹ appears in the spectrum of the complex, which corresponds to the fluctuations of the bound OH- groups [29], which may confirm their participation in the formation of the complex. The spectra lack absorption bands at frequencies of 1700–1760 cm⁻¹, which are characteristic of vibrations of the carboxyl group (-COOH), but there are peaks in the range of 1550–1650 cm⁻¹, which correspond to the vibrations of carboxylate anions (-COO⁻) [29].

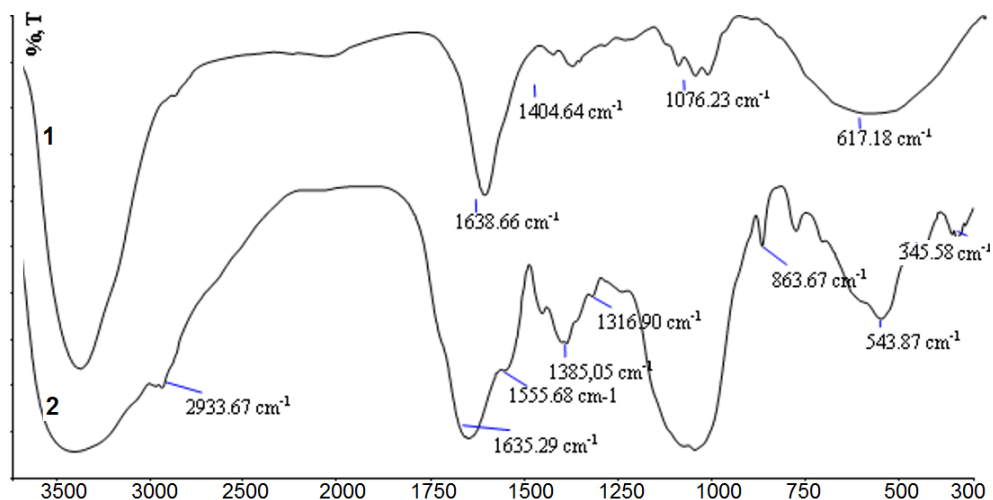


Figure 4. IR spectra of bioligands (1) and iron complex (2)

The presence of functional groups of bioligands in ionic form proves that electrostatic interactions can take part in the formation of the complex. In the spectrum of the complex, unlike the spectrum of bioligands, there is an absorption band at 1040 cm^{-1} . Its presence may indicate the formation of coordination bonds with amides [31]. In the low-frequency region of the complex spectrum there are bands (345 cm^{-1} , 540 cm^{-1}), corresponding to the fluctuations of the ferrum. Moreover, the absorption at frequencies of 540 cm^{-1} is somewhat offset compared to the inorganic forms of the ferrum, for which oscillations in the region $460\text{--}500\text{ cm}^{-1}$ are characteristic [31]. The IR spectra of the studied materials also show that metal ions interact with the lactic acid OH- group. For example, in the complex obtained there is a significant increase in the intensities of the peaks in the region of $1100\text{--}1160\text{ cm}^{-1}$, which indicates the coordination interaction of metal ions with the OH- groups [31].

Thus, since the spectra of the obtained metal complex, as compared to the IR spectrum of the starting material, show an increase in some absorption bands and the formation of new peaks, it can be assumed that the incorporation of ferric ions into the structure of bioligands does not occur mechanically, but as a result of ionic and coordination interactions with their functional groups.

Since chelate complexes of ferric ions with products of metabolism and processing of BM *Lactobacillus delbrueckii subsp. Bulgaricus* B-3964 is planned to be used as dietary supplements and biologically active food ingredients, it is advisable to study their behavior at different pH values (Figure 5) and temperatures (Figure 6).

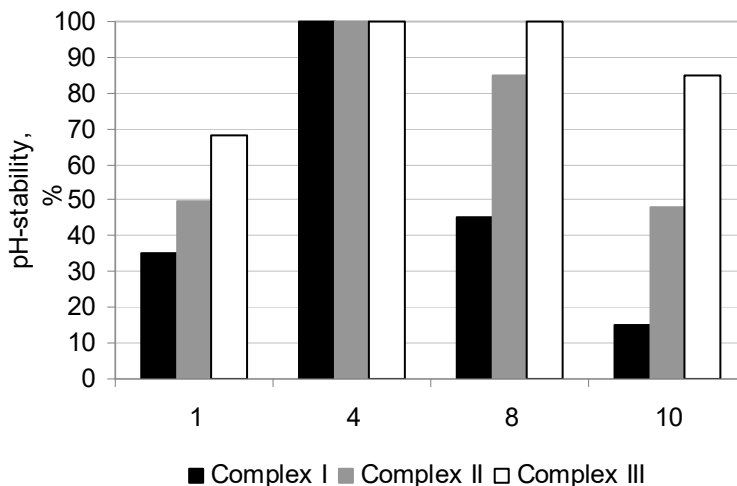
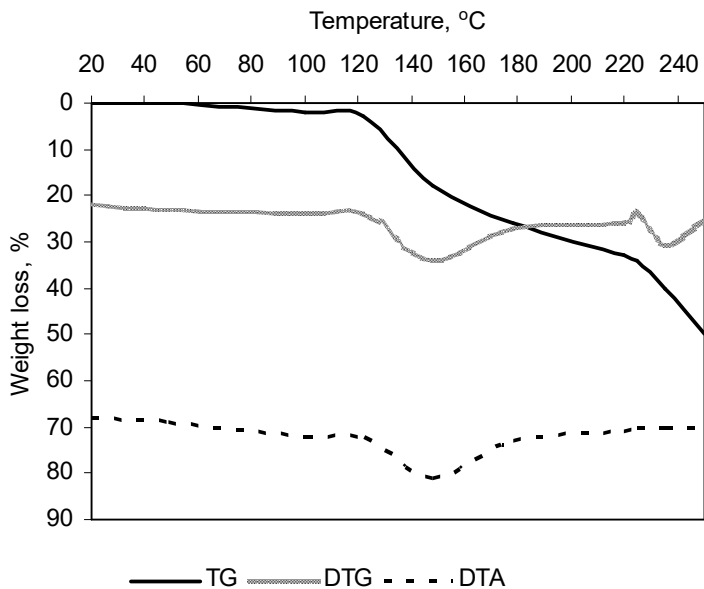


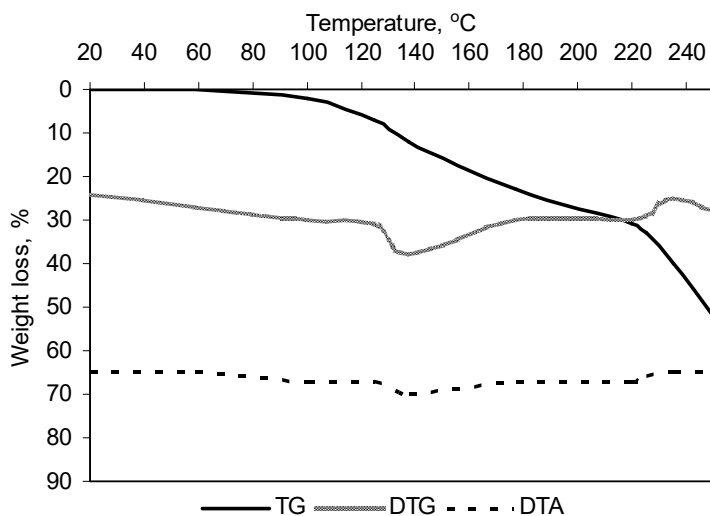
Figure 5. pH-stability of complexes

Based on the data of Figure 5, the complex, formed with the participation of degradation products of peptidoglycans and metabolites of BM, has the greatest stability at different pH values. According to the literature, the mixed ligand polydentate systems exactly can cause a stable “chelating effect”. Complexation of metals with polydentate ligands is more advantageous in terms of thermodynamics than monodentate ligands. The Fe^{3+} complexing agent has d^2sp^3 hybridization of atomic orbitals [30] and may cause the formation of an octahedral complex with bioligands, which also explains the considerable stability of the complexes in environments with different ion activity. The stability of the obtained complexes in the pH range of 8–10 allows us to predict the possibility of the presence of a ferric ion in the dissolved state in the small intestine, where its absorption by enterocytes occurs [2–3]. The stability of chelates at elevated pH values is also due to the competition between metal ions and proton per bioligand anion [30]. Therefore, the resulting chelate structures are stable in the range of pH values inherent in most food systems, which makes the prospect of their use as biologically active food ingredients.

To predict the behavior of the obtained chelate complexes in the composition of food systems that can be subjected to temperature treatment, their analysis was performed by the DSC method (Figure 6a, b). For this study Complex III and mechanical mixture of its components were used.



a



b

Figure 6. Termograms DSC:

a – complex; b – a mechanical mixture of complex components

TG is the thermogravimetric curve that characterizes the mass loss of the sample, depending on the temperature;

DTG is the curve of differential thermogravimetry based on the registration of the rate of change in mass with continuous heating, which is a more accurate interpretation of the TG curve;

DTA is the curve of differential thermal analysis, which serves to fix the presence of certain thermal effects.

In Figure 6a the curves TG, DTG, and DTA obtained from the DSC analysis of the complex are shown, and in Figure. 6b – the curves of the mechanical mixture (MM) of the complex components. When comparing the DSC analysis data, it can be stated that the initial weight loss of the samples begins at a temperature of 49 °C during the thermal treatment of the complex and at 59 °C during the thermal treatment of the MS. These figures show that the first mass loss is not accompanied by thermal effects, indicating that at these temperatures does not destroy the chelate bonds of the complex, which can provoke a change in the enthalpy of the process and the appearance of peaks in the DTA curves. Therefore, the first mass loss is associated with the removal of free moisture in the samples. When the temperature reaches 122–125 °C, the mass loss is 3% for the complex and 14% for the MM. In the temperature range of 122–178 °C, an endothermic reaction is observed during the thermal treatment of the complex, and no thermal effects are observed during the mechanical mixture treatment. The weight loss of the complex in this temperature range is 22%, MM – 16%. The presence of an endothermic peak on the DTA curve of the complex may indicate the presence in its structure of chelate bonds, and during their destruct the enthalpy changes are occurred.

Conclusions

1. The results of studies indicate the effectiveness of the use of polydendant mixed ligand systems of probiotic origin for complexation with ferric ions.
2. The study of the complex of iron (III) and bioligands of probiotic origin by IR spectroscopy showed that electrostatic and coordination interactions are involved in its formation.
3. According to studies, a system of bioligands containing the peptidoglycan degradation products and metabolites of lactobacilli, provides the formation of chelate complexes of ferric ions, stable in a wide range of pH environments (1-10 units).
4. The DSC method shows that the obtained complex is a promising ingredient of dietary supplements and wellness products whose technology involves high-temperature processing, since the complex is stable in the temperature range of 20–122 °C.
5. The presence in the composition of these complexes of low molecular weight muropeptides with high immunotropic activity, allows to classify these compounds to the category of polyfunctional.

The prospect of further research is to study the physiological activity of the resulting complex in animal experiment.

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Application of plant-based natural additives to improve the bioactive properties of organic artisanal cheeses

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Abstract

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Introduction. The aim of research was to increase the content of bioactive substances and improving antioxidative properties of organic artisanal cheeses by application the selected organic vegetable additives into manufacture of cheeses.

Materials and methods. In the produced organic cheeses containing selected organic vegetable additives the antioxidant properties were determined by analyzing the ferric reducing antioxidant power. Series of spectrophotometric measurement were performed to determine the content of polyphenolic substances and other selected bioactive components including: carotenoids, lycopene, chlorophyll, anthocyanins, flavonoids and betalains.

Results and discussion. The findings revealed that the antioxidant activity of the tested products ranged from 1.48 ± 0.11 μmol of Trolox/g (variant of control cheese after ripening) to 4.1 ± 0.3 μmol of Trolox/g (cheese containing tomatoes tested after ripening). In turn, the content of total phenolic compounds in cheeses immediately after ripening ranged from 141.51 ± 2.38 mg GAE/100 g (in the control variant) to 289.9 ± 9.9 mg GAE/100 g (in cheese with addition of dried tomatoes). The final products with the addition of broccoli and tomato after refrigerated storage exhibited the highest antioxidant properties in comparison to other tested products. Furthermore, after the maturation process total carotenoids content in the organic cheeses produced with organic carrot and tomato additives correspond to the results of the antioxidant activity assay. Moreover, cheeses containing the dried tomato additives were characterized by the highest acidity associated with the content of lactic acid after ripening (2.33 ± 0.02 g/100 g of cheese) and after refrigerated storage (2.51 ± 0.03 g/100 g of cheese); simultaneously, these products exhibited the highest values of total phenolic contents. The refrigerated storage of cheeses reduced the hardness and adhesion values only in the case of cheeses produced with the addition of onions, while the other variants showed an increase in this parameter after the end of the refrigerated storage. All vegetable additives contributed to an increase in the red color in the cheeses.

Conclusions. The tested additives increased the level of bioactive components and antioxidant properties of cheeses after ripening and also positively influenced on their color.

Introduction

Due to the fact that transparent packaging is very often used for packaging dairy products, the photo-oxidation reactions of cheeses have become a problem [1]. This issue concerns in particular artisanal organic cheeses, where the manufactured products most often remain unpackageged. An excessive oxidation induces undesirable changes in properties of cheeses including aroma, taste and alterations in external appearance. Moreover, oxidation of proteins and amino acids affects the formation of undesired off-flavour constituents [1]. However, some of the organic products possessing natural antioxidants that might protect from intensive oxidation processes (that are additionally strengthened by photo-oxidation) [2–5]. Also, there is currently a growing interest and demand for healthy cheeses [5–7], nonetheless a limited research is still available on the use of organic fruit and vegetable additives to fortify cheese products. Therefore, the objective of the investigation was to develop a natural method increasing content of bioactive substances and improving antioxidative properties of organic artisanal cheeses. The aim of research is also to evaluate the influence of application the selected, organic vegetables-derived additives on the changes of antioxidant activities and other physicochemical parameters of ripening cheeses. The investigation analyzed also the possibility of using the tested additives as a natural alternative to conventional cheese dyes and also functional components that might improve the health promoting characteristics of organic cheeses.

Materials and methods

Materials

Cow's milk purchased from a certified organic farm (No. PL-EKO-07-04210) from Lublin Province (Krupe, Poland) was used for production of ripened cheeses. The chemical composition of raw milk (g/100 g) was as follows: lactose 4.48, fat 3.45, protein 3.14 (before production, samples of milk were analyzed by infrared spectrophotometry using the MilkoScan 4000 apparatus according to Michaelsen et al. [6]. The average total number of microorganisms in the raw material samples estimated with the Fossomatic 5000 apparatus was below 100,000/ml, while the number of somatic cells was on average 282,000/ml. The hygienic quality of milk obtained from the organic farm and its technological suitability met the requirements for raw materials intended for the production of ripening cheeses.

Organic vegetables (with certificates confirming compliance with the requirements for organic raw material) purchased in organic food stores were used as additives in the production of cheeses (Table 1).

Table 1

List of plant raw materials

Raw plant material	Variety	Name of distribution company
Onion (<i>Allium cepa</i> L.)	Red Baron	Farma Świętokrzyska Sp. z o.o., Warsaw, Poland
Beetroot (<i>Beta vulgaris</i> L.)	Wodan	
Carrot (<i>Daucus carota</i> L.)	Nerac	
Tomato (<i>Lycopersicon esculentum</i> Mill.)	Jack	
Broccoli (<i>Brassica oleracea</i> L.)	Kronos	Przedsiębiorstwo Handlowo – Usługowe, Cedzyna, Poland

Fresh vegetables were individually pre-treated by washing, removing unnecessary parts, shredding (roots of carrots and beet – chips with a thickness of 2 mm, onions and fruits of tomatoes – cubes with sides of 5–7 mm, broccoli – florets not exceeding 15 mm), and blanching (except for tomato) at 95 °C/60 s. Afterwards, shredded plant material was subjected to convection drying (separately each type of vegetable) in a food dehydrator SFD 1205WH (Sencor, Poland) at 50 °C for 36 h.

The content of water (moisture) in all dried vegetables was determined in accordance with the method described by Lucera et al. [7].

Cheese manufacture

Freeze-dried heterofermentative culture Alpha 10 DL 3,5 (Ets A. COQUARD, Villefranche sur Saone, France) containing *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc cremoris*, and *Leuconostoc mesenteroides* was used for production of six variants of cheeses.

Each cheese variant was made with 10 L of pasteurized (74 °C, 5 min) milk supplemented with 2 ml of an aqueous CaCl₂ solution (40%) after cooling (33 °C). Afterwards, the starter culture was added to the milk according to the manufacturer's recommendation and incubated for 45 min. Subsequently, rennet (CHYMOGEN Premium Plus®, Chr. Hansen, Denmark) was added into the milk in a cheese vat (according to the manufacturer's instructions). After the coagulation process (33°C/35 min), when the proper consistency of the curd was obtained, the coagulum was cut into cubes (3 – 5 mm), left for 5 min at 33°C, and gently stirred for 15 min raising the temperature gradually (1 °C/2 min) up to 37 °C. Then, 40% of whey was removed and replaced with the same amount of water at the temperature of 35 °C. The cooking process (with constant stirring of cheese grains) was carried out until the acidity of the curd was pH 6.4–6.5. Then, the whey was drained off and a previously prepared portion of dried organic vegetables (onion, beetroot, broccoli, carrot, or tomato) was added to the cheese mass at a level of 5% (per 1 kg of cheese curd) and thoroughly mixed. Cheeses without any vegetable addition were the control variant. The cheese mass was divided into three equal portions (300 g), transferred into microperforated plastic (round) molds, and rotated every 45 min to drain off the whey effectively.

After molding, the cheeses were pressed at 18°C for 4.5 h by applying 2 kg/h pressure on the top of the round molds (13 x 7 cm) to the final load of 6 kg. Fresh cheeses were salted by immersion in saturated brine (18% NaCl, pH = 4.8; temp 13°C/6 h) and turned over every 45 min. Then, the cheeses were thoroughly dried (13°C/24 h), covered with a polyacetate coating, and transferred to the ripening room for 21 days (13°C/85% relative humidity). After this time, they were placed in a cold chamber (5°C) for six-week storage.

Determination content of selected bioactive components

The contents of selected biologically active substances (characteristic for each type of plant material) in the variants of cheeses containing one type of the tested vegetable additive were determined using the below-mentioned methods.

The lycopene content in cheeses (mg/100 g of product) that contained tomato supplementation was determined with the method described by Fish et al. [8].

The content of chlorophyll (a and b) in cheeses (mg/100 g of product) containing broccoli additive was analyzed according to protocol developed by Lichtenthaler and Buschmann [9]. In turn, the content of betalains in cheeses containing beetroot supplement was analyzed by using the method described by Gościńska et al. [10] involving the simultaneous determination of purple betacyanins and yellow betaxanthins.

The concentration of anthocyanins in variants of cheeses (mg/100 g of product) containing dried onion was determined using the protocol of quantitative methods for anthocyanins developed by Fuleki and Francis [11]. While, the content of flavonoids was analyzed according to Jia et al. [12]. The absorbance was determined (against blank) at 510 nm using Helios Gamma apparatus (Thermo Fisher Scientific, Waltham, MA, USA). The flavonoid content was calculated using the standard calibration curve (prepared with rutin solutions) and expressed in mg/100g of the tested product.

Determination of total phenolic contents in all variants of cheeses was performed according to Jabłońska-Ryś et al. [13]. The absorbance was measured by Helios Gamma apparatus (Thermo Fisher Scientific, Waltham, MA, USA) at $\lambda = 765$ nm. The results were expressed as mg gallic acid per 100 g of cheese.

The sum of carotenoids and β -carotene in all variants of cheeses was determined according to [14].

The titratable acidity was determined according to AOAC (Method 920.124) [15] and expressed as g lactic acid/100 g cheese.

All chemical analyses were performed in triplicate.

Determination antioxidant activities

The antioxidant activity of all produced variants of cheeses was determined by analyzing the ferric reducing antioxidant power (FRAP) of the tested samples to reduce according to the method described by Radzki et al. [16] with some modifications. In brief, the prepared FRAP reagent contained mixture of 300 mM acetate buffer (pH 3.6) with a 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) (Sigma-Aldrich) solution (10 mM TPTZ in 40 mM HCl), and a 20mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (at 10:1:1 ratio). The tested material was subjected to extraction by adding 30 mL of water (distilled, sterile) in to 1 g samples (and mixing in a shaker (Elpan 357; Elpan, Lubawa, Poland) at 50 °C and 5.000xg for 1 h. After centrifugation at 4,800xg for 15 min (MPW350-R; MPW) 100 μL of obtained clear supernatants were thoroughly mixed with 1.9 mL of FRAP reagent and incubated for at 37 °C 90 min in darkness. Then, the absorbance was measured at 593 nm applying the Helios Gamma apparatus (Thermo Fisher Scientific, Waltham, MA, USA). Ferric reducing antioxidant power was calculated using the calibration curve prepared with Trolox aqueous solutions. The results were expressed in Trolox μM or per 1 g of cheese (μM Trolox /g).

Texture profile analysis (TPA)

Measurements were performed with a TA-XT2i Texture Analyser (Stable Micro Systems, Godalming, UK) according to Sołowiej [17]. All analyzed cheese samples had the same size and shape (rolls 1.5 cm high and 1.5 cm wide) and were double compressed to 50% of deformation by a testing set (15 mm diameter). The compression rate was equal to 1 mm/s. The following texture parameters: hardness, adhesiveness, fracturability, cohesiveness, gumminess, and chewiness were evaluated.

Determination salt and fat contents

The salt concentration in the cheeses was determined with the Mohr method according to [18] and expressed as a percentage (%) of sodium chloride.

Determination of the total fat content was performed in accordance with ISO 1735:2004 [19] using a Soxtec Avanti 2055 device (Foss Tecator, Höganäs, Sweden).

Analysis of color parameters

Measurement of color parameters of the cheeses in the CIE L*a*b* system was performed according to Carini et al. [20] using an X-RiteColor 8200 colorimeter (X-Rite Inc.) and X-Rite Color Master software. For each variant of the product, the measurement was made over the entire surface of horizontally cut rings (Ø 12 cm, 5 cm thick) of cheese mass. Every sample was analyzed over the entire surface (evenly) in a ten-fold repetition (the measurements were collected from the surface of the cheese matrix). The L*, a*, and b* values (L*: brightness, a*: redness-greenness, b*: yellowness-blueness) were measured between 390 and 700 nm (D65 illuminant, 10° standard observer, Ø 13 mm port size) using a white standard with the following parameters: L* = 95.87, a* = -0.49, and b* = 2.39.

Statistical analysis

Statistical analysis was performed with the STATISTICA 13.1 program (StatSoft, Inc., USA). The results were presented as mean values with their standard deviations (mean±SD). The analysis of variance (ANOVA) was applied using Tukey's HSD test in order to estimate the significance of the differences between the mean values. The results were discussed based on a significance level set at P < 0.05.

Results and discussion

Content of selected bioactive components and determination of antioxidant activities

The content of selected biologically active compounds was determined in organic cheeses containing addition of dried vegetables (Table 2). The analyses were performed after ripening and after refrigerated storage (Table 3).

Table 2

Moisure level of the dried vegetables used in cheese production

	Type of dried vegetable				
	Onion	Broccoli	Beetroot	Carrot	Tomato
Moisure [%]	6.0± 0.01 ^a	7.6± 0.02 ^b	7.8± 0.03 ^b	8.1± 0.01 ^c	9.1± 0.03 ^d

Explanation notes: The results are given as mean values ± standard deviation ($\bar{x} \pm s/SD$; n = 3). The lowercase letters (a-d) express significant differences (P < 0.05) between values.

Carotenoids are biologically active substances with a high potential of positive effects on the human body. These compounds demonstrate a wide spectrum of health-promoting properties, especially in the prevention and alleviation of cardiovascular disease [21]. Therefore, the selected plant-based raw materials were applied in the production of organic cheeses as a source of the above-mentioned functional substances.

Lycopene is considered as an important component in prevention some cardiovascular diseases and formation of tumors and is estimated that in ripe tomatoes fruits accounts to 80-90% of the total pigment contents [22]. Therefore, high expectations are associated with the possibility of application this bioactive component in the prevention and therapy of prostate cancer [23].

Table 3

Comparison of antioxidant activities and the content of bioactive substances and other components in the variants of organic cheeses

Analyzed parameter/content of substances	Cheese variant	Stage of cheese production	
		After ripening	After refrigerated storage
Carotenoids [mg/100g]	Control	0.43±0.02 ^g	0.38±0.02 ^c
	With onion	0.42±0.03 ^{cd}	0.46±0.04 ^b
	With tomato	6.37±0.20 ^j	6.22±0.10 ^k
	With carrot	5.82±0.20 ⁱ	5.57±0.03 ^h
	With broccoli	0.94±0.05 ^{ef}	0.94±0.04 ^{de}
	With beetroot	0.42±0.02 ^{fg}	0.26±0.01 ^a
Lycopene [mg/100g]	With tomato	6.01±0.23 ^b	5.56±0.18 ^a
Chlorophyll A [mg/100g]	With broccoli	0.86±0.05 ^b	0.52±0.01 ^a
Chlorophyll B [mg/100g]	With broccoli	n.d.	n.d.
Anthocyanins [mg/100g]	With onion	7.71±0.44 ^b	6.61 ±0.16 ^a
Flavonoids [mg/100g]	With onion	12.84±0.22 ^b	9.77±0.80 ^a
Betalains [mg/100g]	With beetroot	0.25±0.03 ^a	0.22±0.02 ^a
Lactic acid [g/100 g cheese]	Control	1.37±0.03 ^a	1.91±0.03 ^{ef}
	With onion	1.92±0.00 ^f	2.07± 0.00 ^{gh}
	With tomato	2.33±0.02 ⁱ	2.51±0.03 ^j
	With carrot	1.85±0.00 ^{de}	1.83±0.03 ^d
	With broccoli	1.5±0.03 ^b	2.00±0.03 ^{fg}
	With beetroot	1.67±0.00 ^c	2.07±0.00 ^h
NaCl [%]	Control	0.73±0.02 ^a	0.74±0.00 ^a
	With onion	0.82±0.02 ^{ab}	0.82±0.02 ^{ab}
	With tomato	0.84 ±0.02 ^{bc}	0.91±0.02 ^{bcd}
	With carrot	0.92±0.00 ^{cd}	0.99±0.00 ^{ef}
	With broccoli	0.86±0.02 ^{bcd}	0.96±0.02 ^{def}
	With beetroot	0.95±0.02 ^{def}	1.03±0.02 ^f
Total fat [%]	Control	26.5±1.8 ^a	26.9±1.9 ^a
	With onion	29.7±2.1 ^a	30.2±2.1 ^a
	With beetroot	29.2±2.0 ^a	29.9±2.1 ^a
	With carrot	25.5±1.8 ^a	25.9±1.8 ^a
	With broccoli	26.8±1.9 ^a	28.1±2.0 ^a
	With tomato	28.7±2.0 ^a	29.8±2.1 ^a
TPC [mg GAE /100g of cheese]	Control	142±2.00 ^a	163±4.00 ^{abc}
	With onion	185±3.00 ^{cde}	157± 9.00 ^{ab}
	With tomato	290±10.00 ^f	294±13.00 ^f
	With carrot	166±7.00 ^{abc}	173.3±3.00 ^{bcd}
	With broccoli	170±7.00 ^{bcd}	200± ^{de} 14.00 ^e
	With beetroot	187±6.00 ^{cde}	194±12.00 ^{de}
FRAP [μmol Trolox/g]	Control	1.48±0.11 ^a	1.76±0.18 ^{ab}
	With onion	1.98±0.32 ^{abc}	1.55±0.22 ^a
	With tomato	4.1±0.30 ^f	2.7±0.10 ^{de}
	With carrot	2.50±0.10 ^{cd}	1.80±0.10 ^{ab}
	With broccoli	2.08±0.29 ^{abcd}	3.28±0.28 ^e
	With beetroot	2.25±0.20 ^{bcd}	2.03±0.28 ^{abcd}

Explanation notes: The results are given as mean values ± standard deviation ($\bar{x} \pm s/SD$; n =3). The lowercase letters (a-f) in the same row that presents the analyzed parameter in all the tested cheese variants (after ripening and after refrigerated storage) express significant differences (P <0.05); n.d.- not detected; TPC – Total phenolic contents; FRAP – Ferric reducing antioxidant power.

The application of vegetable additives such as carrot, tomato and broccoli contributed to enrichment of the organic cheeses with carotenoids (Table 3). After the refrigerated storage the highest content of carotenoids was observed in cheeses containing tomato and carrot additives. Moreover, the lycopene content was analyzed in the cheeses with the addition of tomatoes. The content of this bioactive compound in the cheeses after the ripening process (Table 3) reached the level of 6.01 mg/100 g, but after cool-storage this value slightly decreased (5.56 mg/100g).

It has been reported that milk from pasture feeding may be less vulnerable to light oxidation [24]. Furthermore, some authors have indicated that β -carotene may be a proper biomarker allowing distinguishing dairy products that derive from milk of animals fed in the pasture system from milk of animals subjected to other feeding conventions [25]. However, it is difficult to establish unequivocally that the antioxidant effect is caused only by β -carotene, since milk (especially that obtained from the pasture system) contains high levels of other components exhibiting antioxidant properties e.g. lactoferrin, vitamins C and E or tocopherols [24].

Carotenoids act as singlet oxygen scavengers exhibiting strong antioxidant properties, and β -carotene seems to be an important factor in the prevention of photo-oxidation mainly because it absorbs light depending on the concentration [24].

The results of the determination the total carotenoid content in the organic cheeses containing the carrot and tomato additives after the maturation process correspond to the results of the antioxidant activity assay (Table 3).

It is worth mentioning that tomato lycopene has been perceived as a natural food colorant that is effective in low concentrations, resistant to heat treatment, and stable at extreme pH values occurring in various stages of food processing [23]. Cheeses containing the dried tomato additive were characterized by the highest acidity associated with the content of lactic acid (2.33 ± 0.02 g/100 g of cheese after ripening and 2.51 ± 0.03 g/100 g of cheese after refrigerated storage); simultaneously, these products exhibited the highest values of total phenolic contents (Table 3).

It is suggested that lycopene extract from tomato can be used as a food/dietary supplement e.g. as an antioxidant in products thus contributing to their specific functional value [23]. This is consistent with the present results indicating that the highest antioxidant activity of all the variants of organic cheeses was exhibited (after the ripening period) by products containing the tomato addition (Table 3). Moreover, the intensive color of dried tomatoes and carrots as well as their taste and smell had a decisive influence on the choice of these dried materials in the production of ripening cheeses.

The content of chlorophyll A in cheese with the addition of broccoli after the maturation period reached 0.86 mg/100g; after the cooling storage period, this value decreased to 0.52 mg/100 g (Table 3). Interestingly, no chlorophyll B fraction was found in the ripened and cool-stored cheeses (this fraction was found only in dried broccoli), which suggests that the fraction may have been decomposed in the technological process of cheese production.

It has been suggested that the absence of blanching (as it happens with fermentation) as well as the enzymatic activity of lipases, lipoxygenases, and peroxidases contribute to changes in chlorophyll enhancing formation of pheophytins, which results in a decrease in the content of chlorophyll and development of brown color [26]. It corresponds to the present results, which indicate that the technology of organic cheese manufacture used (lactic acid fermentation) changes the chlorophyll content in products supplemented with broccoli. This negative effect may probably have been limited by blanching the raw material before further preparation thereof as a dried additive.

The content of anthocyanins and flavonoids was analyzed in cheeses with the addition of red onions. The content of anthocyanins in the ripened cheeses slightly decreased after the cooling storage and amounted to 6.61 mg/100 g. Similarly, the content of flavonoids decreased in the stored product (Table 3). Also, in cheeses containing dried red beetroot the content of betalain pigments decreased after storage (in comparison to samples of this cheeses variant collected after reining process) however the changes in these values were not statistically significant ($P>0.05$).

Obtained results indicate that the process of refrigerated storage contributes to the reduction of the content of some bioactive components used as natural cheese dyes.

It has been suggested the use of phenolic compounds as nutrients to enhance the functional properties of milk as well as various dairy products, including cheeses [27, 28] therefore, the obtained in the investigations results are relevant and have considerable practical application.

The application of the tested vegetable additives in cheese manufacture contributes significantly to an increase in the total phenolic content in ripened cheese (compared to the control cheese variant) (Table 3). A similar effect was described by Lucera et al. [7] who enriched spreadable cheese with flours from by-products derived from red and white grape pomace, broccoli, corn bran, and artichokes. They showed that the additives used influenced increased the content of flavonoids and TPC in the products.

The cheese storage had a varied effect on the content of phenolic compounds (Table 3). In case of the cheeses with broccoli and control products, a slight increase in the content of polyphenolic compounds was noted. In turn, a decrease in the total content of phenolic compounds was observed in the case of cheeses with the addition of onion. Moreover, the results indicate that the addition of vegetables did not result in significant differences in the fat content of the cheese (Table 3). A similar finding was reported in studies in which green tea extract was added to full fat cheese [29]. The addition of the extract significantly improved the antioxidant properties of the products (which is also consistent with the results of the antioxidant assay obtained for the cheeses at the end of the ripening process) without affecting the characteristics of the composition of the cheese.

The fat content in the cheeses analyzed directly after ripening ranged from 25.5% (for the cheese with dried carrots) to 29.7% (for the cheese with onion). While, after refrigerated storage, the fat content in the products was higher as a result of evaporation of part of the water in the product during this period. The highest fat content in the final product after the end of storage was determined in the cheese with the onion addition, while the lowest fat concentration was recorded for the carrot-supplemented cheese (Table 3). However, the differences between the products were not statistically significant ($P>0.05$).

The addition of the vegetables contributed to an increase in the NaCl content of the products (Table 3). The higher salt content recorded in the stored products was most probably related to biochemical conversions (including proteolysis) and intensified water loss (compared to the first analyzed stage of cheese production) contributing to a higher concentration and diffusion of NaCl in the cheese mass.

Determination of the antioxidant potential (Table 3) measured by FRAP indicated that the antioxidant activity of the tested products ranged from 1.48 ± 0.11 μmol of Trolox/g (variant of the control ripened cheese) to 4.1 ± 0.3 μmol of Trolox/g (ripened cheese containing tomatoes).

The results of the analyses showed that each of the applied vegetable additives contributed to an increase in the antioxidant properties of the cheese products. Similar effects were demonstrated by Lee et al. [28], who reported higher antioxidant properties of Cheddar-type cheese fortified with an *Inula britannica* flower extract.

The antioxidant properties of the ripened cheeses were highly correlated with the content of total phenolic compounds ($R=0.94$; $p=0.05$). This is in agreement with findings described by Lee et al. [28] suggesting that the scavenging activity of cheeses increased proportionally to the total phenolic content. While in case of the cool-stored cheeses, the correlation between total content of phenolic compounds and antioxidant activity was lower and it was not statistically significant (i.e. $R=0.6$; $p=0.05$). A slight decrease in the antioxidant activity was observed in cheeses supplemented with tomatoes, onions, and carrots, whereas increased values of bioactivity was noted for cheeses with the broccoli addition (Table 3).

The results showed that the variants of organic cheeses exhibited antioxidant properties. This corresponds to the findings described by Han et al. [27] suggesting that supplementation of cheese curd with some plant additives e.g. whole grape extract, green tea extract, or dehydrated cranberry powder, which contain a wide range of bioactive compounds (including polyphenols), may significantly enhance the antioxidant properties of final cheese products.

Texture profile analysis (TPA)

The analysis of the TPA results showed differences among the tested cheeses in terms of textural parameters (Table 4). The highest hardness after ripening and refrigerating storage was observed for cheeses obtained with the addition of dried onion, whereas the lowest values of this texture parameter were recorded for the carrot-supplemented cheeses.

The refrigerated storage reduced the hardness and adhesion values only in the case of cheeses produced with the addition of onions, while the other variants showed an increase in this parameter after the end of the refrigerated storage.

The results of research performed by some authors indicate that hardness of cheeses increases together with the time of ripening [30], while others have observed an opposite tendency [31,32]. Whereas, Delgado et al. [33] indicated that hardness and adhesiveness significantly increased during ripening of raw goat milk cheeses (Ibores cheese), which may be explained by the progressive loss of moisture in the process of maturation. Similarly, Pinho et al. [34] indicated a decrease in hardness during ripening of Terrincho raw ewe milk cheese. The decrease in the hardness parameter in samples of cheeses supplemented with carrots or onions observed in our research corresponds to the results obtained by Van Hekken et al. [35], who analyzed Monterey Jack goat milk cheese and analyses of Torta del Casar raw ewe milk cheese described by Delgado et al. [36].

We observed that the refrigerated storage reduced the fracturability of the control cheeses as well as products with addition of onions or tomatoes (Table 4). The other variants of the final products analyzed after the refrigeration storage showed an increase in the value of this texture parameter. Moreover, the refrigeration storage reduced the values of springiness, cohesiveness, and chewiness of all the organic cheeses with the exception of products containing dried onions, which exhibited a higher cohesiveness value after the refrigerated storage. Similar findings have been described by Delgado et al. [33], who noted a significant decrease in the values of cohesiveness and springiness up to day 60 of Ibores cheese maturation. This is apparently connected with the fact that cohesiveness decreased through breakage of the casein network, while the lower values of springiness were a consequence of moisture loss and an increase in the fat concentration in the cheese matrix [37]. Moreover, a decrease in cohesiveness and springiness might also be a result of increased content of polypeptide nitrogen with simultaneous reduction of casein nitrogen occurring in the process of cheese maturation [33].

Table 4

Comparison of the texture parameters of cheese variants analyzed after the ripening process and in the final products.

Cheese variant	Stage of cheese production	Texture parameter						
		Hardness [g]	Fracturability [g]	Adhesiveness [J]	Springiness	Cohesiveness	Gumminess [g]	Chewiness [g]
Control	AR	1061.5 ±62.2 ^c	7.68 ±0.20 ^l	17.9 ±4.3 ^a	0.454 ±0.033 ^{ef}	0.244 ±0.029 ^f	254.8 ±24.5 ^f	115.8 ±15.1 ^g
	ARS	1158.7 ±109.1 ^d	6.30 ±1.18 ^j	22.8 ±7.5 ^c	0.327 ±0.014 ^{bc}	0.170 ±0.014 ^{abc}	192.5 ±3.9 ^c	61.7 ±4.5 ^c
With onion	AR	1893.9 ±138.5 ^l	5.02 ±0.67 ^e	59.7 ±13.6 ^h	0.585 ±0.052 ^h	0.179 ±0.013 ^{bcd}	255.9 ±25.2 ^g	149.7 ±15.3 ^k
	ARS	1768.6 ±131.6 ^k	4.51 ±0.53 ^d	38.7 ±12.4 ^f	0.435 ±0.025 ^{de}	0.192 ±0.034 ^{de}	331.5 ±76.1 ^k	128.6 ±30.1 ^h
With beetroot	AR	1183.6 ±136.2 ^c	3.77 ±0.61 ^b	38.8 ±7.6 ^g	0.491 ±0.061 ^g	0.200 ±0.019 ^{de}	225.1 ±34.9 ^c	109.4 ±19.7 ^f
	ARS	1642.9 ±174.5 ^j	6.22 ±0.70 ⁱ	110.7 ±14.9 ^j	0.341 ±0.039 ^{bc}	0.162 ±0.019 ^{ab}	224.1 ±83.2 ^d	99.4 ±20.4 ^d
With carrot	AR	1412.1 ±49.1 ^h	4.04 ±1.03 ^c	18.4 ±6.6 ^b	0.409 ±0.019 ^d	0.209 ±0.031 ^c	255.9 ±49.8 ^g	143.9 ±28.4 ^j
	ARS	1399.1 ±142.7 ^g	5.36 ±0.72 ^f	35.2 ±6.3 ^e	0.406 ±0.064 ^d	0.195 ±0.019 ^{cde}	315.4 ±83.2 ⁱ	138.1 ±31.8 ⁱ
With broccoli	AR	1283.7 ±60.5 ^f	6.06 ±0.95 ^h	27.9 ±2.0 ^d	0.477 ±0.011 ^{fg}	0.253 ±0.001 ^f	318.4 ±22.1 ^j	152.1 ±13.7 ^l
	ARS	1416.3 ±277.5 ⁱ	7.07 ±0.87 ^k	117.9 ±24.4 ^k	0.352 ±0.069 ^c	0.181 ±0.011 ^{bede}	274.9 ±62.5 ^h	105.5 ±7.9 ^e
With tomato	AR	882.3 ±137.2 ^b	5.79 ±0.53 ^g	106.8 ±3.2 ⁱ	0.314 ±0.036 ^{ab}	0.166 ±0.004 ^{abc}	134.6 ±14.1 ^a	44.3 ±6.9 ^a
	ARS	983.3 ±115.4 ^a	2.82 ±0.41 ^a	142.1 ±11.9 ^l	0.288 ±0.059 ^a	0.146 ±0.007 ^a	140.2 ±22.8 ^b	44.4 ±9.4 ^b

Explanation notes: AR- cheeses analyzed directly after finishing the ripening process; ARS- final products analyzed after refrigerated storage; The parameters were calculated from texture profile analysis (TPA). The results are given as mean values ± standard deviation ($\bar{x} \pm s/SD$; n = 6). Means followed by different lowercase letters (a-l) in the same column (texture parameter referring to all tested cheeses variants after ripening and after refrigerated storage) denote significant differences (P < 0.05).

Changes in gumminess were noticed after the ripening and refrigerated storage period. The cold storage reduced the values of this texture parameter in the control cheeses and the broccoli-supplemented products, whereas an increase in the values of this parameter was noted in the other tested variants.

The differences in the texture of the final products can be attributed to the different properties of the additives influencing the activity of the starter culture, which was reflected in further biochemical processes occurring during maturation and refrigerated storage.

Analysis of color parameters

The results of the instrumental analysis of the color of the cheeses are presented in the Table 5.

The L* parameter determines the brightness of the tested samples (the higher the value of this parameter, the lighter the color of the sample). In the analyzed cheeses, the L* parameter ranged from 46.38 (cheese with beetroot) to 83.74 (cheese without additives – control). After the period of cold storage, the value of this parameter decreased in all the samples except for the cheese with carrots, for which a slight increase in the value of L* was observed. An opposite tendency was reported by Delgado et al. [33] in Ibores cheese during ripening (samples were analyzed on maturation days 1, 30, 60, and 90), in which the lightness parameter (CIE L*) significantly increased throughout the ripening process. However, these differences might be related to the differences in the specificity of goat's and cow's milk and the longer period of Ibores cheese ripening, where proteolysis was more intensive.

The present results showed that the use of the vegetable additives decreased the L* parameter. A similar effect was observed in studies conducted by Golmakani et al. [38], who assessed the effects of Spirulina concentrations on the survival of *L. casei* strains in bacteriologically acidified feta-type (BAF) cheese. They indicated that the addition of Spirulina significantly reduced the brightness of the cheese; moreover, the L* values decreased by increasing the Spirulina concentration.

The a* parameter reflects color changes in the scale from green (negative values) to red. The highest red color value was noted for the control cheese samples (4.00) and the beetroot-containing cheeses (21.96) after the process of maturation. All vegetable additives contributed to an increase in the red color in the cheeses. The process of refrigerated storage of the control cheeses contributed to a slight increase in the value of the a* parameter in these products. In addition, increased values of this parameter were also observed in samples of cheeses supplemented with red onion or tomato, while a decrease in the a* parameter value was recorded in the other samples.

It has been reported that the β -carotene (belonging to carotenoids group) level in dairy products is correlated with the yellow color [24], which is in agreement with the present results, especially in the case of the tomato- or carrot-supplemented products (Table 3 and Table 5).

The b* parameter determines the color changes from blue (negative values) to yellow. In all cheeses with vegetable additives, except for the cheese with the beetroot addition, the values of the b* parameter were similar to the values noted for the control product and ranged from 29.02 (carrot-supplemented cheese) to 30.68 (broccoli-containing cheese). In turn, the beetroot-supplemented cheese was characterized by blue color (-5.69).

These results indicate that the process of refrigerated storage induced a slight change in the value of the b* parameter in most of the analyzed samples. An increase in the proportion of yellow color was observed in the stored control samples and cheeses with the beetroot and tomato additives, whereas reduced values of this parameter were observed in cheeses containing onion, carrot, and broccoli.

Table 5
Comparison of the color parameters in the variants of cheeses analyzed after the ripening process and refrigerated storage

Cheese variant	Stage of cheese production	Color parameter		
		L*	a*	b*
Control	After ripening	83.74±1.27 ^k	4.00±0.39 ^b	29.39±2.19 ^g
	After refrigerated storage	79.5±0.21 ⁱ	4.89±0.08 ^d	31.35±0.36 ^j
With onion	After ripening	78.17±3.77 ^h	5.11±0.39 ^c	29.95±5.59 ^h
	After refrigerated storage	77.63±0.5 ^g	6.61±0.69 ^f	27.03±0.8 ^d
With beetroot	After ripening	46.38±2.96 ^b	21.96±2.28 ^k	-5.69±1.52 ^a
	After refrigerated storage	41.92±0.71 ^a	19.07±2.96 ^j	2.04±4.19 ^b
With carrot	After ripening	75.9±1.86 ^f	11.97±3.39 ⁱ	29.02±2.36 ^c
	After refrigerated storage	77.63±0.5 ^g	6.61±0.69 ^f	27.03±0.8 ^d
With broccoli	After ripening	73.07±2.5 ^e	4.63±1.46 ^c	30.68±3.61 ⁱ
	After refrigerated storage	69.1±2.58 ^d	2.62±0.55 ^a	25.66±1.64 ^c
With tomato	After ripening	81.48±4.47 ^j	7.1±2.54 ^g	29.23±2.44 ^f
	After refrigerated storage	68.8±1.45 ^c	11.38±0.9 ^h	32.19±0.8 ^k

Explanation notes: The results are given as mean values ± standard deviation (x±s/SD; n =10). Means followed by different lowercase letters (a-k) in the same column (color parameter) denote significant differences (p <0.05).

Conclusion

1. The applied vegetable additives had a varied effect on cheese texture parameters. The highest hardness after ripening and refrigerating storage was observed for cheeses obtained with the addition of dried onion, whereas the lowest values of this texture parameter were recorded for the carrot-supplemented cheeses.
2. The used plant-derived additives significantly increased the level of bioactive components and enhanced the antioxidant properties of cheeses after process of ripening.
3. After refrigerated storage the highest antioxidant properties exhibited the cheeses produced with the addition of broccoli and tomato.
4. The tested vegetable-derived additives contributed to increase in the red color of cheeses mass, all vegetable additives (especially carrot, tomato and beetroot) had a positive effect on the colouring of the cheese mass exhibiting a potential as an alternative to conventional cheese dyes.

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Influence of processing parameters on the techno-functional properties of berry coagulants

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Abstract

Keywords:

Milk
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Introduction. The aim of the research is to determine the influence of the parameters of hydrothermal treatment and rubbing of berry raw materials on the content of soluble pectins in berry purees. Attention is paid to the compliance of the purees with the requirements for berry coagulants, which can be used to obtain milk-protein co-precipitates from the buttermilk.

Materials and methods. Fresh cranberries and viburnum berries were chosen as the subjects of study. The treatment of the berries with acute vapor was made with the help of the electric steam-convection oven of the injector type at a temperature of 105–107 °C during 5–60 seconds. The berries rubbing was made with the help of a crushing-rubbing machine using sieves with hole diameters of 0.4–1.2 mm.

Results and discussion. The content of pectin substances in both berry purees increases in direct proportion to the extension of hydrothermal treatment from 5 s to 60 s. The viburnum puree is characterized by the slower increase in the amount of pectin substances comparing to the cranberry puree, which can be explained by the difference in the structure of protopectin molecules.

The losses of vitamin C for both berry purees are relatively low in the first 15 s of treatment, while then they are increasing rapidly due to the acceleration of hydroxyl bonds breaking in the ascorbic acid structure.

Processing berry raw materials with an acute vapor is considered rational at a temperature of 105–107 °C for 15–30 seconds for cranberries and 5–15 seconds for viburnum berries, which corresponds to an increase in the amount of pectin substances on 19–22% and 11–19% in cranberry and viburnum purees respectively.

The largest outcome of cranberry puree was achieved using a sieve with a holes diameter of 0,8–1,2 mm, whereas for the production of viburnum puree it is rational to use a sieve with a holes diameter of 0.6–0.8 mm. The need to use sieves with larger holes to increase the yield of cranberry puree can be explained by the more pronounced susceptibility of the coarse viburnum fibre towards mechanical stress compared to the finer cranberry fibre with regard to particle size reduction.

The chemical composition of obtained purees comparing to the berry raw materials is characterized by an increase in the content of pectin substances by 20 and 21%, and a decrease in the content of vitamin C by 30 and 29 for cranberry and viburnum purees, respectively. The obtained coagulants are characterized by an organic acid content of 2.0 and 1.7% for cranberry and viburnum purees, respectively.

Conclusions. The significant increase in the content of soluble pectins and the presence of organic acids allow to use the obtained berry purees as coagulants in the technology of milk-protein co-precipitates from the buttermilk.

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Introduction

In the global market current conditions the demand for protein and protein-rich foods, in particular those obtained using dairy protein concentrates, is spreading steadily. This tendency is explained by a wide range of properties of milk proteins, in particular, the proximity of their amino acid composition to the proteins of human body tissues, which indicates their ability to meet the needs of human body more quickly and fully [1].

Despite the variety of methods used for protein precipitation, a large part of the obtained concentrates contains only casein fraction of milk proteins or is produced using various chemical substances of artificial origin during precipitation [2]. As a result, they are characterized by a number of organoleptic deficiencies, not to mention the content of substances for the utilization of which the enzyme system is not genetically foreseed. The exceptions are presented only by microparticulates obtained using membrane technologies, but the equipment needed for their obtaining is very complex and can not be applied in the establishments of restaurant industry [1–3].

The disadvantages given above indicate the need to improve the existing technologies of proteins coagulation for the purpose of reaching the appropriate harmonization of the obtained concentrates quality with international benefits to the foods that can be used in the enterprises of restaurant industry. Therefore, an urgent and promising task today is the research of ways of using natural domestic raw materials which will enable obtaining of protein substances by precipitation methods and will lead to the production of milk-protein concentrates with new techno-functional and consumer properties.

Literature analysis

Nowdays, the development of a new method of protein substances precipitation will be considered expedient when it increases its effectiveness compared to traditional methods of protein concentration. It is possible to achieve the efficiency of the new technology by reducing the amount of raw materials, energy or technical resources required for the production process. This causes the need for modification of existing technologies by introducing additional factors that can reveal the potential of techno-functional properties of dairy proteins.

In recent years, a number of improved methods for milk-protein concentrates obtaining was proposed, and each of this methods included the modernization of separate stages of thermoacid and thermocalcium coagulation or membrane methods [4–8]. In our opinion, the most expedient component capable to accelerate and simplify the concentration of milk proteins is the use of natural plant products or components that are characterized by rich chemical composition, sorption and radioprotective properties [9–11].

However, it should be noted that the current world and national experience of the use of dietary supplements in nutritional diets indicates a lack of efficiency in the use of the potential of food plant raw material components, not to mention a limited number of studies on the use of plant components to stabilize the processes of protein precipitation [12–14].

An additional factor in improving the efficiency of innovation technology is the use of available domestic raw materials. From this perspective, it is expedient to use buttermilk as the main raw material, as it contains almost the entire protein, carbohydrate and mineral complex of whole milk and is characterized by significant volumes of production within the country. As a plant material it is expedient to use cranberries and viburnum berries, which, unlike food additives, synthesized in an industrial way, are characterized by low cost and high content of different Biologically Active Substances, necessary for maintenance of

normal homeostasis, satisfaction of energetic and plastic needs of the human organism, as well as reduce the risk of diseases caused by oxidative stress, such as cancer and cardiovascular diseases [9, 15].

In the proposed technology of protein coagulation [16] it is expedient to use cranberries and viburnum berries as coagulants, additional coagulation centers and source of structure stabilizing and enrichment components. In the first case, the plant material is considered as a source of organic acids that can regulate pH and, accordingly, provoke the coagulation and denaturation of protein substances. This determines the need to ensure the use of the maximum content of organic acids of berry raw materials and to investigate the optimal amount of berry component required by the system to achieve pH, neighbor to the isoelectric point of dairy proteins.

Regarding the influence of the berry pectinates on the structural characteristics of received products, it should be noted that for today mixtures of biopolymers, in particular proteins and polysaccharides, are widely used to stabilize foam and emulsions, which in most cases are unstable polydisperse systems due to the large surface of the disperse phases [17]. In this context, the mechanism for increasing the stability of named disperse systems is based on the creation of protective layers around the emulsion drops or foam bubbles by biopolymers.

It has been experimentally established [18] that the long-term stability of the emulsions and the foam is increased if additional polysaccharides, capable of creating a spatial structure of the dispersion medium, are added to the proteins. That strengthens the disperse phase and prevents its destruction. Hence the need for transferring of the pectin substances of berry raw materials into a soluble state. This will cause the activation of their techno-functional properties and the provision of the necessary conditions for the creation of complexes, which becomes possible with additional technological processing of berries.

The dispersed state of plant materials, as a component of the dispersion medium of foam-emulsion systems, plays a significant role in the formation of organoleptic parameters of the final product [19]. To facilitate the whipping process, to prevent the coalescence of air bubbles and, as a consequence, the formation of a subjective perception of the whipped products «velvety», it is advisable to ensure the homogeneity of the system components structure, which requires the most diligent grinding of plant materials. In this case, it is necessary to preserve the maximum amount of Biologically Active Substances of fresh raw materials, in particular organic acids and pectin substances. Similar studies were done in the work [13], where the technological parameters of protein substances precipitation of skim milk were substantiated and determined due to the effect on them of the acids contained in purees of cornel and blackthorn berries treated in a special way and addition of 0.9% of the phosphates mixture «Biofos 90».

Analyzing the presented data, it can be concluded that in order to effectively use pectin substances in the process of proteins precipitation in order to improve the structural-mechanical and techno-functional properties of received products, it is expedient to finely shred plant materials till the state of berry puree. In addition, considerable attention needs to be paid to the study of ways to increase the content of soluble pectins in purees.

Taking into account the aforementioned, the **aim** of research is to determine the influence of parameters of acute vapor processing and rubbing of the berry raw materials on the content of soluble pectins in berry purees. Attention is paid to the compliance of the obtained puree with the requirements for berry coagulants, which can be used to obtain milk-protein co-precipitates from the buttermilk.

Indicated aim can be achieved through a number of **tasks**, namely:

- Study of the chemical composition of the berry raw materials (cranberries and viburnum), in particular the content of organic acids and pectin substances
- Determination of influence of the technological parameters of processing on the properties of berry purees;
- Justification of the rational method and modes of plant raw materials processing.

Materials and methods

Materials

Raw materials. Fresh cranberries and viburnum berries harvested in the Obukhiv and Vasylykivsky districts of the Kyiv region in 2015–2016 were chosen as the subjects of study.

Methods

Study of chemical composition of berry raw materials and purees

Mass fraction of water. The mass fraction of water was determined by drying the samples to constant weight at a temperature of 105–110 °C (AOAC 7.003) [20].

Protein content. Nitrogen content was determined by the Kjeldahl method, followed by conversion to crude protein, conversion factor 6.25 (AOAC 2.057) [20].

Fat content. Study of fat content in berry raw materials and purees was determined by Soxhlet method [21].

Crude fiber. The samples for carbohydrates investigation were prepared by rapid grinding of berries, adding weighed samples to hot alcohol and H₂O and successive heating on H₂O bath during 30 min, stirring frequently (AOAC 3.002 b) [20]. The content of crude fiber was determined by the weight method after successive boiling of the analyzed weight in the medium of sulfuric acid and sodium hydroxide with following drying [22].

Soluble sugars. The content of soluble sugars was determined by the titrimetry method by Bertrand [23].

Pectin substances. The content of pectin substances in berry purees was determined by the titrimetry method based on the titration of the alkaline pre-selected and prepared pectin substances before and after hydrolysis [24].

Mineral constituents. The samples for mineral constituents investigation were prepared by oven drying and grinding (AOAC 3.002 a) [20]. The mineral constituents were determined by X-ray fluorescence method using a spectrophotometer SF-2000 (AOAC 3.015, 3.019, 3.065) [20].

Organic acids. The content of organic acids was determined by Bitartrate method (AOAC 22.062) [19] in recalculation on the malic acid.

Polyphenols. The content of polyphenols was determined by a method based on the extraction of colored substances with concentrated hydrochloric acid and the optical determination of their concentration in the samples in comparison with a standard solution of cobalt sulfate [24].

Study of vitamin C content in berry raw materials and purees

The content of vitamin C was determined by the Indophenol Titration Method. 50 cm³ of berry puree was pipette into 100 cm³ volumetric flask. 25 cm³ of 20% acetic acid was added as stabilizing agent and was diluted to 100 cm³. 10cm³ was pipette into a conical flask, and 2,5 cm³ acetone was added. It was titrated with 2, 6-Dichloroindophenol (DCIP) Standard solution. A faint pink colour that was persisted for about 15 seconds was observed. The amount of dye used in the titration was determined volumetrically and used in the calculation of the vitamin C content mg/ 100 ml in the berry puree samples [25].

Technology of berry raw material processing into purees

The proposed method of berry raw materials processing should include the following technological operations:

1. Inspection;
2. Washing;
3. Hydrothermal treatment – processing with acute vapor at the temperature of 105–107 °C during 5–60 seconds;
4. Rubbing with the help of a crushing-rubbing machine.

Study of the influence of parameters of acute vapor processing on the raw materials chemical composition

The treatment of the berries with acute vapor was made with the help of the electric steam-convection oven of the injector type «Convotherm 4 EasyTouch 6.10 ES». The analysis were made in the «wet air» mode at a temperature of 105–107 °C during 5–60 seconds.

The berry treatment involved two stages: the first stage – in which a layer of berries was heated to sufficient temperature and the second stage – held to allow the temperature at the centre of each piece to increase to that needed for conversion of pectin substances into a soluble state.

Determination of berry rubbing parameters

The berries rubbing was made with the help of a crushing-rubbing machine with a speed of 1500 rpm at the step and the height of teeth in a crusher of 2.5 mm.

Experimental researches for berry purees were made using sieves with hole diameters of 0.4; 0.6; 0.8; 1.0 and 1.2 mm.

Statistical analysis

Data were expressed as means ± standard deviations. All laboratory studies and experiments were conducted with a fivefold repetition. The obtained data are given in the unit of the international SI system.

Statistical analysis was performed using Microsoft Excel 2010.

Results and discussion

Chemical composition of berry raw materials

At the first stage, the chemical composition of berry raw materials was investigated. The study was conducted because the chemical composition of the berry raw materials depends on many factors [23, 25–27], in particular climatic conditions and the place of growth (Table 1).

Based on the data obtained, it can be stated that cranberries and viburnum berries are the valuable source of macro- and micronutrients necessary for human beings, such as mono- and polysaccharides, dietary fibers, proteins, organic acids and pectin substances.

Generally, the highest share in total solids of berries is contributed by carbohydrates, i.e. sugars. Organic acids, together with sugars, play an important role in the sensory characteristics of berries. The results show that higher sugar-to-acid ratio among studied berries had cranberry, when viburnum is characterized by lower content of this substances.

Table 1

Chemical composition of berry raw materials, g/100 g of berries (n = 5, P ≤ 0,05)

Name of the indicator	Content in berries	
	Cranberry	Viburnum
Water	85.2	86.1
Proteins	0.4	0.3
Fats	0.1	0.2
Including pufas	-	0.1
Carbohydrates	10.2	8.8
Including sugars	4.1	2.7
dietary fibers	3.8	3.2
pectin substances	1.0	1.4
Ash	0.1	0.1
Organic acids (in recalculation on the malic acid)	2.1	1.8
Polyphenols	0.5	0.8
Vitamin c, mg	13.3	12.7
Calcium, mg	8.2	7.4
Potassium, mg	85.0	34.2
Ferum, mg	0.3	0.3
Phosphorus, mg	13.0	0.3

The content of soluble pectin substances in cranberries is 1.0, which is 0.4 g lower then in viburnum berries. This result can be explained by the firmness of cranberries comparing to the viburnum berries.

The organically bound mineral elements in the studied berries are presented by Ferum, Calcium, Potassium and Phosphorus, and the vitamin content is high in Vitamin C. Between the other substances such polyphenolic compounds as anthocyanins, leucoanthocyanins, catechins, flavonols and phenoxy acids can be noted, which indicates the high antioxidant properties of the studied berries.

Influence of the time of processing with an acute vapor on the chemical composition of raw materials

Hydrothermal treatment of berry raw materials with acute vapor was made with the purpose of giving the determined techno-functional properties to the final product and ensuring its protection against microbiological contamination.

The key part in the obtaining of the desired techno-functional properties of berry purees plays the conversion of pectin substances into a soluble state. This transformation can be explained by the fact that pectin substances of studied berries involve high amount of protopectin – an insoluble high-molecular-weight pectin complex which gives soluble pectin when treated with high temperature [28].

In addition, this method of treatment was aimed on the other technological tasks as well: reducing microbiological contamination, inactivating enzymes and facilitating subsequent rubbing.

The literature analysis [29–30] defined that the most rational was the hydrothermal method of berry raw materials processing. It allows not only to satisfy the conditions for the technological tasks implementation, but also to preserve the maximum amount of valuable nutrients of berries. At the same time it is expedient to do the hydrothermal treatment of berries at a temperature of at least 80–85 °C for the conversion of pectin substances into a soluble state, and to maintain microbiological purity using a temperature of 95–100 °C.

Regarding the duration of hydrothermal treatment, it is well known that its increase contributes to significant losses of thermally unstable nutrients of berry raw materials. Therefore, the next step was to study the effect of the duration of raw materials processing on the content of target components in berry purees. The evaluation of the quality of the obtained purees was made by determining the content of pectin substances and ascorbic acid, as the most thermoplastic vitamin, in them (Figure 1, 2).

According to the data of the Figure 1, the content of pectin substances in both berry purees increases in direct proportion to the extension of hydrothermal treatment from 5 s to 60 s.

With the further processing the increase in the amount of pectin substances is rather slow, but the content of vitamin C has a marked tendency to decrease because of its sensibility to high temperatures (Figure 2). This occurs due to the high temperature effect on vitamin C which is thermally labile. Therefore, it was decided to limit the time interval to 60 s during the studies.

The hydrothermal treatment of viburnum puree is characterized by the slower increase in the amount of pectin substances comparing to the cranberry puree (Figure 2), which can be explained by the difference in the chemical composition, i.e. difference in the structure of protopectin molecules.

The losses of vitamin C (Figure 2) for both berry purees are relatively low only in the first 15 s of treatment, while then they are increasing rapidly. Such dynamics is explained by the fact that temperature increase accelerates the break of hydroxyl bond in the ascorbic acid structure, thus enlarging rate of its destruction [24].

As prolonging the berries processing time will reduce the vitamin C content in the berry purees, in our opinion, it is rational to treat berry raw materials with an acute vapor at a temperature of 105–107 °C for 15–30 seconds for cranberries and 5–15 seconds for viburnum berries, which corresponds to an increase in the amount of pectin substances on 19–22% and 11–19% in cranberry and viburnum purees respectively, and can prevent significant loss of vitamin C.

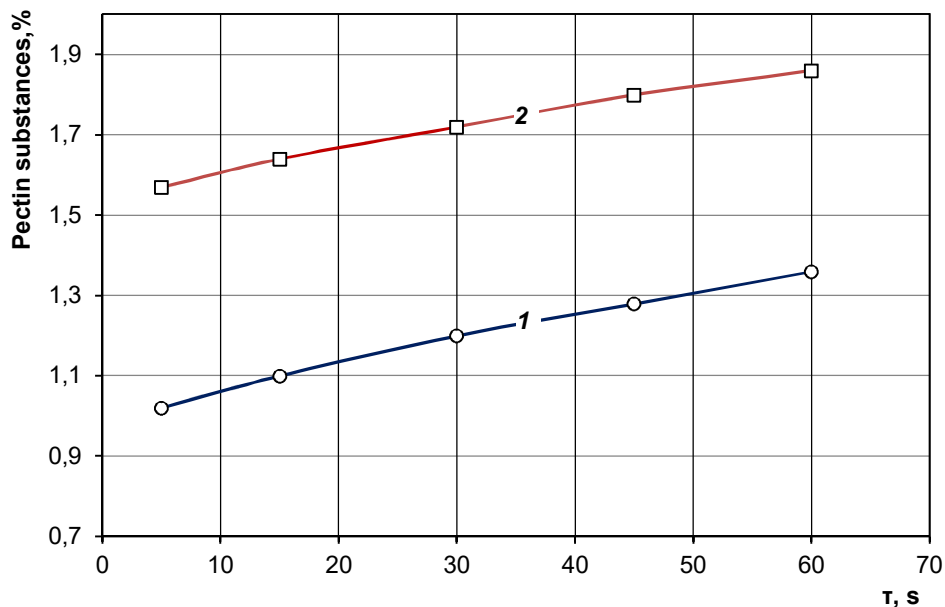


Figure 1. Impact of hydrothermal treatment duration on the content of pectin substances in berry purees:
1 – cranberry puree; 2 – viburnum puree.

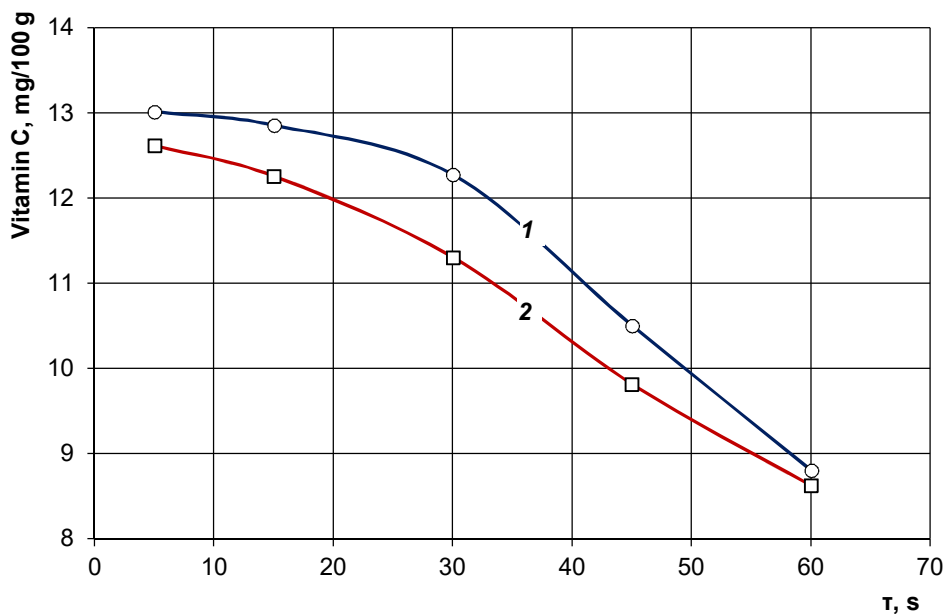


Figure 2. The impact of hydrothermal treatment duration on the content of vitamin C in berry purees:
1 – cranberry puree; 2 – viburnum puree.

Rational hole diameters of the crushing-rubbing machine sieves

Berries rubbing with the help of the crushing-rubbing machine was aimed at ensuring the necessary degree of vegetable raw materials grinding, high productivity of the process and quality of the final product. Experimental researches were made to determine the rational hole diameters of the crushing-rubbing machine sieves, subject to the use of which the highest yield of homogeneous puree, which will not flake off during storage, can be achieved (Figure 3).

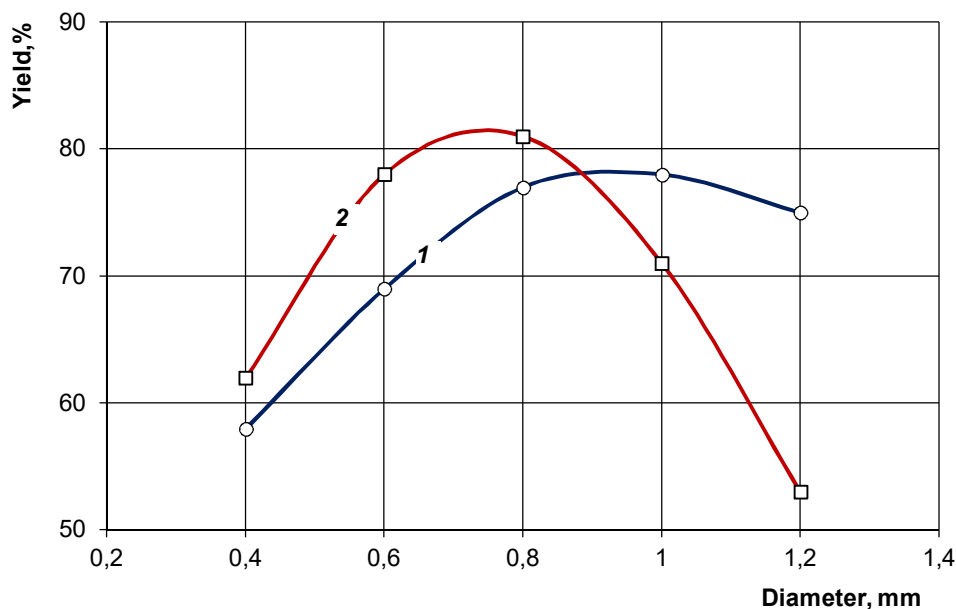


Figure 3. Dependence of the berry puree yield on the hole diameters of the crushing-rubbing machine sieves:
1 – cranberry puree; 2 – viburnum puree.

From the data obtained, it was found that the largest yield of cranberry puree was achieved after using a sieve with the hole diameters of 0.8–1.2 mm, whereas for the viburnum puree production it is rational to use a sieve with the hole diameters of 0.6–0.8 mm.

The need to use sieves with larger holes to increase the yield of cranberry puree can be explained by the more pronounced susceptibility of the coarse viburnum fibre towards mechanical stress compared to the finer cranberry fibre with regard to particle size reduction. Similar results are presented in the work [31].

The study of the dynamics of changes in the yield of berry purees from changing the diameter of the sieve holes in the rubbing process was not the purpose of our work and needs further researches.

Chemical composition of the berry purees

Taking into account the conducted researches, while berry raw materials processing into purees the following parametrs were used: processing with acute vapor at the temperature of 105–107 °C during 15–30 seconds for cranberries and during 5–15 seconds for viburnum berries; rubbing with the help of a crushing-rubbing machine with the diameter of sieve holes of 0.8–1.2 mm for cranberries and 0.6–0.8 mm for viburnum berries.

The chemical composition of berry purees obtained by the proposed method was investigated (Table 2).

Table 2

Chemical composition of berry purees, g/100 g of puree (n = 5, P ≤ 0,05)

Name of the indicator	Content in purees	
	Cranberry	Viburnum
Water	87.8	89.1
Proteins	0.3	0.2
Fats	0.1	0.2
Carbohydrates	7.4	6.9
including pectin substances	1.2	1.7
Ash	0.1	0.1
Organic acids (in recalculation on the malic acid)	2.0	1.7
Vitamin C, mg	9.3	9.0
Calcium, mg	6.3	6.0

According to the table, berry purees contain a small amount of proteins and fats, but they differ by the significant content of carbohydrates – 7.4% for cranberry puree and 6.9% for viburnum puree.

The content of pectin substances increases by 20 and 21% for cranberry and viburnum purees, respectively, compared to the content of this substances in raw materials (Table 1). The obtained results could be related to the berries softening while treatment with an acute vapor which was accompanied with solubilization of pectins due to the temperatue effect.

Vitamin C decreases by 4 mg for cranberry puree and 3.7 mg for viburnum puree comparing to untreated berries, which is permissible in view of the further use of the obtained semi-finished products.

It is particularly important to note a significant amount of organic acids of 2.0% and 1.7%, respectively, for cranberry and viburnum purees, which is sufficient to regulate the pH of the protein-carbohydrate dairy raw materials. That is, the obtaines purees can perform a coagulation function according to a modified method of obtaining of milk-protein concentrates from buttermilk.

Conclusion

The modes of treating of cranberries and viburnum berries with an acute vapor and their fine shredding to the state of purees were investigated.

1. Processing of berry raw materials with an acute vapor at a temperature of 105–107 °C during 15–30 s for cranberries and during 5–15 s for viburnum berries was found rational. This corresponds to an increase in the amount of pectin substances in 19–22% and 11–19% for cranberry and viburnum purees respectively, and allows to prevent significant losses of vitamin C.
2. For the obtaining of finely shredded berry purees by rubbing raw materials in the crushing-rubbing machine, it is expedient to use a sieve with hole diameters of 0.8–1.2 mm for cranberry puree and 0.6–0.8 mm for viburnum puree.
3. The study of the dynamics of changes in the yield of berry purees from changing the diameter of the sieve holes in the rubbing process was not investigated and needs further researches.

The significant increase in the content of soluble pectins and the presence of organic acids allow to use the obtained berry purees as coagulants in the technology of milk-protein co-precipitates from the buttermilk.

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Quality characteristics and antioxidant activity of goat milk yogurt with fruits

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Abstract

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Introduction. The aim of the research was to evaluate the physico-chemical, microbiological sensory characteristics and antioxidant potential of goat milk yogurt with fruits.

Materials and methods. The yogurt was prepared from goat's milk with the addition of scald fruits (10%) of aronia (Chokeberry L., Nero variety), peaches (*Prunus persica*, variety Moldova), raspberries (*Rubus idaeus*, Cusma de Guguță variety), strawberries (*Fragaria xanassa*, Selva variety), apples (*Malus domestica* variety, Golden). Quality indices and antioxidant potential was determined according to standard methods.

Results and discussions. The added fruits type had a strong impact on the values of titrable acidity and pH. The peache yogurt had a pH of 4.68 ± 0.019 . Higher acidity was obtained for raspberry yogurt, 103 ± 0.076 °T. The amount of dry matter indicates $20.40 \pm 0.45\%$ in strawberry yogurt. The dry matter content is inversely proportional to the value of the water activity, and maximum values were detected of 0.904 ± 0.038 for apples, peaches, raspberries yogurt. The minimum viscosity values were obtained for aronia yogurt, 5450 ± 4.85 Pa·s, and maximum for strawberry yogurt 8960 ± 4.45 Pa·s. The results obtained for determining the total number of germs in the yogurt are satisfactory, the highest result was for peaches yogurt, $1.8 \log$ cfu/ml. The maximum amount of lactic acid is in apple yoghurt $7.16 \pm 0.40 \log_{10}$ cfu/ml. No yeasts and molds were detected. Aronia yogurt has the highest total content of polyphenols ($187.15 \text{ mg GAE } 100\text{g}^{-1}$), anthocyanins ($56.45/100\text{g}$) and antioxidant activity (3.9%), the maximum carotenoid content $0.452 \text{ mg}/100 \text{ g}$ was obtained for peaches yogurt and ascorbic acid $25.77 \text{ mg}/100 \text{ g}$ for strawberry yogurt. Yogurt samples sensory properties show that strawberry yogurt has the best characteristics, obtaining 19.25 points of 20.

Conclusions. The addition of aronia fruits, strawberries and raspberries and peach positively influences the biological value and the quality indexes of goat milk yogurt with fruits.

Introduction

Goat milk and dairy products occupy a significant place in the rational diet of humans, due to their high chemical composition and the easy assimilation of the most accessible animal origin proteins [1]. The nutritional interest for the benefit of goat milk yogurt is generally associated with the biological value, such as proteins, calcium, phosphorus and vitamin A, D, in a relatively high percentage [2].

The fruits are compatible with dairy products [3]. Harnessing the bioactive potential of biologically active fruit compounds, with health benefits, is constantly expanding, the main purpose being the rational use of these compounds in yogurt [4].

To provide a more acceptable look and mask the specific smell of goat's milk, fruit can be added to the recipes, not only to provide attractive color to the product but exert an anti-inhibitory effect, due to the fruits biological components, which could extend the yogurt shelf life, hypotheses also supported by other researchers [5]. Some fruit fillers contain tannins, which react with milk proteins, forming a thick precipitate [6].

The aronia is rich in anthocyanins, minerals, antioxidants and vitamin C, thus contributing to the fortifying of the immune system [7].

Raspberries and strawberries can be substitutes for sweets, containing daily carbohydrate dose for an adult body [8], but also an important portion of fiber and water [9], which helps to clean and moisturize tissues [10].

Peaches are rich in beta-carotene and fiber, especially if they are consumed with shell, and in the composition of yogurt they enhance their benefits for the body [11].

Apple is a particularly valuable food for all ages. Apples contains the main vitamins and mineral salts, substances that give a surplus of vitalizing energy [12].

Processing and obtaining new products can substantially affect the quality and properties of bioactive fruit compounds, and the study of these effects is of fundamental and applicative importance in order to optimize processes and improve the quality of yogurt [13].

The use of aronia, strawberries, raspberries, and other bleached fruits in the manufacture of yogurt is less studied. The scald process can diminish the biological activity and nutritional value of the fruits [14]. In this context it is important to know how the biological value of the scald fruits in combination with the yogurt is highlighted, considering the process of yogurt manufacturing. In addition to the negative effects of heat treatment of fruits, scald also has positive effects: enzymes inactivation, air elimination of tissues, which consequently fixes, maintains and even accentuates the color of the finished products, as well as fixing and preserving vitamins during the following processes [15].

The aim of the research was to evaluate the physico-chemical, microbiological sensory characteristics and antioxidant potential of goat milk yogurt with fruit.

To achieve the goal, the following objectives were proposed:

1. Justification for choosing the fruits of aronia, apples, peaches, strawberries and raspberries;
2. Determining and arguing the quality indices of the yogurt (sensory, physico-chemical and microbiological indices);
3. Determination and argumentation of the antioxidant potential of the yogurts samples.

Materials and methods

Preparation of fruits pulp

Freshly ripened fruits (aronia, apples, peaches, raspberries and strawberries) were gently washed under water and cleaned with an aseptic knife. The fruits were processed by scalding at 95°C for 5 minutes, packed in glass jars and stored under optimum conditions.

Preparation of fruit yogurt

To prepare fruit yogurt the goat milk sample was received from the local goat farm. The goat's milk was pasteurized at 90°C for 10 minutes, after which it was cooled to the inoculation temperature. For the yogurt manufacture the Lyofast YAB 352 starter culture was used for inoculation, which contain *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Lactococcus lactis* subsp *Lactis* biovar *diacetylactis*, sucrose and maltodextrin. In the yogurt mixture, the scald fruit pulp was added in 10% concentration. The samples were thermostated at 37 °C for 6 hours. The end of the coagulation process was determined by the pH value and firmness of the coagulum. The yogurt samples were packaged in 180 g containers and stored at 8±2 °C.

The yogurt was prepared by the thermostat method, being an effective method to study the formed clot and its firmness [2].

The assortment of yogurt is presented in Table 1.

Table 1

Assortment of manufactured yogurt

Raw material	Sample					
	Classic yogurt	Yogurt with aronia	Yogurt with apple	Yogurt with peach	Yogurt with raspberries	Yogurt with strawberry
Goat milk	CY	ArY	ApY	PY	RY	SY

Methods

Titration acidity determination consists in neutralizing the acidic milk substances with 0.1n NaOH (KOH) solution using phenolphthalein as an indicator. The calculation formula is

$$\text{Acidity (}^{\circ}\text{T)} = 10 \cdot V,$$

where: V- volume used for titration [16].

pH determination. the pH value of the milk was determined using pH metre (glass electrodes) [16].

Viscosity determination of acid dairy products was determined using the "Brookfield DV – III" rheometer, with indicator no. 04, 250 rotations/min, data were read after 30 seconds of rotations [17].

Dry matter content determination. Method use the Radwag MAC moisture analyzer [18]. which consists in IR sample drying on an apparatus aluminum support until a constant mass of the dry residue is obtained.

Water activity determination. It is measured the vapor pressure of the water around the food and divided by the vapor pressure of the pure water to give a value between 0.0 and 1.0 [19].

Fat content determination – made by the acid-butyrometric method and consists in the separation of the fat using isoamyl (amyl) alcohol by centrifuging the milk, previously mixed with sulfuric acid [20].

Determination milk protein content by formaldehyde titration -consists of blocking the proteins amyl groups with formic aldehyde and the release of the carboxylic groups, which are neutralized with 0.1n NaOH solution [21].

Determination of the total number of microorganisms, lactic bacteria. The number of bacteria is estimated indirectly, based on the number of colonies generated by the cells of microorganisms after thermostatzation at 37 °C, for 48 hours [22, 23].

Determining the number of yeasts and molds. The homogenized samples were diluted in series by adding 1 ml of sample in 9 ml of pepton water, usind agar medium according to the instructions, the result was expressed as colony units per ml (CFU/ml) [23].

Determination of total phenolic compounds – estimated according Folin-Ciocalteu method [24]. A volume of 1 ml of methanolic extract of each sample was added to 1 ml of Folin-Ciocalteu's solution in a test tube. After 3 minutes, 1 ml of 20% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The mixture was allowed to stand at room temperature in a dark environment for 30 min. Absorbance was measured against the blank reagent at 725 nm. Gallic acid was used for the calibration curve with a concentration range of 50-1000 $\mu\text{g}\cdot\text{mL}^{-1}$. Results were expressed as mg GAE \cdot 100g $^{-1}$.

Determination of anthocyanin content was measured by spectrophotometric method at 540 nm, extracted with a solution of 95% ethyl alcohol and 1.5 n HCl to discoloration [25].

Antioxidant properties was determined by using a platinum and silver electrodes pattern model B90417 AVL – 1M the thermostatic cell, according to SM EN 12857:2014 [26].

Vitamin C – prepared extract titration with 0.001 N indicator solution of 2,6 diclorfenolindofenol [27].

Carotenoids determination – the method is based on the photometric determination of the mass carotene concentration in the solution obtained after the carotenoids extraction with an organic solvent and purified of the accompanying substances using dye by column chromatography [28].

Sensory quality assessment based on the score scale. Evaluation of each sensory characteristic by comparison with score scales and obtaining the average score of the tasting

group [29]. The mean score of the sensory analysis was passed in the centralized results sheet. The average of the total score is calculated based on the weighted average scores. Except for the control sample, the yogurt assortment was evaluated as "very good" and characterized as follows: "Product with pleasant, specific, well defined sensory characteristics, does not present any noticeable defects".

$$P_{mp} = P_{mnp} \times f_p,$$

where: P_{mnp} – unweighted average score (the arithmetic average of the results);

f_p – the weighting factor (shows how much a sensory characteristic participates in the total sensory quality of the product).

$$P_{tp} = \sum P_{mp}$$

Statistical analysis. The variance analysis of the results was carried out by least square method with application of Student test. The differences were considered statistically significant if probability was greater than 95% (p-value <0.05). All assays were performed in triplicate. The experimental results are expressed as average±SD (standard deviation).

Results and discussions

Analysis of the yogurt samples physico-chemical indices

Table 2 presents the composition of goat milk yogurt samples. The values obtained for fat and protein content were slightly influenced by the addition of fruits [30]. The protein content of yogurt changes with the incorporation of strawberry, raspberry and peach. The fat content is lower in the yogurt samples, compared to the control sample, possibly due to the increase of water content in the product with the fruits addition, also it should be mentioned the fruit low fat content. Similar observations were obtained by [31].

Table 2

Composition of goat milk yogurt samples

Nr.	Sample	Fat content,%	Protein content,%	Dry matter,%
1.	CY	3.4±0.035	6.03±0.052	16.03±0.42
3.	ArY	2.0±0.032	5.31±0.049	17.20±0.49
4.	ApY	2.6±0.038	5.14±0.051	18.93±0.45
5.	PY	2.4±0.040	5.43±0.047	18.73±0.51
6.	RY	2.3±0.039	5.62±0.050	19.11±0.50
7.	SY	2.1±0.037	5.51±0.048	20.40±0.45

The dry matter plays an important role in forming the texture of the finished product [32]. The addition of fruits in yogurt significantly influenced the total dry matter content. The dry matter content of fruit yogurt has decreased with the fruits addition, due to the high content of water contained in fruits which significantly increases in the process of scald fruit. Similar observations were reported by [33], who found that the total dry matter content decreased with the addition of fruit. For strawberry yogurt, the highest content of dry matter was obtained 20.40±0.45%.

Table 3 presents the physico-chemical indices of yogurt samples. The acidity of a food product is one of the first quality indices that demonstrate its freshness [34]. Acidification of the milk leads to the destruction of the internal structure of the casein micelle due to the solubilization of κ -casein [35], as a result of acid coagulation, resulting in the formation of the lactic gel. The type of added fruit had an impact on the values of titrable acidity and pH. Higher acidity values (103 °T) were obtained for the raspberry yogurt. The lowest values were obtained for the yogurt with aronia, 94 °T. A similar observation was reported by [36], who reported that the acidity of the yogurt increased due to the type of added fruit.

Table 3

Physico-chemical indices of yogurt samples

Nr.	Sample	Titrable acidity, °T	pH	a_w	Viscosity, Pa·s
1.	CY	90±0.073	5.06±0.015	0.895±0.029	5090±4.54
3.	ArY	94±0.075	4.65±0.018	0.901±0.035	5450±4.85
4.	ApY	95±0.071	4.62±0.016	0.904±0.038	7650±4.12
5.	PY	98±0.078	4.68±0.019	0.904±0.038	8500±4.19
6.	RY	103±0.076	4.61±0.015	0.904±0.038	8640±4.38
7.	SY	98±0.075	4.67±0.011	0.903±0.030	8960±4.45

Water activity (a_w) can participate in various chemical and biochemical reactions (eg Maillard reaction) that can affect the nutritional value of the yogurt, in terms of decreasing the storage time. If the water activity value is in the range 0,8-1, the product is slightly perishable with the risk of rapid development of microorganisms [37], for which minimum values of 0.895±0.029 were detected in the control sample and maximum values of 0.904±0.038 for apples, peaches, raspberries yogurt.

Yogurt is a soft solid product, and its network is a relative dynamic system, inclined towards rearrangement. The physical properties of yogurt can be explained using the model of interactions in casein micelle that includes the balance between attraction and rejection forces [38]. Rheological properties are important indicators of the quality of the yogurt. The samples were investigated at a temperature of 15 °C, the viscosity depends directly on the sample temperature [39]. This behavior is typical for non-newtonian liquids and is clearly highlighted for fruit yogurt samples. The minimum viscosity values were obtained for the control sample 5090±4.54 Pa·s, followed by the aronia yogurt 5450±4.85 Pa·s, apple yogurt 7650±4.12 Pa·s, peache yogurt 8500±4.19 Pa·s, raspberries yogurt 8640±4.38 Pa·S, strawberrie yogurt 8960±4.45Pa·s.

Analysis of the yogurt samples microbiological characteristics

The use of starter culture favors obtaining fermented dairy products. In the process of lactic fermentation, under the influence of the starter culture bacteria, the conversion of lactose into lactic acid occurs [40], which leads to some changes in the manufactured product: the pH decreases to the isoelectric point of the proteins (4.6), during the reduction of pH begins the process of forming the gel network specific to fermented milk [41]. On the other hands, biologically active fruit substances can catalyze oxidation reactions, thus being added to the yogurt composition could serve as a natural preservative, contributing to the pathogenic bacteria inhibition [42].

Table 4

Microbiological characteristics of yogurt samples

Nr.	Sample	Total number of microorganisms, log cfu/ml	Lactic bacteria (log ₁₀ CFU/ml)	Yeast and mold
1.	CY	2.1	7.22±0.03	Absence
3.	ArY	1.5	7.16±0.40	
4.	ApY	1.7	7.05±0.01	
5.	PY	1.8	7.08±0.03	
6.	RY	1.7	7.14±0.01	
7.	SY	1.6	7.06±0.01	

The microbiological characteristics of yogurt samples is presented in Table 4. The highest total number of microorganisms was obtained for the peach yogurt sample (1.8 log cfu/ml), the highest number of lactic bacteria (7.16 log₁₀ cfu/ml) was obtained for the aronia yogurt sample and the smallest value (7.05 log₁₀ cfu/ml) for the apple yogurt sample. Similar findings were also reported by [43], who stated that the number of lactic bacteria increases optimally in slightly acidic conditions when the pH is between 4.5 and 6.4.[44].

No yeasts and molds were detected.

Antioxidant potential of the yogurt samples

Yogurt in combination with fruits has a functional role in the human body, due to the supply of fiber, vitamins, minerals, phytonutrients, polyphenols, anthocyanins [45]. The incorporation of processed fruits into yogurt is a popular approach to increase the phenolic content and improve the antioxidant profile. Fortifying yogurt with naturally antioxidants also responds to consumers' demands for "clean label" foods [46, 47].

Some fruits, such as aronia, are good sources of phenolic compounds, especially anthocyanins. Polyphenols are known to interact with milk proteins and form insoluble complexes that reduce the total free polyphenol content [48].

The content of total polyphenols, anthocyanins, ascorbic acid, carotenoids, antioxidant properties of yogurt samples are shown in Figure 1–6. There were significant differences between the samples. The aronia yogurt had the highest polyphenol content (187.15 mg GAE 100g⁻¹). Peach and apple yogurt recorded the following values for the total polyphenols content: 184.15 mg GAE 100 g⁻¹, respectively 173.93 mg GAE 100 g⁻¹.

Due to antioxidant properties, anthocyanins play an important role in determining the color of fruit yogurt and differ from other compounds due to their ability to form different structures depending on the pH of the environment. Due to this property, anthocyanins can offer the body, protection against the intense harmful reactions of free radicals and can be used in the manufacture of yogurt as natural dyes [49]. In addition to their coloring properties, anthocyanins exhibit a wide range of biological activity, including antimicrobial, antimutagenic, anticancer, antitumor and antioxidant activities [50]. Anthocyanins are important, being the alternative of synthetic dyes, are considered safe because they have been consumed for centuries in fruits and vegetables without any health risk [51].

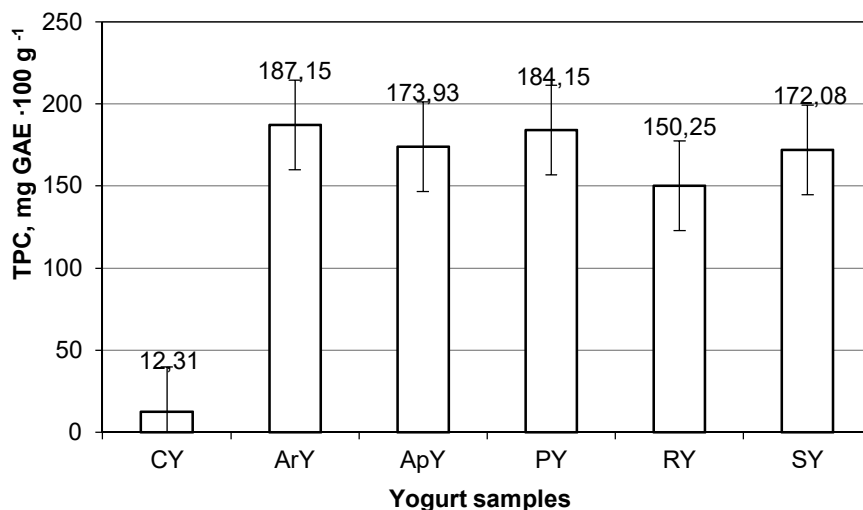


Figure 1. Total polyphenol content of yogurt samples

Using a simple method for quantifying total monomeric anthocyanins, the content of anthocyanins in fruit yogurt was determined. Values ranged from 19.36–56.45 mg/100g (Figure 2). The highest content of anthocyanins was in aronia yogurt (56.45 mg/100g), followed by the raspberry yogurt (48.34 mg/100g), the strawberry yogurt (40.47 mg/100g). low value was obtained for apple yogurt (19.36 mg/100g).

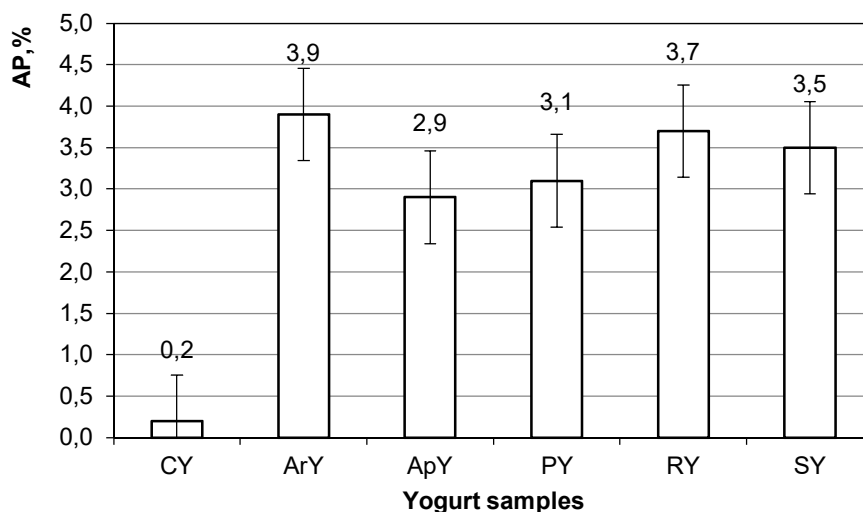


Figure 3. Antioxidant properties of yogurt samples

Antioxidants are bioactive substances that prevent oxidation reactions promoted by oxygen or peroxides and thus protect cells from the oxidative stress effect [52]. Fruits are a natural source of antioxidants and therefore their effectiveness in protecting against oxidative stress has been demonstrated by some researchers [53].

Natural antioxidants with a positive effect on oxidative stability have the role of preventing rancid [54], an undesirable fact in the obtained yogurt samples. Aronia yogurt registered the highest antioxidant property of 3.9%, followed by raspberries (3.7%) and strawberries (3.5%) yogurt.

There are numerous data [55] that show that fruits can provide more vitamins, more important being vitamin C (Figure 4) and β -carotene (Figure 5) [56].

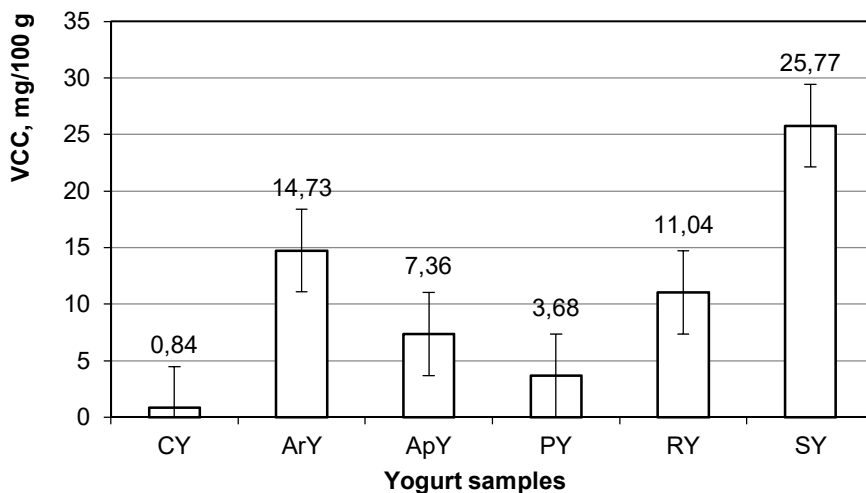


Figure 4. Vitamin C content of yogurt samples

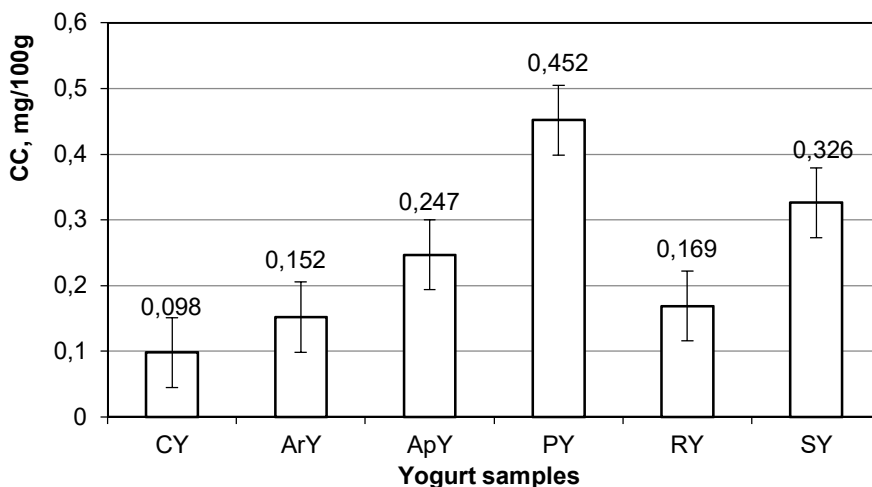


Figure 5. Carotenoids content of yogurt samples

The vitamin C content of the fruit yogurt samples ranges from 3.68 to 25.77 mg/100g. The strawberry yoghurt had the highest value (25.77 mg/100g), results discussed also by [57].

Carotenoids are a natural antioxidant that is present in yellow and green vegetables and fruits [58]. As a plant-based antioxidant, carotenoids can control the excess formation of free radicals and increase the capacity of antioxidants, as well as replace synthetic carcinogens that cause liver damage. Carotenoids, for example, are located in the membranes of fat globules, where they prevent the automatic oxidation of fat [59].

The carotenoid content ranged between (0.152–0.452 mg/100g) peach yogurt had a maximum value. Similar results were reported by [60].

Analysis of the sensory characteristics of the yogurt samples

The characteristics of the sensory quality are the parameters appreciated by the consumers, being the most important factor in determining the acceptance of food products [61]. The sensory properties of fermented dairy products should be examined in the following order: appearance and consistency, taste and smell, color [62,63]. The results of the sensory evaluation are shown in Figure 6.

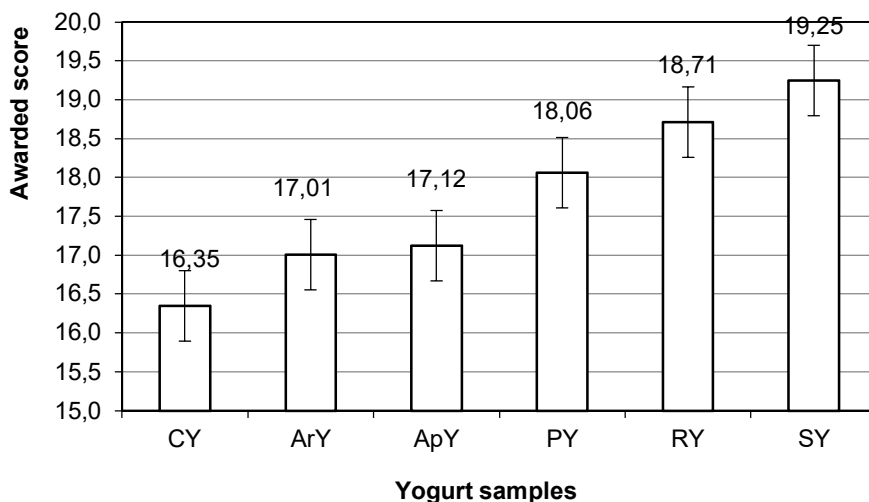


Figure 6. Total point average of the yogurt samples.

According to the results of the sensory analysis, the strawberry yogurt sample obtained the highest score, 19.25 points of 20; followed by raspberry yogurt, similar results being obtained by [64].

Conclusion

1. Goat milk due to its curative properties, high digestibility, technological properties (high quality of the formed coagulum) is a recommended raw material in yogurt fabrication.
2. High biological value, antibacterial properties of fruits used as additives make the end product – goat milk yogurt with fruits – safe functional food for consumption.
3. The addition of scald fruit improved the yogurt quality index, compared to the control sample, positively influencing the content in bioactive compounds.

4. In the yogurt samples with aronia fruits, there was a significant increase in the content of polyphenols, anthocyanin's, antioxidant properties compared to other samples.
5. Strawberry yogurt obtained the highest values for sensory characteristics and the vitamin C content.
6. Peach yogurt has an important content of carotenoids and polyphenols.

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Effect of aeration on physicochemical, color and texture characteristics of confectionery foams

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Abstract

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Introduction. The aim of this research was to determine the aeration process effect and the importance of raw materials on the quality characteristics of foams used in confectionery.

Materials and methods. The application of two different techniques and using raw materials of different origin (cream – S2, S4 and vegetable cream – S1, S3) were the basis for obtaining the foam samples, which have been analyzed in terms of chemical composition, porosity, color parameters and texture properties. The samples porosity was measured using ImageJ software (NIH Image) and the primary and secondary texture parameters were achieved by texture profile analysis test with Mark 10-ESM 301 Texture Analyzer.

Results and discussion. The highest fat content of confectionery foams was observed for cream-based samples (20.35%), while the vegetable cream-based samples presented lower value (15%). The moisture content of analyzed confectionery samples varied from 43.59 to 47.68%, the difference of moisture content according to sample classification (S1-S4) being significant ($p < 0.01$). The highest concentrations of soluble substances, 26.25 and 26.40, were presented by vegetable cream-based samples; S1 and S3 foam samples belonging to the same statistical group. The water activity of foam samples ranged from 0.804 to 0.824, the S4 cream-based samples presented the highest value. The samples porosity varied between 15.27 and 7.04%. The diameter of the air bubbles ranged between 4.36 mm to a few micrometers, the samples porosity having a negative influence on both primary ($r = -0.946^*$) and secondary ($r = -0.967^*$) texture properties. The brightness of vegetable cream-based samples presented the highest values (92.77 and 93.64), which means that this samples are whiter and brighter than the cream-based foams (89.74 and 86.89); while, b^* color parameter, which represents yellow–blue axis showed high values for cream-based samples (28.71 and 34.09). The texture profile analysis results showed that the highest hardness is shown by S3 sample – 3.48 N, the other foam samples showing close values ranging between 1.52 and 1.76 N. The samples with a low porosity had high hardness values, high levels of fracturability (0.24 N), which implies a more compact and brittle product, with a high viscosity (0.64), gumminess (1.53), chewiness (1.31) and cohesiveness (39.82).

Conclusions. Besides the influence on the firmness and other texture parameter, the aeration process changes the product appearance, color properties and mouth-feel.

Introduction

Many food products, especially those of bakery, pastry and confectionery [1, 2], depend on the aeration process to develop a certain structure, which can be obtained by biological, mechanical or chemical methods. An alternative to these methods is represented by the continuous aeration, in which the air phase is dispersed by mechanical action (shear and elongation flow forces) into the continuous phase [3]. A great importance in the continuous aeration process of food matrix is that the air phase must be concomitantly dispersed and stabilized [4, 5]. Air bubbles are an integral part in many confectionery products such as: meringues, marshmallow, mousses, whipped cream, nougat or aerated chocolate [6]; the aeration process being used to generate a range of novel textural properties [7–9]. Along with increasing volume, lowering density, improving the palatability and sensory appeal, the aeration process determines an increase in the viscosity and a decrease in fluidity of the product during processing, reduces stickiness and, ultimately, modifies the appearance, structure, textural characteristics, lightens the color of the products and changes the sensory properties (a lighter mouth-feel) of the finished product [10]. The air phase used in aeration processes must comply with certain conditions, such as free from all contaminants, without particulates, without oil droplets, odorless, dry and without microorganisms [10, 8]. The stability and comporment of confectionery foams are closely connected to their microstructure, and more precisely, to the air void dimension, distribution and volume fraction; the foams with smaller bubbles and with a uniform distribution of them exhibit the best textural parameters, flow behavior and stability over time, being creamier and more interesting to consumers [11]. Air bubbles are influenced by the process conditions, such as the residence time, the mixing head geometry and its operating conditions, the mechanism of the air bubbles can be applied also to assess the shelf life of whipped dairy products, since the textural properties and mouth-feel are a direct result of the elaborate interactions between bubble mechanics and human senses [4, 11]. Fixing air in the food structure is of great importance for producers which see air bubbles as a novel and versatile food ingredient used to produce a range of new textures from crunchy or crisp to soft one. To the authors knowledge there are few research about aerated confectionery. Therefore, the purpose of this research is to determine the effect of the aeration process and the importance of raw materials on the quality characteristics, color and texture of foams used in confectionery.

Materials and methods

Materials and experimental products preparation

The confectionery foams samples were produced in industrial conditions at SC. MOPAN S.A. company (Suceava, Romania). For samples preparation cream, vegetable cream, milk, white chocolate, sugar, gelatin and yolk were used (Figure 1 and 2). The gelatin was used as a stabilizing agent for the foam's air cells. The samples aeration was performed by mechanical whipping process using a planetary mixer (KitchenAid), the speed being set to 1000 rotations/min, using a six wire whip attachment and the whipping time was set to 5 minutes.

The foam samples were prepared as follows: for S1 and S2 samples (Figure 1) the sugar and yolk was whipped and mixed with hot milk, tempered chocolate and hydrated gelatin; this mixture was incorporated in the whipped cream and vegetable whipped cream. For S3 and S4 samples (Figure 2) in the whipped cream and vegetable whipped cream were

incorporated other ingredients starting with the mixture between sugar syrup and whipped yolk and followed by tempered chocolate and hydrated gelatin.

The S1 and S3 samples are vegetable cream-based foams, while S2 and S4 are cream-based foams. Prior to the analysis, the samples were kept under refrigeration conditions for 12 hours.

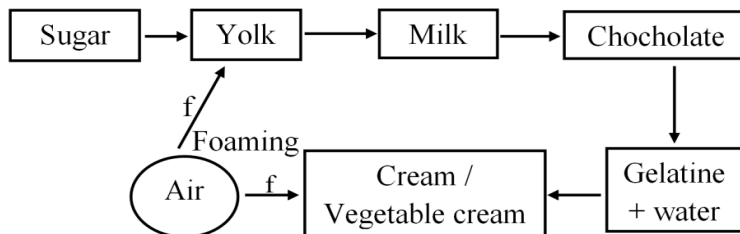


Figure 1. The structural design of S1 and S2 confectionery foams: s-solution; f-foaming.

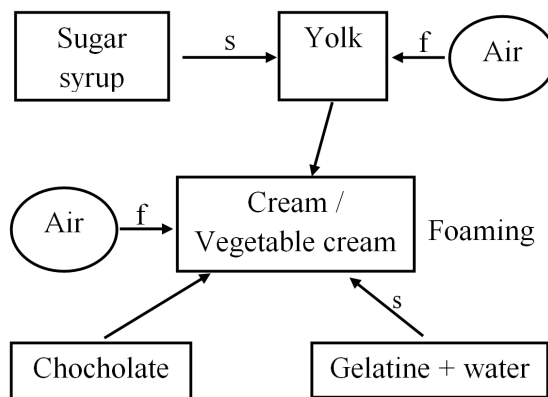


Figure 2. The structural design of S3 and S4 confectionery foams: s – solution; f – foaming.

Physicochemical parameters

The moisture content of foams samples were determined by oven drying method (103± 2°C), [12] and then expressed as percent, the protein content was determined by Kjeldhal method [13] and expressed as percent of wet basis. The Soxhlet method [14] was applied to determine the fat content and the results were expressed as percent of wet basis. Total acidity was measured by direct titration with NaOH 0.1 N, expressed as cm³NaOH/100g sample and the pH was determined using a Metter Toledo pH-meter [15]. The confectionery foams' soluble substances concentrations (°Brix) were determined by a Leica Mark II Plus refractometer, with an accuracy of 0.1% and the water activity was measured with a water

activity meter (AquaLab Lite). The foam porosity was measured using the ImageJ software (NIH Image) and expressed as percentage. The color measurements were achieved with a CR-400 Chroma meter from Konica Minolta (Konica Minolta, Japan) using the CIE $L^*a^*b^*$ uniform color space method, where: L^* represents brightness, a^* represents red–green axis and b^* represents yellow–blue axis. Hue angle or tone (h^0), chroma or color intensity (C^*) and yellowness index (YI) was calculated as follows (eq.1-3), [16, 17].

$$h^0 = \tan^{-1} (b^* / a^*) \quad (1)$$

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$YI = \frac{142.86b^*}{L^*} \quad (3)$$

Texture profile analysis

Texture measurements were carried out on confectionery foams samples at ambient temperature with Mark 10-ESM 301 Texture Analyzer (Mark 10 Corporation, USA) with a loading-unloading speed of 10 mm/min using compression discs of 5 cm diameter, the foam samples being compressed to 50% of its original size [18]. The confectionery foams samples had cubic shape with side of 30 mm and subjected to a texture profile analysis – TPA test [19]. The experimental data obtained from TPA test were used to calculate primary textural parameters like: hardness (H), viscosity (V), adhesiveness (A), cohesiveness (Co), springiness (S), and secondary textural parameters gumminess (G), chewiness (Ch) and fracturability (F), [20].

All physicochemical measurements were carried out in triplicate. All the reagents were of analytical grade.

Statistical analysis

The analyzed foams results were subjected to analysis of variance ANOVA using STATGRAPHICS CENTURION XVI (Trial Version) and Pearson correlation with SPSS 13.0 (SPSS Inc. Chicago, IL).

Results and discussion

Physicochemical analysis

The physicochemical parameters (fat, protein, moisture content, Brix concentration, a_w , free acidity, pH) and color parameters (L^* , a^* , b^* , chroma, yellowness index, hue angle) of confectionery foam samples are shown in Table 1. The confectionery foams' fat content varied between 15.00 and 20.35%, the highest fat content was observed for cream-based foams (S2, S4); while the vegetable cream-based foams (S1, S3) presented lower value. The results of one factor analysis of variance (ANOVA) indicated that the difference is statically significant ($p < 0.05$). The protein content of analyzed confectionery foams is in the same range (9.70 – 10.20%), the statistical difference being insignificant ($p > 0.05$).

Water is an important component of confectionery products, one of the main functions being to solubilize the raw materials and help mixing them. The water content influences the

product texture properties and is one important factor affecting the shelf life [21]. Moisture content has a significant contribution on the texture of confectionery products; the moisture content of analyzed confectionery samples varied from 43.59 to 47.68%, the difference of moisture content according to sample classification (S1-S4) was significant at a level of $p < 0.01$. According to *Fontana, 2006* [22] the water activity of confectionery products has a significant role in quality assurance, processing, shelf life, sensory and texture properties evaluation. Confectionery products such as boiled sweets, toffees, caramels, jellies, creams or coatings cover a wide range of water activities (a_w) from 0.2 to 0.9 [22]. The water activity of foam samples ranged from 0.804 to 0.824, the S4 cream-based samples having the highest value. The concentration of soluble substances expressed as °Brix ranged between 23.50 (S2) to 26.40 (S3), the highest Brix concentration was presented by vegetable cream-based samples (S1, S3 – samples belonging to the same statistical group), one-factor analysis of variance highlighted this difference at a level of $p < 0.01$. The measured pH values of confectionery foam samples were in the slightly acidic zone, showing values close to each other (6.67-6.78). The total acidity of the confectionery products is due to the organic acids used (the citric, malic, tartaric, lactic or acetic acid are commonly used), [23] or due to the raw materials used in the production process and it has the role to extend or enhance tartness or sour perception in addition to the product specific functions and also to control the textural characteristics of the product [24]. The total acidity of the analyzed foams belongs to the same statistical group, (ANOVA), $p > 0.05$, the difference being insignificant. A relatively novel approach to create new products is fixing air into food structure, thus obtaining products with a low caloric intake, new texture characteristics, new appearance, color and a modification in the mouth feel [9]. The samples porosity varied between 15.27 and 7.04%, also the difference being insignificant ($p > 0.05$). However, introducing air or another gas into a food matrix makes the finished product lighter; increasing the final volume and the texture becomes softer.

In Table 1 there are presented also the color parameters of confectionery foam samples. Color is an important characteristic of food products, being the first quality attribute evaluated by the consumers and especially of confectionery products; consumers choosing products according to their color characteristics and appearance [25]. The confectionery products come in a wide range of colors from the ingredients used or from the addition of food coloring. In terms of brightness – L^* , we can observe that S1 and S3 foam vegetable cream-based samples present the highest values, which means that these samples are whiter and brighter than the cream-based foams (S2 and S4), $p < 0.001$. Instead, b^* color parameter, which represents yellow–blue axis showed high values for S2 (28.71) and S4 (34.09) foam samples. In case of a^* color parameter (red–green axis) all foam samples presented negative values ranging between -7.17 and -5.35, more toward green. The lowest color intensity (C^*) values were observed for vegetable cream-based samples, while the cream-based sample (S4) had the highest values. The cream-based samples S2 and S4 presented also the highest yellowness index. One way ANOVA analysis highlighted that all color parameters were different accordingly to the ingredients used and the technology applied in the production process ($p < 0.001$).

Table 1

Physicochemical and color parameters of confectionery foams

	S1	S2	S3	S4	F – ratio
Physicochemical parameters – mean (SD)					
Fat [%]	15.00 (0.35)c	19.82 (0.20)ab	18.61 (0.14)b	20.35 (0.70)a	34.44**
Protein [%]	10.10 (0.20)a	9.70 (0.25)a	10.20 (0.20)a	9.90 (0.32)a	0.80 ^{NS}
Moisture [%]	47.68 (0.21)a	45.68 (0.18)b	44.48 (0.52)bc	43.59 (0.61)c	17.42**
Brix	26.25 (0.25)a	23.50 (0.90)c	26.40 (0.30)a	25.00 (0.50)b	31.34**
a_w	0.815 (0.01)a	0.814 (0.08)a	0.819 (0.05)a	0.824 (0.02)a	0.03 ^{NS}
Acidity [cm³NaOH/100g]	0.011 (0.001)a	0.011 (0.001)a	0.012 (0.001)a	0.011 (0.001)a	0.028 ^{NS}
pH	6.78 (0.01)a	6.72 (0.01)b	6.67 (0.01)c	6.73 (0.01)b	27.11**
Porosity [%]	15.27 (2.56)a	14.67 (1.98)a	7.04 (2.10)a	12.23 (3.30)a	1.95 ^{NS}
Color parameters – mean (SD)					
L*	92.77 (0.58)a	89.74 (0.10)b	93.64 (0.70)a	86.89 (0.10)c	127.05***
a*	-6.64 (0.03)c	-7.17 (0.01)d	-5.35 (0.09)a	-6.16 (0.14)b	239.47***
b*	21.93 (0.25)c	28.71 (0.18)b	21.04 (0.29)d	34.09 (0.23)a	193.76***
C	22.92 (0.15)c	29.59 (0.20)b	21.71 (0.3)c	34.65 (0.22)a	121.35***
h°	106.848 (0.12)a	104.03 (0.19)b	104.26 (0.10)b	100.25 (0.16)c	112.05***
YI	33.78 (0.21)c	45.70 (0.09)b	32.09 (0.25)c	56.06 (0.18)a	125.73***

Different lowercase letters (a–d) in a row show significant differences between the groups ($p < 0.05$). NS – not significant ($p > 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

In Figure 3 there is presented the structure of the analyzed foams samples used in confectionery industry. Foams samples were made through the whipping process, which has the role of introducing air bubbles into a network of fat droplets to generate the foam. As we can see the pore size varies widely, vegetable cream-based samples showed larger air bubbles trapped in the structure of foam, with a heterogeneous distribution of air voids. The diameter of the air bubbles ranging between 4.36 mm (S1) to a few micrometers (S2 and S4). According to Goralchuk, 2019 [26] the bubbles shape differs depending on the continuous phase content, thus low concentration phases generate spherical forms while high concentration phases generate dodecahedral forms [26]. Compared to vegetable cream-based samples, the cream-based samples presented smaller air bubbles trapped in the structure of foam and with a more homogeneous distribution of air voids.

Texture properties measurement

Texture Profile Analysis (TPA) is a double compression test and it is used more and more often for texture measurements due to the fact that it can quantify a large number of texture parameters in a single analysis [27]. Both primary (hardness (H), adhesiveness (A), viscosity (V), cohesiveness (Co), springiness (S)) and secondary (gumminess (G), resilience (R), fracturability (F) and chewiness (Ch)) texture profile analysis (TPA) parameters were determined using a texturometer, the results being presented in Table 2. The TPA profile of design confectionery samples are shown in Figure 4 (S1, S2, S3 and S4), and as we can see the highest mechanical strength is shown by S3 sample (Figure 4) 3.48 N, the other foam samples showing close values ranging between 1.52 N and 1.76 N. The high hardness value of the sample S3 is due to a low porosity (Figure 3). The level of sample aeration influences the texture parameters, thus obtaining products with a soft or tough texture. Besides the influence on the firmness and other texture parameter, the aeration process changes the product appearance, color properties and mouth-feel; providing a sense of fullness in comparison to the non-aerated food [28].

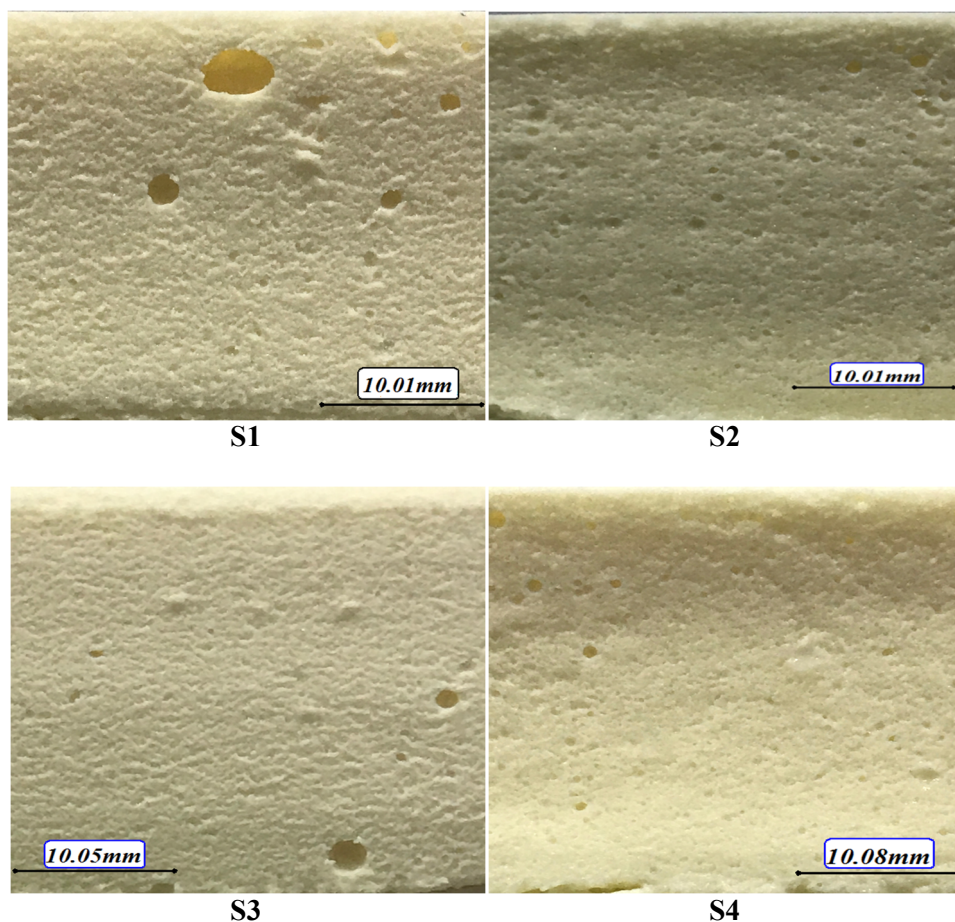


Figure 3. Structure of the analyzed foams used in confectionery

Table 2

Texture parameters of confectionery foam samples

Texture parameters – mean (SD)	S1	S2	S3	S4
Hardness [N]	1.70 (0.25)	1.76 (0.12)	3.48 (0.45)	1.52 (0.10)
Viscosity [N]	0.40 (0.09)	0.38 (0.10)	0.64 (0.21)	0.26 (0.05)
Fracturability [N]	0.22 (0.05)	0.03 (0.03)	0.24 (0.01)	0.06 (0.01)
Cohesiveness[%]	26.45 (0.50)	35.28 (0.40)	39.82 (0.45)	31.64 (0.65)
Adhesiveness [N·s]	18.50 (0.23)	23.80 (0.38)	35.27 (0.85)	7.497 (0.25)
Springiness[%]	83.89 (1.85)	90.53 (2.55)	85.73 (2.25)	47.25 (2.90)
Gumminess [N]	0.45 (0.10)	0.59 (0.11)	1.53 (0.10)	0.48 (0.10)
Chewiness [N]	0.37 (0.21)	0.54 (0.11)	1.31 (0.65)	0.22 (0.09)
Resilience	1.23 (0.50)	1.04 (0.45)	0.52 (0.35)	0.21 (0.10)

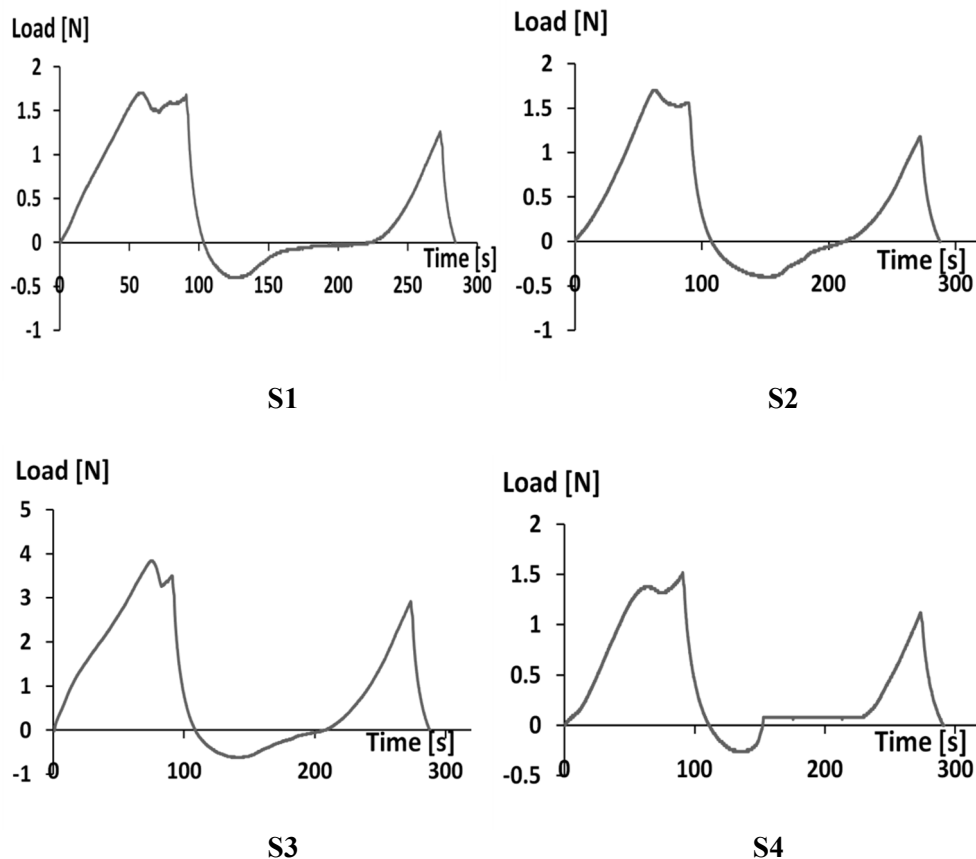


Figure 4. Texture Profile Analysis (TPA) curves of the analyzed foams used in confectionery

Another important aspect is the fact that in case of aerated products the amount of ingested food is reduced, thus decreasing the caloric intake. In some studies, it is shown that [29] food consumption is influenced by both weight and volume of food products. Apart from a high hardness the S3 sample also shows high levels of fracturability (0.24 N), which implies a more compact and brittle product, also a high viscosity, gumminess, chewiness and cohesiveness. The highest TPA springiness of aerated samples was recorded by S2 (90.53%) followed by S1 and S3 samples, which had close values; the lowest springiness being recorded by the S4 sample. The TPA resilience could be defined as how well a sample regains its original position [30]; the analyzed confectionery samples' resilience ranged from 1.23 to 0.21.

Table 3

Pearson Correlation matrix between physicochemical and texture properties

	Fa	P	M	B	aw	A	pH	Pr	H	V	F	Co	A	S	G	Ch	R
Fa	1	-.569	-.866	-.629	.499	.049	-.604	-.226	.009	-.217	-.720	.595	-.127	-.417	.119	.033	-.684
P		1	.116	.986*	.165	.676	-.150	-.611	.653	.661	.959*	.051	.409	.101	.590	.546	-.072
M			1	.167	-.818	-.331	.692	.531	-.252	.036	.356	-.601	.105	.617	-.349	-.196	.943*
B				1	.199	.551	-.004	-.492	.520	.533	.936*	-.105	.265	.009	.453	.401	-.064
aw					1	.147	-.276	-.351	.037	-.240	-.122	.119	-.447	-.900	.100	-.097	-.962*
A						1	-.813	-.974*	.994**	.921*	.634	.768	.809	.297	.993**	.963*	-.274
pH							1	.873	-.800	-.641	-.065	-.984**	-.643	-.118	-.860	-.813	.505
Pr								1	-.946*	-.810	-.509	-.804	-.679	.093	-.967*	-.897	.484
H									1	.956*	.641	.774	.869	.401	.994**	.985**	-.174
V										1	.730	.650	.950*	.631	.924*	.968*	.120
F											1	.010	.536	.359	.560	.572	.207
Co												1	.705	.254	.831	.816	-.370
A													1	.793	.844	.933*	.278
S														1	.345	.522	.795
G															1	.981*	-.255
Ch																1	-.069
R																	1

Fa – fat content; P – protein content; M – moisture content; B – Brix concentration; aw – water activity; A – Acidity; Pr – porosity; H – hardness; V – viscosity; F – fracturability; Co – cohesiveness; A – adhesiveness; E – springiness; G – gumminess; Ch – chewiness; R – resilience.

*Correlation is significant at the 0.05 level (1-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Pearson correlation

Table 3 presents the Pearson correlation matrix of the physicochemical and texture properties of design confectionery samples. It can be observed that a positive correlation is between the protein content and the concentration of soluble substances ($r = 0.986^*$) and a negative correlation is between sample acidity and the level of aeration (porosity). The TPA texture properties are highly positively correlated among them such as: hardness with viscosity ($r=0.956^*$), gumminess with hardness ($r=0.994^{**}$) and hardness with chewiness ($r=0.985^{**}$); another positive correlation is between adhesiveness and chewiness ($r=0.933^*$). Regarding the correlation of texture properties and physicochemical parameters of analyzed samples it can be observed that the fracturability is positively influenced by the protein content ($r=0.959^*$) and the concentration of soluble substances ($r = 0.936^*$). The moisture content and water activity have a significant influence on the foam samples resilience ($r=0.943^*$ and $r=-0.962^*$) while a significant negative influence on the foams hardness is given by the sample porosity ($r=0.946^*$). According to *Fontana, 2006* [22] food products with a high value of water activity are characterized as having a soggy, juicy, chewy and soft texture. A significant positive correlation was recorded between gumminess and chewiness with total acidity ($r=0.993^{**}$, $r=0.963^*$), hardness ($r=0.994^{**}$, $r=0.985^{**}$), viscosity ($r=0.924^*$, $r=0.968^*$), while the porosity was negatively correlated with this two secondary texture parameters (G and Ch).

Conclusion

The origin of the raw materials (cream or vegetable cream) used in the production process had an influence on the physicochemical characteristics and color parameters (L^* and a^*); one-factor analysis of variance ANOVA highlighted these differences at least at a level of $p < 0.05$.

The confectionery samples based on vegetable cream presented a whiter and brighter color, larger air bubbles, with a heterogeneous distribution of air voids, while the cream-based foams were more yellow, with smaller air bubbles and a more homogeneous distribution of air voids.

The aeration process of confectionery samples had a negative influence on both primary (hardness) and secondary texture properties (gumminess), thus the introduction of air bubbles led to a lighter and softer texture and the increased volume of the product provides a sense of fullness. Fixing air into the structure of confectionery products represents an alternative in creating new products with special textures and new appearance, which also have a lower caloric intake.

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Nature of water bonding in hydrated milk-protein systems

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Abstract

Introduction. The nature of the connection and the characteristics of the process of relaxation of clusters and water domains in milk and protein systems was investigated in order to predict their functional and technological properties.

Materials and methods. The relaxation features of water clusters and domains in colloidal solutions of milk proteins were studied by the thermo-stimulated depolarization method (TSD). Electrophoretic analysis of the fractional composition of milk proteins was performed according to modified Laemmli method.

Results. The peculiarities of water distribution in bulk protein matrices in milk and in hydrated milk protein concentrates by TSD were studied. On the basis of comparative analysis of TSD spectra of relaxation of dipole structures of water in low-temperature and high-temperature regions in samples of fresh skimmed milk, reconstituted skimmed milk powder and sodium caseinate solution, a significant difference in the nature of water cluster formation in these systems is proved. Milk protein concentrates with reduced energy of activation of depolarization of water in the hydrated state are found to form spatial grids with smaller cell sizes than proteins of natural milk. The revealed effect is explained by the fact that under the influence of heat treatment and drying there are significant conformational changes of protein compounds caused by the denaturation of the majority of serum proteins, in particular, immunoglobulins, serum albumin, β -lactoglobulin and α -lactalbumin. It is relevant to use in composition of foods milk protein concentrates, whose protein matrices have spatial limitations and hold water clusters smaller than that of natural milk. The results of the research are of practical importance as they allow the purposeful formation and stabilization of the spatial structure of protein-containing food systems.

Conclusions. The peculiarities of the nature of water bonding in hydrated milk-protein systems of different degrees of heat treatment have been established.

Introduction

In biological systems, water is distributed in the form of fragments of structures of different sizes – clusters (<1 nm) and domains (≥ 1 nm) [1]. The change in the distribution in the protein-containing systems of water domain domains leads to a shift in the ratio between "free" and "bound" water, and thus significantly affects the structure of these protein-containing systems [2]. Spatial protein gels are capable of holding water domains in the sizes from 1 to 100 nm, thereby counteracting the gradual phase separation over time as well as the growth of ice crystals in the process of freezing [3]. This effect is extremely important for the purposeful influence on the processes of formation and stabilization of the structure of food systems [2].

Identify the content of "bound" water and the distribution of fragments of its structure can be by studying the dependence of the relaxation response of the molecules (depolarization) on the physical and chemical properties and the content of the filler in the disperse system [1]. Large-sized water domains with a number of water molecules > 1000 are relaxed in the high-temperature region of the spectrum (HT, $95\text{ }^{\circ}\text{C} < T < 0\text{ }^{\circ}\text{C}$). The smallest clusters ($R < 0.5$ nm) can be attributed to structures that contain 4-6 molecules of water and are localized between adjacent primary particles in aggregates [2], their relaxation is observed in the low-temperature (LT) region of the spectrum. The distribution of the cluster radius with the number of molecules up to several dozen occurs within $0.5 < R < 1$ nm. The spatial constraints of relaxing dipole structures lead to the dependence of the relaxation characteristics on the sizes of these structures [3].

Despite the fact that the properties of bound water in milk and milk products were studied by many scientists [4, 5], the distribution of bound water in these systems as fragments of the structure has not yet been investigated. It should also be noted that such physical properties of milk proteins as solubility, the nature of the interaction between themselves and the aquatic environment, and the ability to form spatial structures are significantly dependent on the conditions of the previous thermal, mechanical and biochemical treatment of milk [6]. It is clear that the main structural factor affecting the relaxation properties of water in protein solutions is the size of its domains, which depend on the content of micelle casein (average size $0.1\text{ }\mu\text{m}$) and their distribution in the volume of hydrated systems [7]. Thus, the study of the peculiarities of water distribution in the matrices of milk proteins of various composition and degree of purification is an actual direction of scientific research. The practical significance of the results of such a study is to use them as a tool for predicting the behavior of proteins of varying degrees of processing in food systems.

The aim of the research was to study the effects of water state in milk protein systems on their physico-chemical characteristics.

The tasks of the research are:

- to justify applying the method of thermo-stimulated depolarization to study the peculiarities of water distribution in bulk protein matrices in protein-containing food systems;
- identify the impact of pre-treatment on the physico-chemical properties of milk proteins and milk protein concentrates.

Materials and methods

Materials

Reconstituted skimmed milk powder [5] and hydrated caseinat sodium [8] were selected. The mass fraction of dry matter in both samples of solutions of milk-protein concentrates was 10%. Fresh skimmed milk with a mass fraction of fat 0,05% was used as control.

Methods

Method of thermostimulated depolarization

TSD spectra of the samples studied in the form of tablets of 30 mm in diameter and ≈ 1 mm in thickness were measured at a temperature range of $-183-0$ °C for polarization voltage of 200 V and a heating rate of 3 °C / min on a device for the production of SKB in the city of Angarsk (RF). In the process of heating with an electrode, the strength of the depolarization current was measured in the range $10^{-14}-10^{-7}$ A, which was fixed on the computer. The errors were: for temperature – $\delta_T = \pm 2$ °C; for current – $\delta_I = \pm 5\%$; for the heating rate – $\delta_h = \pm 5\%$ [1, 3].

The activation energy of the proton conductivity (E_a) for the TCD was calculated from the Arrhenius equation [10]:

$$\frac{d \ln I}{dT} = \frac{E_a}{RT^2};$$

where I – current of proton conductivity, A; T – temperature, K; R – universal gas constant.

TSD spectra were analyzed both in low temperature (LT) and in high-temperature (HT) areas.

The decomposition of the TSD spectra into components of the Gauss function was performed according to the Peakfit Origin 7.0 program [3]. On the drawings of the TSD-spectrum points, the experimental values of the depolarization current value were obtained, the thin lines denote individual relaxation peaks, and the fat curve is the result of all obtained by the decomposition of individual peaks. The degree of correlation between experimental data and mathematically expanded curves in all cases was within the range from 0.96 to 0.99. Calculations of the distribution of clusters (<1 nm) and domains (1–100 nm) of bound water in size were performed using the TSD-cryopometry method [9, 10].

Method electrophoretic analysis of the fractional composition of milk proteins

An electrophoretic analysis of the fractional composition of milk proteins was performed using the modified Laemmli method using a concentration gel for protein distribution in the range of molecular weights of 10000 Da to 150000 Da [11]. Treatment of electrophoregrams obtained by disc-electrophoresis was performed using the ImageMaster TotalLab v.2.01 (Amersham Biosciences) program [3].

Results and discussion

Investigation of the bond nature and the peculiarities of the water molecules relaxation in milk-protein systems by the TSD method

A comparative analysis of the obtained TCD spectra of fresh skimmed milk, reconstituted skimmed milk powder and colloidal sodium caseinate solution (Figure 1) convincingly proves a significant difference in the nature of the cluster formation of water in these systems.

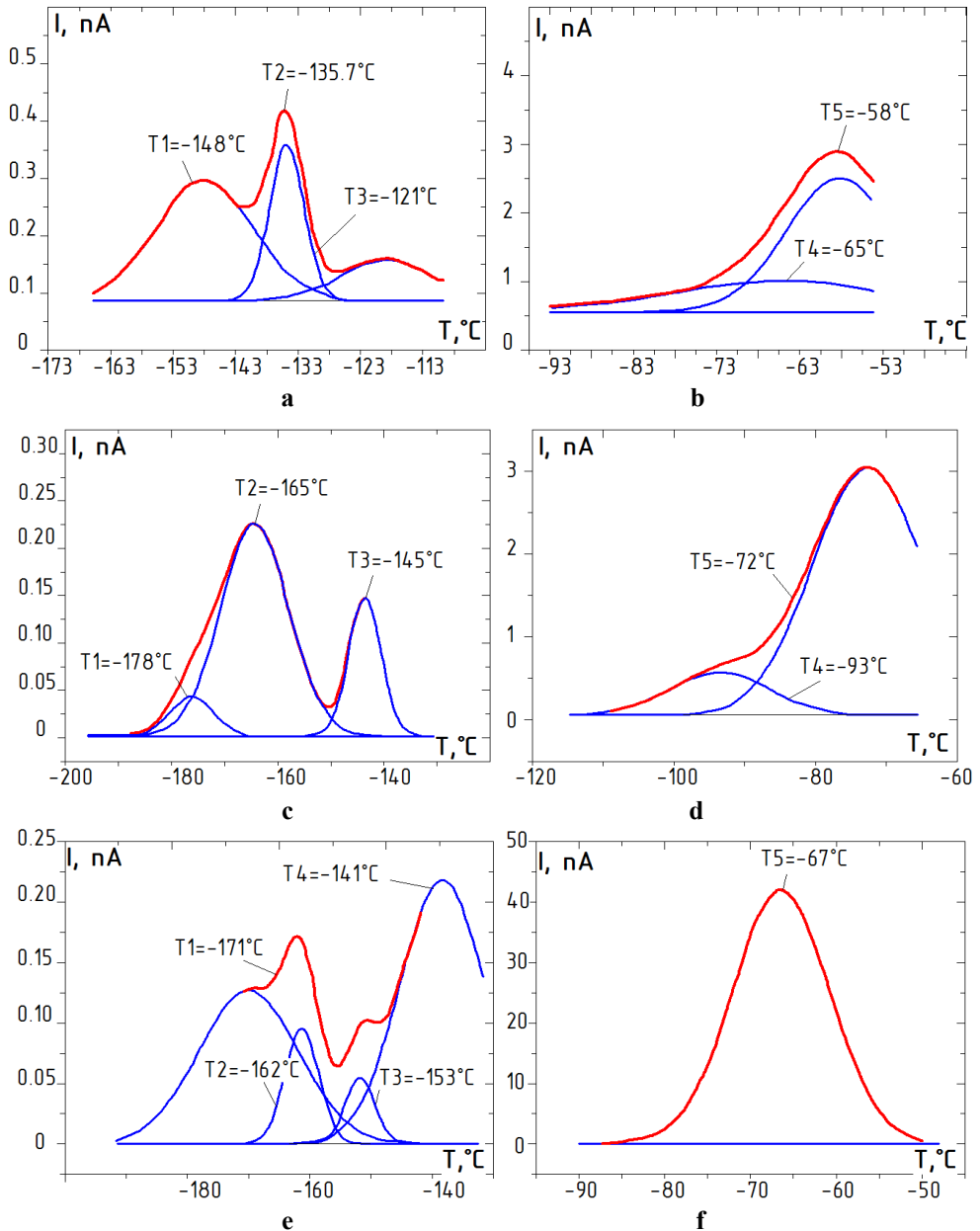


Figure 1. LT and HT areas of the TSD spectra:
a – high-temperature regions in samples of fresh skimmed milk;
b – low-temperature region in samples of fresh skimmed milk;
c – high-temperature regions in samples of skimmed milk, reconstituted;
d – low-temperature region in samples of skimmed milk, reconstituted;
e – high-temperature regions in samples of aqueous sodium caseinate;
f – low-temperature region in samples of aqueous sodium caseinate of fresh skimmed milk (a, b), skimmed milk, reconstituted (c, d) and aqueous sodium caseinate (e, f).

Thus, in the low temperature area of the spectrum, the relaxation maxima for domains (clusters) of water molecules are observed in reconstituted milk and sodium caseinate solution at lower temperatures than in a sample of fresh milk. In this temperature range, their E_a depolarization is in the range of 5 to 10 kJ/mol, indicating the relaxation of water molecules in small clusters (up to 10 molecules). Instead, such clusters are not observed in natural milk.

The third relaxation maximum coincides with similar peaks of relaxation in natural milk. In the BW region of the spectrum, 2 relaxation maxima are observed at -93 and -72 °C, which is also characteristic of natural milk, but they are somewhat shifted to a lower temperature region, indicating the formation of water molecules smaller in size in the aqueous solution of reconstituted skimmed milk.

Comparison of the integral intensities for peaks observed in the TCD spectrum of skimmed milk powder (Figure 1, b) in the LT area of the spectrum indicates, firstly, the symbiosis of the relaxation processes occurring in both systems and, secondly, on the essential differences in the nature of the distribution of water inside the protein matrix of dry milk compared with natural milk. In view of the detected effect, it can be assumed that the "cells" of the net formed by the milk proteins of dry milk, when reconstituted in water, become less than in natural milk.

The TSD spectrum of aqueous suspension of sodium caseinate (Figure 1e, f) differs from the spectrum of the reconstituted skimmed milk by the presence (Figure 1 c, d) in LT area of 4 relaxation maxima at temperatures of -171, -162 -153 and -141 °C, the integral intensities of which are approximately one order higher than for the peaks of reconstituted dry milk. This indicates that the number of relaxation structures (clusters and water domains) formed in the presence of casein is an order of magnitude larger than that of reconstituted milk (in the region of the spectrum of the LT). The same dependence was observed in the HT area of the spectrum, in which there is one relaxation maximum at -67 °C of an almost ideal Gaussian species with an integral intensity of an order of magnitude higher than that of reconstituted milk.

The explanation of the intensity of the TSD spectrum in both HT and LT areas may consist in the fact that during heat treatment, especially at temperatures of 100 °C, dephosphoric and dehydration of casein, its complexation with denatured whey proteins, lactose, etc., occurs. as a result of which casein micelles disintegrate or increase the size. During hydrolysis, in the first place, χ -casein, for high temperatures glycemacopeptides are released, reducing the degree of hydration of casein mixtures, thermal dephosphorylation reduces the ability of casein to bond calcium, micelles destabilize and polymerize [12, 13].

Electrophoretic analysis of the fractional composition of milk proteins

With another study of the change in the fractional composition of milk proteins under the influence of technological treatment, the electrophoregram of milk was analyzed, which was shown in Figure 2.

Table 1 shows the fractional composition of fresh milk and reconstituted milk, which confirm the decrease in total protein in milk and significant changes in the fractional composition of proteins under the influence of technological treatment.

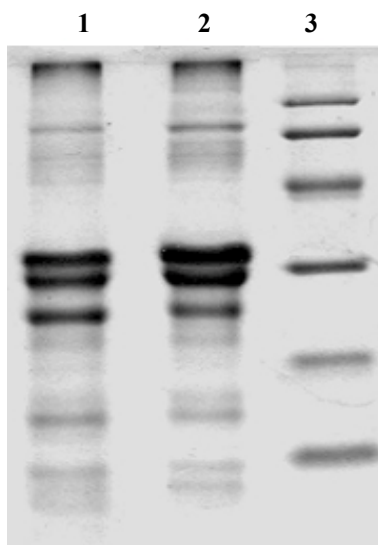


Figure 2. Electrophoregram of milk samples
 1 – fresh milk; 2 – reconstituted milk; 3 – markers of molecular weight
 (94, 67, 43, 30, 20, 14.4 kDa).

Table 1

Fractional composition of fresh milk proteins and reconstituted milk

Molecular weight fraction, kDa	Concentration of the fraction, mg / cm ³	
	Raw milk	Reconstituted milk
150 – 137	0.624	0.09
118 – 114	0.42	0.382
91 – 89	0.85	0.801
86 – 83	2.732	2.479
76 – 74	0.839	0.606
67 – 63	1.271	–
30 – 29	11.623	5.712
28 – 27	8.755	6.386
25 – 23	4.09	3.1
18	2.63	0.602
15 – 14	3.857	–
12 – 10	2.81	–
Cyта	40.5	21.45

The listed relative content of the main fractions of milk proteins to the total protein content in the investigated samples before and after heat treatment is given in Table 2.

Table 2

Fractional composition of milk proteins

Fraction name	Content, %	
	Raw milk	Reconstituted milk
Casein	60.4	70.8
β -lactoglobulin	6.4	2.8
α -lactalbumin	9.5	6.0
Lactoferrin	2.07	2.8
immunoglobulins of class G	1.54	–
albumin	3.1	–
γ -casein (12-10 kDa)	6.9	–
minor proteins	10.09	12.48

It is confirmed that in samples of the reconstituted milk separate fractions disappear, namely albumin, class G immunoglobulins and γ -casein. The content of β -lactoglobulin and α -lactalbumin is reduced by about 40%. The content of casein fractions is almost unchanged. Consequently, under the influence of heat treatment and drying, there are significant conformational changes in proteinaceous compounds caused by the denaturation of most serum proteins, in particular, immunoglobulins, serum albumin, β -lactoglobulin and α -lactalbumin. Almost constant content of casein against the background of a significant decrease in the total protein in milk can be attributed to other scientists by the process of complexation between denaturated β -lactoglobulin with α -lactalbumin and thermostable χ -casein with disulfide bridges, although other types of bonds are not denied [12, 13, 14, 15]

The dispersion of protein particles after aggregation and drying on average decreases, respectively, by 2.3 and 2.5 times. Pre-treatment and drying of milk cause slight changes in the structure of casein micelles, but after the restoration of dry milk in water there is a denser, compared with the initial milk, packaging micelle. This effect can be related to the denaturation of serum proteins and the interaction of casein micelles with calcium salts, which leads to more structuring of the system [13].

Consequently, skimmed milk, and casein, can form "cells" in spatial networks of smaller sizes than those observed in the TSD spectra of natural milk [16]. This is confirmed by the fact that the intensity of the relaxation maxima observed at -176 and -165, -170 and -162 °C, are correlated with each other and higher than the relaxation peaks in natural milk in the LT area.

The revealed effect has practical significance for understanding the patterns of formation of the structure during low-temperature processing of protein-containing systems [17]. Excessive freezing of free water with the formation of ice crystals in excess of 60 microns leads to the appearance of a coarse-crystalline structure in food systems [18, 19]. Therefore, it is expedient to use in the composition of foodstuffs milk-protein concentrates, protein matrices which have spatial constraints and keep clusters of water smaller than those for natural milk.

Conclusions

It was found using the method of TSD that dry milk protein concentrates (dried skim milk and sodium caseinate) in hydrated form produce spatial mesh with smaller cell sizes than natural protein, due to conformational changes of protein macromolecules under high temperature during the process of thickening and drying.

The TSD method allows obtaining information on the interaction of dairy proteins with a dispersion medium as moisture-retaining bulk matrices in food systems for the purposeful regulation of their physico-chemical characteristics.

Reference

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Effect of ultrafine grinding and high pressure technology on functional properties of soybean by-products

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Abstract

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Introduction. The content of soluble dietary fiber (SDF) and the taste and quality of soybean by-products can be improved by combination of ultrafine grinding-high pressure technology.

Material and methods. Soybean by-products; superfine grinding KCW-701S; high static pressure processing device FPG5620YHL; ultrafine grinding-high pressure technology set pressure:0, 50, 100, 150, 200 and 300MPa; material-liquid ratio: 1:3, 1:5, 1:7, 1:9 and 1:11; time: 5, 10, 15, 20 and 25 min.

Result and discussion. Ultrafine grinding has a significant impact on SDF of soybean by-products. As the frequency decreases, the SDF content gradually increased. When the frequency was 30 Hz, it reached the highest value of 27.11%, and it increased by 8.1% compared with control. When the frequency was less than 30 Hz, the SDF content drops sharply to 24.12%. Therefore, the ultrafine grinding frequency was the best at 30Hz, and SDF of soybean by-products was the highest.

When the pressure at 150MPa, the material-liquid ratio was 1:7, at 10min, the content of SDF had reached a maximum of 28.76% in soybean by-products, which was increased by 12.76%, it compared with the control group.

The water solubility, expansion, water and oil holding capacity of soybean by-products were lower, it compared with the control group. When the pressure at 150 MPa, water soluble content was minimum 11.24%. But with the rose in time, the minimum was 10.39% at 15 min. At 150 MPa for 10min, the expansion was 8.2mL/g, but when the processing time was 20 min, the expansibility was up to 8.8mL/g. The water and oil holding capacity of soybean by-products have similar trends. At 100 MPa, the pressure achieved the highest value, at 150 MPa, the water and oil holding properties was the smallest, but when the treatment time exceeds 10 min, the water and oil holding properties slightly increased.

Conclusion. The ultrafine grinding frequency at 30 Hz, and SDF of soybean by-products was the highest value of 27.11%. When the pressure was 150M Pa, the material-liquid ratio was 1:7, at 10 min, the SDF of soybean by-products has reached maximum, and it was 28.76%.

Introduction

Soybean residue is rich in nutrients, containing 50% dietary fiber, 25% protein, 10% fat, 33% isoflavones, slightly higher amino acid content than soy milk, as well as calcium, phosphorus, iron and B vitamins. Therefore, soybean by-products have the nutritional characteristics of high fiber, high protein, low fat and low reducing sugar, and is rich in mineral elements of potassium, calcium and magnesium et al [1–4]. Bean by-products had a certain health care function and were a good dietary fiber raw material [5–6]. With the development of nutrition and related disciplines, more and more studies had found that dietary fiber plays a very important role in human health. It was an indispensable nutrient in human healthy diet, especially in the health of digestive tracts. Comprehensively, dietary fiber has physiological functions, such as lower blood fat and blood sugar, improved the intestinal environment, and controlled body weight [7–8]. The bean by-products dietary fiber was mainly composed of cellulose, hemicellulose (dry weight content 40–60 g/100 g) and lignin. Dietary fiber includes soluble and in SDF. However, SDF has a potential "prebiotic" label [9–10]. SDF has high viscosity and strong water holding capacity. It can be used by intestinal microorganisms and slow down the digestion rate. And delay the body's absorption of water compounds by carbon bodies, have a role in decreasing postprandial blood glucose, and improve insulin sensitivity. Ingesting abundant SDF can also accelerate cholesterol excretion, control blood sugar and cholesterol levels, and prevent cardiovascular disease, obesity, diabetes, and other diseases.

The content of SDF and the taste and quality of soybean by-products can be improved by different processing technologies. Nowadays, ultrafine grinding technology can effectively improve the functional properties of bean by-products dietary fiber [11–12]. Ultrafine grinding technology has been extensively used in various fields such as food, chemical, pharmaceutical, cosmetic pesticides, dyes, coatings, electronics and aerospace. High pressure technology refers to the sealing of food materials in an elastic container or pressure-resistant device system, the pressure conditions are generally (100–700 MPa), it often used water or other fluid medium as a medium to achieve sterilization, and change materials, the purpose of physical and chemical properties. In recent years, high pressure technology has been used in the food industry as a new technology, but high pressure was commonly used in meat products, vegetables, beverages, jam and so on. There are few studies on high pressure in cereal products, and most of them are concentrated on cereal starch. It has a modification effect on starch slurry, which can destroy the surface and internal structure particles of starch [13–14]. However, there is likewise a small part in the research of soybean by-products. For example, soybean by-products were processed by high pressure technology, SDF content of soybean by-products can be increased [15–17].

However, the combination of ultrafine grinding-high pressure technology for soybean by-products, there has been no research report. This paper uses soybean by-products as raw materials by ultrafine grinding-high pressure technology to treat soybean by-products. The SDF and functional properties of soybean by-products were analyzed by ultrafine grinding-high pressure technology. It provides theoretical reference to further research and application of soybean by-products in food.

Materials and methods

Material

Soybean by-products.

Reagent

Reagents such as absolute ethanol and acetone are of analytical grade.

Instrument

Superfine grinding KCW-701S; high static pressure processing device FPG5620YHL; multifunctional vacuum packaging machine DRZ-420; electric thermostatic water bathed HWS-26; low-speed desktop centrifuge TDL-40B; electric heating constant temperature blast drying box DHG-9140A.

Experiment methods

Operating procedures. Soybean by-products → hot air drying → ultrafine grinding → vacuum packaging → high pressure → dry → reserved.

The soybean by-products powder was diluted with water, and the material-liquid ratio was about 1:7. Put it in the packaging bag and seal it, and put it into the machine for high pressure treatment. Freeze the processed raw materials for 24 hours, freeze dry for 48 hours, pass through 80 meshes of sieves and reserve.

The preparation of bean by-products was slightly modified: Used 500g of clean soybeans, add 1000g of water, soak for 3h, put in a soybean milk machine to squeeze, and filter out excess water to obtain fresh wet bean by-products [18].

Experimental design. The SDF in soybean by-products as the main index, and determine the optimal frequency of ultrafine grinding. On this basis, high pressure processing, and determined the best conditions. Pressure: 0, 50, 100, 150, 200 and 300 MPa, in order to save time, for 10 min respectively, material-liquid ratio: 1:7. According to the highest SDF content, the pressure conditions are selected. Under a fixed pressure, analyze different material-liquid ratios: 1:3, 1:5, 1:7, 1:9, 1:11, and select the material-liquid ratio of the highest SDF content; Then determine the different processing time: 5, 10, 15, 20 and 25 min. It can be concluded under what conditions the bean dregs has the highest SDF content, and analyzed the functional properties in soybean by-products of ultrafine grinding- high pressure technology.

Effect of ultrafine grinding – high pressure technology and ultrafine grinding frequency on SDF of soybean by-products. Effect of different pressure, material-liquid ratio and processing time on SDF of ultrafine soybean by-products powder. Determination of SDF: GB/T 37492–2019 «Inspection of grain and oils – Determination of SDF in cereals and cereals products — Enzyme gravimetric method».

Effect of high pressure on functional properties, different pressures on water solubility content, treatment time on water solubility content of ultrafine soybean by-products powder. Refer to [19]. A sample of 0.500 g of soybean by-products was weighed and placed in 200 mL beakers. 50 mL of distilled water was weighed and placed in a constant temperature water bath at 90 °C for constant stirring. After 30 min, centrifuge at 3000 r/min for 15 min, pour the resulting supernatant into a Petri dish, and dry to a constant weight at 105 °C to weigh the residue (the total mass of the Petri dish and residue – Petri dish quality).

$$\text{Water solubility} = \frac{\text{Residue mass}}{\text{Sample quality}} \times 100\%.$$

Effect of different pressures and treatment time on expansion of ultrafine soybean by-products powder. Measurement method was referred to [20–22]. Weigh 1.000 g of the sample of a container with a graduated surface, add 10 mL of distilled water, stir, and let it stand at room temperature for 24 h. Record the volume of the sample at this time.

Calculation method: set the sample mass to N_0 , and the expanded volume was N_1 expansion = Volume after expansion ÷ Sample mass × 100%.

$$\text{Expansion} = \frac{N_1}{N_0} \times 100\%.$$

Effect of different pressures and treatment time on expansion of ultrafine soybean by-products powder. Determination of water holding capacity (*WHC*): weigh about 0.2 g of soybean by-products into a centrifuge tube, add 10 mL of water, stir evenly, place at room temperature for 1 h, centrifuge at 3000 r/min for 20 min, discard the supernatant, and weight of the centrifuge tube [20, 21].

$$\text{WHC} = (m_1 - m_2 - m_3) / m_3$$

where m_1 – centrifuge tube and residue mass after centrifugation; m_2 – centrifuge tube mass; m_3 – dry weight of sample.

Effect of different Pressures treatment time and on oil holding capacity (*OHC*) of place it in a constant weight centrifuge tube, add 5 mL of soybean oil, mix well, and let it stand for 30 min, shaking once every 5 min. After that, centrifuge at 4500 r/min for 25 min to remove the upper loose fat and weigh the total mass of the centrifuge tube and residue [23].

$$\text{OHC} = (m_1 - m_2 - m_3) / m_3$$

where m_1 – centrifuge tube and residue total mass; m_2 – centrifuge tube mass; m_3 – sample mass.

Statistical designs and data analysis. All data were assayed at least three times and the results were expressed as mean standard deviation ($\bar{x} \pm \text{SD}$). Data and mapping were analyzed used statistical software WPS (Excel), and SPSS analysis software was utilized to test. The level on which significant differences were reported was setting $p < 0.05$.

Results and discussion

Effect of ultrafine grinding frequency on SDF of soybean by-products

As can be seen from Figure 1, ultrafine grinding significantly increased the content of SDF in soybean by-products, compared with ordinary pulverization technology. When the frequency at 40 and 30Hz, the SDF content was 27.03% and 27.11%, and the difference was significant, compared with other results ($p < 0.05$). When the ultrafine grinding frequency at 20–10Hz, the content of SDF suddenly drops. This was because part of the structure of the bean by-products was damaged during crushing. Insoluble hemicellulose and insoluble pectin compounds, melting phenomenon or partial bond breakage. Thereby, it was transformed into a small soluble molecule substance, which leads to increase to SDF in soybean by-products [24–26]. Below 30Hz, the structure of the soybean by-products was severely damaged, and the powder particles reached a certain fineness, which causes the small particles to aggregate with each other, adhere to the inner wall of the machine cavity, and the output rate decreases, which hinders the release of SDF in the soybean by-products. At this time, the molecular structure of soybean by-products tended to be stable, and there was no further changed, and the content of SDF also remained basically unchanged [27]. Therefore, when the ultrafine grinding frequency at 30Hz, the SDF content reached a maximum of 27.11%, which was 8.1% higher than ordinary pulverization. Coupled with the previous research results [28],

When the frequency at 30Hz, the soluble dietary fiber content of soybean by-products was the highest.

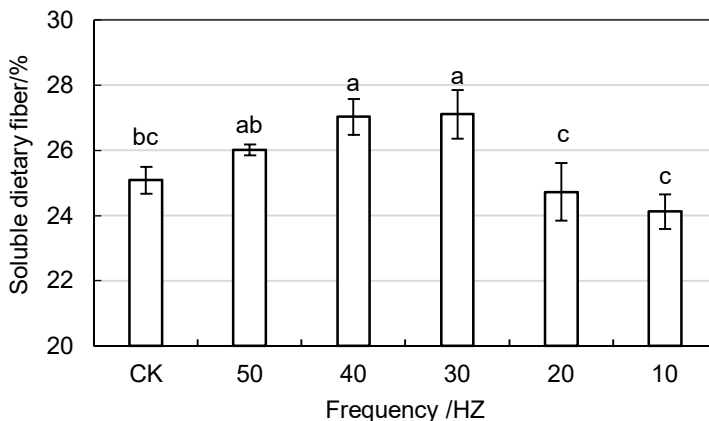


Figure 1. Effect of ultrafine grinding Frequency on SDF of soybean by-products
^{a-c} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of ultrafine grinding – high pressure technology on SDF of soybean by-products. Effect of different pressure on SDF of ultrafine soybean by-products powder

It generally considered that the pressure exceeding 100MPa was high pressure. It can be seen from Figure 2 that different pressure has a greater impact on the solubility of soybean by-products.

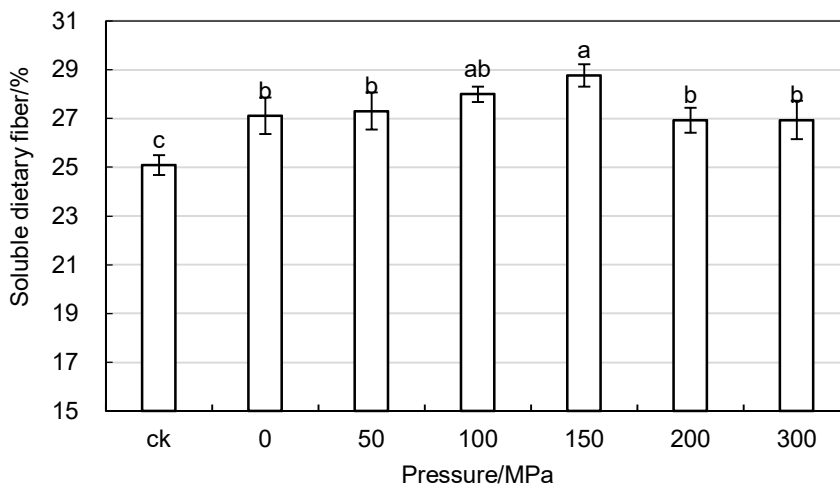


Figure 2. Effect of different pressure on SDF of ultrafine soybean by-products powder
^{a-d} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

With increasing pressure, the content of SDF in soybean by-products gradually increased. When the pressure was 150 MPa, the content of SDF reached a maximum of

28.76%, an increase of 12.76% compared with the control group, and an increase of 5.7% compared with ultrafine grinding (0 MPa). When the pressure at 300 MPa, the content of SDF in soybean by-products dropped to 26.94%, and it was located in a stable trend. When the bean by-products were subjected to ultrafine grinding, the cohesive force inside the bean by-products was broken, and the particle size becomes fine. On this basis, high pressure processing and instantaneous processing have produced extremely high static pressure and accompanying forces, which can change the dense tissue structure of bean by-products, so that SDF was made public from the cells [29]. Therefore, ultrafine grinding-high pressure technology can significantly increase the content of SDF in soybean by-products. Among them, SDF content of soybean by-products obtained was the highest at 150 MPa.

Effect of material-liquid ratio on SDF of ultrafine soybean by-products powder

It can be seen from Figure 3 that different material-liquid ratios have a great influence on the SDF of soybean by-products. Due to the strong water absorption of soybean by-products, at 1:3, the soybean by-products did not get sufficient water absorption, and the degree of destruction under high pressure was insufficient, so the SDF content was low. However, with the increase of water content, the content of SDF gradually increased. The value was the highest and reached 28.76%. However, with the increase of water content, when the material-liquid ratio exceeds 1:9, the paste was thin and susceptible to precipitation. Precipitation may occur in the compression process. The structure of the space where the bean by-products gather with each other changes, and the release of SDF content was limited. Therefore, SDF content was the highest by high pressure, when the ratio of material to liquid was 1:7. Treated soybean by-products under high pressure, used water as a pressure transmitting medium, thereby destroy the internal structure of soybean by-products. The combined effect of pressure and moisture was the main reason for the increase in SDF content [30].

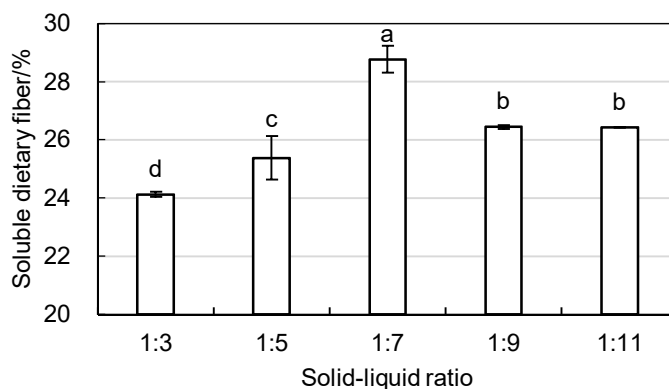


Figure 3. Effect of material-liquid ratio on SDF of ultrafine soybean by-products powder
^{a-d} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of processing time on SDF of ultrafine soybean by-products powder

It can be seen from Figure 4 that different times has a greater influence on the solubility of soybean by-products. When the processing pressure at 150 MPa and the material-liquid ratio was 1:7, when the cycle of 1, With the continuous extension of the high pressure treatment time, the SDF content of soybean by-products gradually increased. When the time for 10 min, the maximum content of SDF in soybean by-products was 28.76%. Compared with ultrafine grinding (0 MPa), it was 5.7% higher. When the treatment time exceeds 10 min, the content of SDF in soybean by-products gradually decreases, and after 20 min, it was basically in a stable trend. Therefore, treatment for 10 min has a great influence on the content of SDF of soybean by-products.

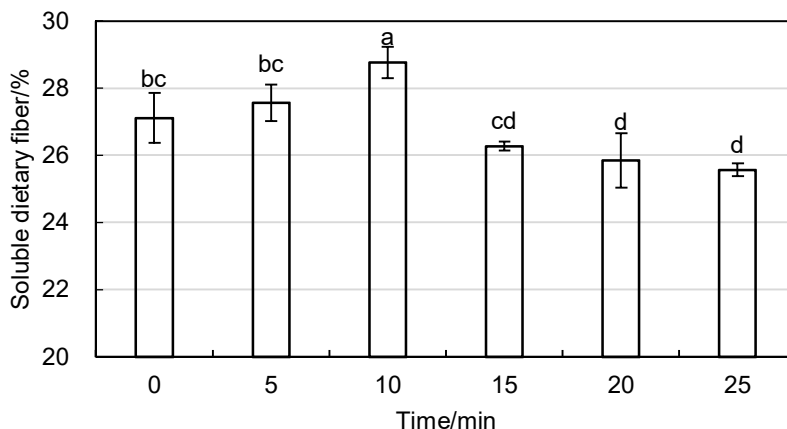


Figure 4. Effect of processing time on SDF of ultrafine soybean by-products powder
^{a-d} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of high pressure on functional properties of ultrafine soybean by-products powder. Effect of different Pressures on water solubility content of ultrafine soybean by-products powder

It can be seen from Figure 5 that when the pressure 0 MPa (30 Hz), the water solubility content reached 20.84%, which was significantly different from the other groups ($p < 0.05$). Compared with ordinary soybean by-products (CK), the water solubility was increased by 73.38%. However, with the increase of pressure, the water solubility content gradually showed a downward trend. At 150 MPa, the effect was the greatest, and the lowest water solubility content was 11.24%. This may be explained by the bean by-products were rubbed by mechanical force during crushing. The resultant force was applied to the high pressure treatment. The soybean by-products were damaged under a certain pressure, the protein existing on the bean by-products was denatured, the hydrophobic groups were turned out, and the hydrophilicity was greatly reduced, which leads to the easy precipitation of the proteins and affects the water solubility of the soybean by-products. Therefore, different pressure can affect the water solubility of soybean by-products of ultrafine grinding-high pressure technology treatment.

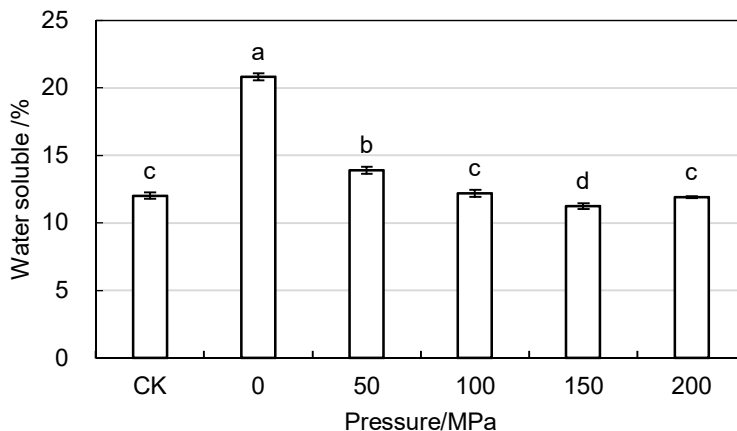


Figure 5. Effect of different Pressures on water solubility content of ultrafine soybean by-products powder

^{a-d} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of different Pressures on expansion of ultrafine soybean by-products powder

The expansibility forced means that the fibrous substance in the material will increase to volume after absorbing water, forming a certain feeling of satiety in the human stomach and intestines. As can be seen from Figure 6, the expansion of soybean by-products reached the minimum by ultrafine grinding-high pressure technology treatment. However, with the increase of pressure, the expansibility of the bean by-products powder gradually increased. The expansibility property was 7.8 g/mL, at 50 MPa, but there was almost no obvious change in the range of 100-150 MPa. Therefore, change of expansibility properties of superfine grinding-high pressure treated soybean by-products was mainly affected by pressure.

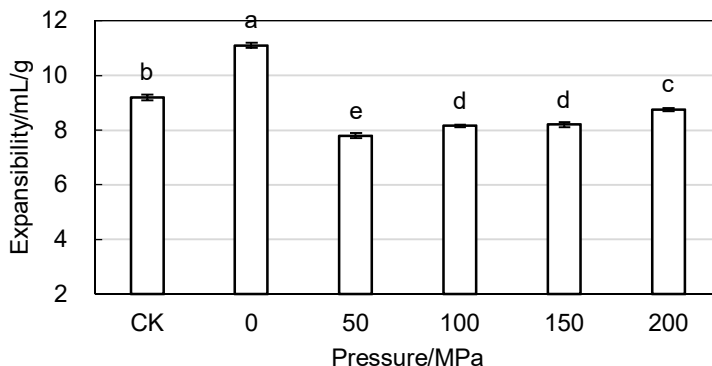


Figure 6. Effect of different Pressures on expansibility of ultrafine soybean by-products powder

^{a-c} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

This phenomenon may be that the soybean by-products undergo a secondary change in the spatial network structure. Under certain pressure conditions, and some of the capillary structure was damaged, which reduces its ability to restrain water molecules and causes the powder to expand weakly. It was the same as Cheng Jiao-Jiao research conclusion [31].

Effect of different pressures on water holding capacity of ultrafine soybean by-products powder

The water holding capacity of soybean by-products indicates its ability to absorb water. It can be seen from Figure 7 that the maximum water holding capacity of ordinary dregs (CK) was 10.92 g/g, which was significantly different from other groups ($p < 0.05$). The ultrafine grinding – high pressure treatment makes the overall capacity of water retention of soybean by-products lower, but when the pressure was 100 MPa, water holding capacity reaches 5.73 g/g, when it was above 150 MPa, it gradually shows a stable trend. This may be the ultrafine grinding treatment, so that more long-chain molecules in the dietary fiber molecules were converted into short-chain small molecules, adding more hydrophilic groups. After high pressure treatment again, the binding force of the molecular hydrophilic groups was instantly weakened, and the water cannot be blocked. Under high-speed centrifugation, water molecules in the material cannot be found. Therefore, ultrafine grinding-high pressure treatment reduces the water holding capacity of soybean by-products [32].

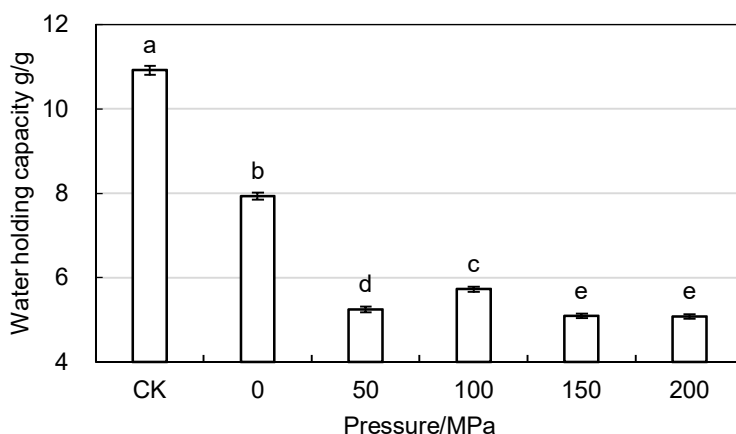


Figure 7. Effect of different Pressures on water holding capacity of ultrafine soybean by-products powder

^{a-c} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of different Pressures on oil holding capacity of ultrafine soybean by-products powder

The dietary fiber in soybean by-products can absorb the fat in food and excrete it from the body, reduce saturated fatty acids in the human body, and achieve weight loss. It can be seen from Figure 8 that under high pressure treatment, the oil holding capacity and water holding capacity of soybean by-products powder were similar, which were smaller than those of other groups, and the overall maximum were 51%, a significant difference ($p < 0.05$).

Under the action of ultrafine grinding (0), the space structure of bean by-products was opened, and the fat is retained in the gap. The high pressure force once again destroys the inside of the soybean by-products, forming a multi-level space. At this time, the structure was relatively loose, causing the oil inside the soybean by-products cells to flow out, attach to the surface of the bean by-products, and weaken the interaction with the vegetable oil. Under the action of centrifugal force, the ability of soybean by-products to bear fat becomes weak. Therefore, ultrafine grinding-high pressure treatment makes the oil holding power of soybean by-products powder weaken, which has a greater relationship between the pressure.

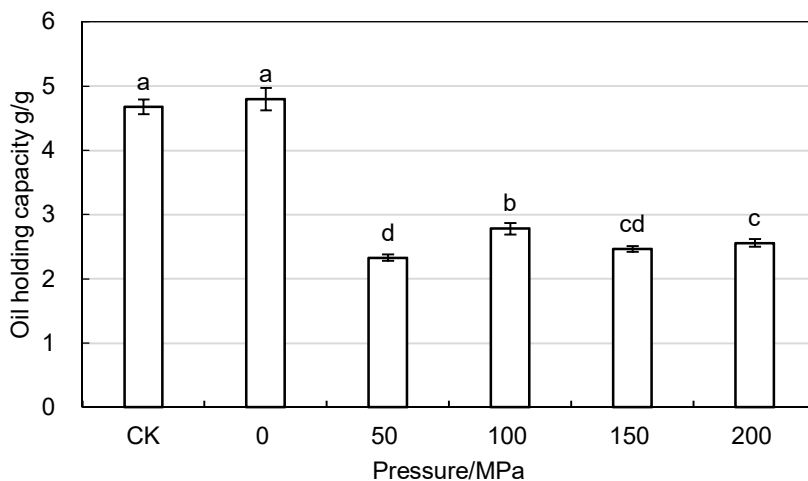


Figure 8. Effect of different Pressures on oil holding capacity of ultrafine soybean by-products powder

^{a-d} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of high pressure treatment times on functional properties of ultrafine soybean by-products powder. Effect of treatment times on water solubility content of ultrafine soybean by-products powder

It can be seen from Figure 9 that the soybean by-products undergo a high pressure treatment, and the water solubility content changes drastically with the increase of the treatment time. When the treatment time was 5min, the water solubility content was 11.51%, which was a decrease of 44.77%, compared to other treatments, which were significant ($p < 0.05$). When the processing time was 10 min, the highest water soluble content was 11.24%, and when the processing time was 15 min, the lowest water solubility content was 10.39%. When the treatment time was above 20 min, the water solubility content does not change significantly. It shows that the processing time was between 10–15 min, the structure of the soybean by-products changes, the protein was denatured, and the hydrophilicity was weakened, which affects the water solubility of the bean by-products.

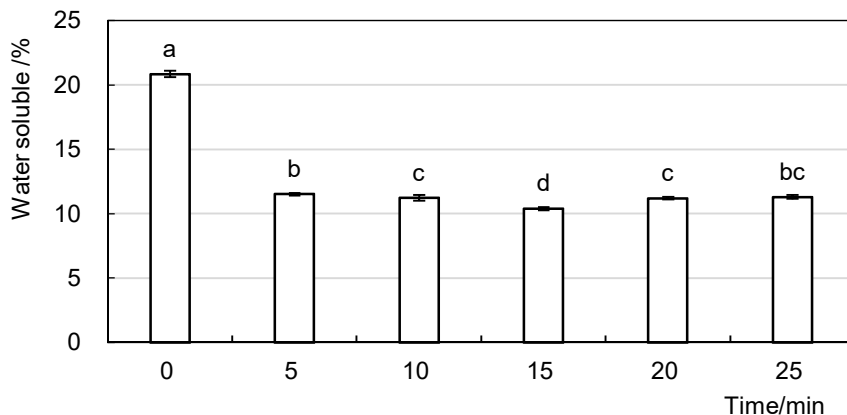


Figure 9. Effect of treatment time on water solubility content of ultrafine soybean by-products powder

^{a-d} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of treatment times on expansion of ultrafine soybean by-products powder.

It can be seen from Figure 10 that expansibility property of the soybean by-products was lower than 0 MPa as a whole, and the difference was significant ($p < 0.05$). It was demonstrated that when soybean by-products enter the high pressure cavity for 5 min, an instantaneous pressure difference can be generated, and the structure of soybean by-products begins to loosen. As time is there, a multi-dimensional network structure was gradually formed into the soybean by-products, which can absorb more moisture, which results in an increase in expansibility. The expansion at 20 min was the highest at 8.8 mL/g. But more than 20 min, the space structure of soybean by-products gradually closed, the bearing moisture weakened, resulting in reduced expansibility.

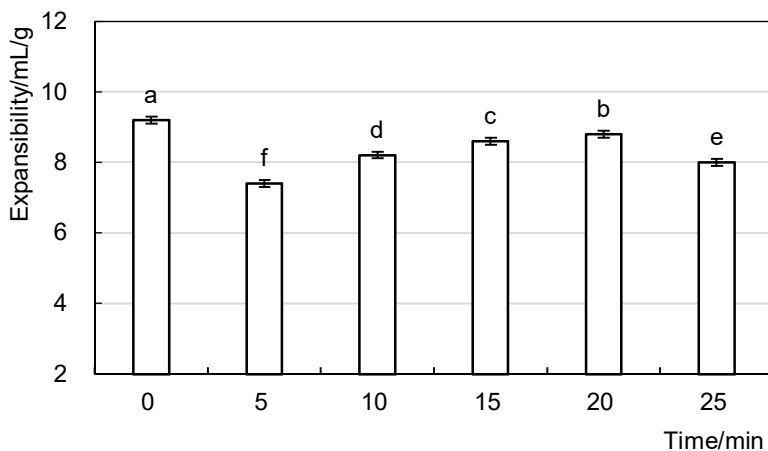


Figure 10. Effect of treatment time on expansion of ultrafine soybean by-products powder

^{a-f} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of treatment time on water holding capacity of ultrafine soybean by-products powder

It can be seen from Figure 11 that after different treatment time, the water holding capacity of the soybean by-products was lower than 0 MPa. However, as time is there, the water holding capacity of soybean by-products was picking up. At 10 min, the water holding capacity was the weakest, and the structure of the soybean by-products was probably the most loose. The soybean by-products cannot support the absorbed moisture, especially under the action of centrifugal force. The bearing capacity was weak, resulting in poor water holding capacity. But at 25min, the water holding capacity maximum was 7.21 g/g, which indicates that under this condition, the structure of soybean by-products changed from loose and porous to aggregate, the particles condensed with each other, and the binding force of water was tight.

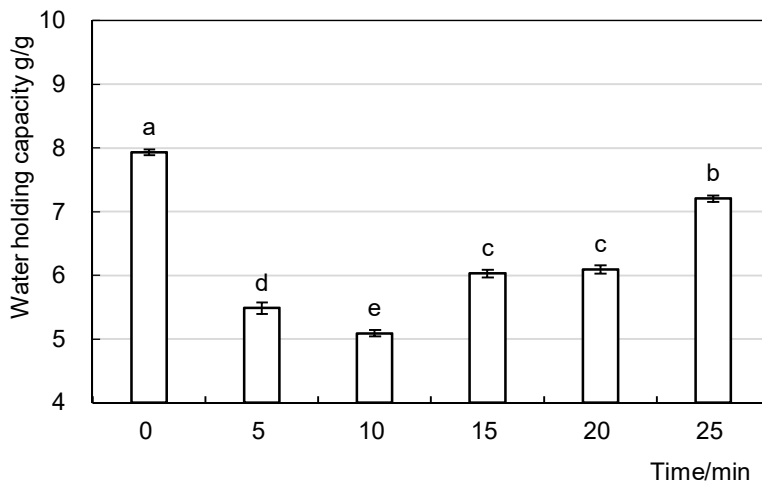


Figure 11. Effect of treatment time on water holding capacity of ultrafine soybean by-products powder

^{a-d} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Effect of treatment time on oil holding capacity of ultrafine soybean by-products powder

It can be seen from Figure 12 that weakest oil holding capacity was 2.47 g/g at 10 min, which was 48.54% lower than that of the control group, but the oil-holding capacity increased significantly when the treatment time was above 15 min. At 25min, the oil retention was 5.96 g/g. It shows that the structure of ultrafine soybean by-products powder has undergone a series of changes for 5–25 min compression. After processing for 5–10 min, the structure of the soybean by-products was gradually opened. At this time, a small amount of oil enters the inside and the surface of the soybean by-products, but the bearing capacity of the soybean by-products was weak, and it was difficult to block the oil. At 15–25 min, the spatial structure of soybean by-products gradually changed from open to closed. With the extension of time, when the processing time was 25 min, the soybean by-products particles were aggregated with each other, which causes the internal oil to be restrained, and the oil holding capacity for the highest.

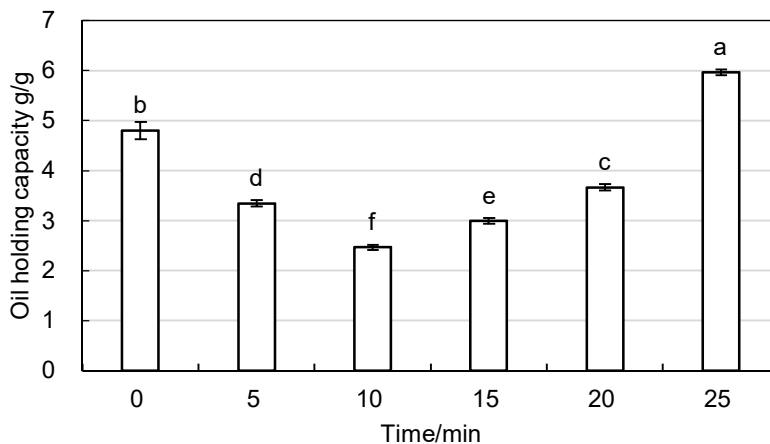


Figure 12. Effect of treatment time on oil holding capacity of ultrafine soybean by-products powder

^{a-f} Different parameter superscripts in the figure indicate significant differences ($p < 0.05$)

Conclusion

The SDF of soybean by-products has a significant effect by ultrafine grinding. As the frequency decreases, the SDF content gradually increased. When the frequency was 30Hz, the highest value was 27.11%, which was 8.1% higher than the control. This could be caused by the structure of soybean by-products was damaged in a specific frequency range. Insoluble hemicellulose, and insoluble pectin compounds undergo melting or partial bond breakage, converted into soluble small molecular substances, increasing the SDF content. When the frequency was less than 30 Hz, the content of SDF dropped sharply to 24.12%. When the frequency reached a certain fineness, the small particles aggregate with each other, hindering the release of SDF in the soybean by-products, and a part from the soybean by-products powder adheres to the inner wall of the machine cavity, and the discharge rate was reduced. Therefore, the ultrafine grinding frequency at 30 Hz, and the content of SDF in soybean by-products was the highest.

High pressure treatment was performed on the basis of ultrafine grinding. With the increased in pressure, the content of SDF in soybean by-products moderately increased. When the over pressure at 150 MPa, the content of SDF in soybean by-products reached maximum was 28.76%, which was 12.76% higher than the control group, and 5.7% higher than ultrafine grinding (0MPa). When the pressure was greater than 150 MPa, the content of SDF in soybean by-products decreased to 26.94%, and it was stable at 300 MPa. When the soybean by-products were subjected to ultrafine grinding, the cohesive force inside the bean by-products was broken, and the particle size becomes fine. On this basis, high pressure processing and instantaneous processing have produced extremely high static pressure and accompanying forces, which can change the dense tissue structure of bean by-products, so that SDF was released from the cells. With the increased in pressure, the cell wall of soybean by-products was a process from relaxation to destruction. Above 200 MPa, the soybean by-products were severely damaged, which instead affects the dissolution of the SDF content, it was not that the higher the pressure, the higher the SDF content. Therefore, ultrafine grinding-

high pressure technology treatment can significantly improve the SDF of soybean by-products at 150 MPa.

When the material – liquid ratio was 1:3, the soybean by-products do not get sufficient water absorption, and the SDF content was low. The material-liquid ratio was 1:7, and the viscosity of the paste was moderate. At this time, the highest value was 28.76%. However, with the increased of water content, when the material-liquid ratio exceeds 1:9, the paste was thin and susceptible to precipitation. At this time, the content of SDF in soybean by-products gradually decreased. Therefore, the optimum condition of the material-liquid ratio was 1:7. Used water as a balanced transmission medium for pressure, the combined effect of pressure and moisture was the main reason for the increased in SDF content of soybean by-products.

With the continuous increased in treatment time, the content of SDF of soybean by-products gradually increased. When the processing time was 10min, the SDF content reached a maximum, it was 28.76%, longer than 10min, it gradually decreased, and it was basically stable after 20min. Therefore, the processing time was 10 min, and the content of SDF was the highest in soybean by-products.

The water solubility, expansibility, water and oil holding capacity of soybean by-products were lower, by ultrafine grinding and high pressure technology, than those of the control group. When the pressure at 150 MPa, the minimum water-soluble content was 11.24%, but with the increased of processing time, the minimum was 10.39% at 15min. The expansibility of bean dregs was 8.2 mL/g at 150MPa for 10min. Nevertheless, when the processing time was 20min, the maximum expansibility was 8.8 mL/g. The water and oil holding capacity of soybean by-products have similar trends. In the high pressure range, the pressure reached the highest value at 100 MPa. At 150 MPa, the water and oil holding capacity were the smallest. However, when the treatment time at 150 MPa exceeds 10 min, the water and oil holding capacity gradually increased.

Therefore, the pressure and time has a significant impact on soybean by-products. Comprehensively shows that the pressure at 150 MPa, the material-liquid ratio was 1:7 for 10 min by ultrafine grinding-high pressure technology, the content of SDF in soybean by-products reached maximum, it was 28.76%. Compared with the control group, it was increased by 12.76%, and it was increased by 5.7%, compared with ultrafine grinding (0 MPa). This conclusion can provide an important basis of the development of SDF food.

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Effect of cereals milling on the contents of phytic acid and digestibility of minerals and protein

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Abstract

Keywords:

Cereal
Debranning
Mineral content
Phytic acid
Milling
Bioavailability

Introduction. The aim of the study was to the examination of effects of the milling process which were applied differently to cereals for commercially flour production, on the phytic acid, microelement, and *in-vitro* digestibility.

Materials and methods. The nutritional consequences of the milling processes of the cereals (wheat, barley, rye, oat, paddy) were evaluated by examining protein, ash, phytic acid, mineral contents, and protein/mineral digestibility rates. Mineral contents were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES), protein content by Kjeldahl method, and mineral digestibility and phytic acid were determined by *in-vitro* assays.

Results and discussion. The dehulling/debranning process increased the protein digestibility rate over to 65% and decreased phytic acid content significantly ($p < 0.05$) of the cereal grains except for rye. The mineral digestibility raised for all grain samples, but it was evaluated statistically insignificant ($p > 0.05$). The rice flour was the sample that had the lowest phytic acid content (921.87 mg/100g) and the highest mineral digestibility rate (58.35%). Although the contents of total ash and some of the minerals (Na, Ca, K, Mg, Zn, Fe, Ba, and P) decreased in flours especially in wheat and paddy, the rate of some minerals (Na, Mg, Zn, Fe, and Al) increased due to their distribution and localization in the grain layers, especially in rye and oat. The result of the study has shown that the level of the minerals changed depending on the bran/hull content of the grains, and the milling process was more effective on phytic acid content and on protein digestibility than on the mineral digestibility. While the phytic acid content was located on the outer layer of the grain, it was decreased in the kernel.

Conclusions. Although the phytic acid content which affects the digestibility of the cereals were reduced by the milling process, the combination of different treatments such as soaking, fermentation could be suggested for improving the nutritional quality of the cereal.

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Introduction

Cereals have been the staple foods in many countries and they are the main carbohydrate and energy sources in the human diet. They are also a major source of proteins, minerals, dietary fiber and some micronutrients which are located in the grain fractions at different rates [1]. The main fractions of the wheat grain and other cereals are endosperm, bran and germ. Moreover, the barley, oat and rye have a hull layer which has a high level of ash content. The endosperm consists of starch granules embedded in a protein matrix. A large part of the storage proteins in cereals, especially in rye and oats, are found in the endosperm. The part called bran which is found at the rate of 14-16% in the grains, consists of pericarp, testa, hyaline and aleurone layers. Aleurone layer, which is located just above the endosperm and constitutes an important part of the bran layer, is very rich especially in terms of mineral content such as K, Mg, Ca, P, Na, Al, Fe and Zn [2,3]. The different minerals distribute in different layers across the grain. This change come together with the factors of debranning and milling process, it may be seen an increase or decrease in various minerals after milling [4]. For example, in the milling process of the wheat, the aleurone layer which rich in mineral contents is usually removed along with the other external layers from the endosperm, and a flour which has lower mineral content than whole grain flour is obtained [5]. The rate of the ash in the different fractions with respect to grain variety changes like as the minerals, and the rate increases from the inner towards external layers [6]. Therefore, the dehulling/debranning process make the ash content of the grain decrease, and the rate of ash (%) is accepted as an indicator which is exhibited the separation efficiency of the bran from the endosperm [7].

Since their better technological and sensory properties, the refined cereals may be preferred more than whole grain or bran-enriched products. However, the growing interest to natural, functional and minimally processed foods is influencing the preference of customers in recent years. Especially the cereal grains which taken an important part of the daily diet have the potential usage in the production of enriched functional foods [8]. On the other hand, the antinutrients which are another aspect of the outer fraction of the cereals may be seen as a negative factor for daily diet. For instance, the bran layer is much richer in minerals than endosperm. However, its minerals have low digestibility. Because the minerals located in bran are physically entrapped in strong cell wall structures and they also occur as phytate form in bran and germ [4]. Therefore, the fractions of the cereal grains should be evaluated separately with respect to the advantage of nutritional components and disadvantage of antinutrients. Because, while the nutrient compounds such as mineral and protein located in different layers in the grain and their location and rates changed according to cereal types, the level and distribution of antinutrients such as phytic acid also affect from same factors. In several studies were reported that the bran layer of the several kinds of cereal such as wheat and rice contains a high amount of phytic acid which acts as an antinutrient substance [9, 10]. The phytic acid which is the main antinutrient compound found in cereals reduces mineral bioavailability and protein absorption of them thanks to its chelating properties. It causes micronutrient malnutrition and mineral deficiencies which are a widespread global health problem in many countries. Dehulling/debranning methods as well as other pretreatment and processing techniques as soaking, fermentation, germination, can reduce the phytic acid which is especially located in the outer layer of the cereals. [11].

The debranning/dehulling process is the pre-milling stage which is removed the external layers (hull and/or bran) along with the aleurone layer of the kernel [6] in modern mills. Moreover, allowing to obtain endosperm layer that is produced refined flour. However, the level of the bioactive compounds, such as fiber and phenolic compounds and minerals

deployed in the outer layer may decrease [8]. On the other hand, the whole grain of some cereals such as rye and barley is ground in the stone mills commercially, and then the bran/hull particles are separated from the flour. Even if optimum conditions are provided, a little amount of bran may mingle to the flour in the milling process. This situation affects the ash content and the composition of the minerals of the flours [12].

The aim of the study was to evaluate the relationship between the nutritional content of some grains (wheat, barley, rye, oats, and paddy (whole rice)) and the debranning/dehulling treatment which was the main stage of the milling process. The effects of these treatments were examined by ash, protein and mineral content as well as indirect methods such as mineral and protein digestibility. The data obtained before and after the milling process were evaluated statistically.

Materials and methods

Materials

In this study, the wheat sample named as "Ekiz wheat" (*Triticum aestivum*), were supplied from the grains which harvested in July 2017 at Devrekani, Kastamonu (Turkey). It was milled in a modern milling factory (Üçbaşak Milling, Devrekani, Kastamonu) to produce flour. Before milling, the wheat grains were cleaned (by used differences in size, shape, color, specific weight and magnetic force to separate foreign material from the grains). The kernels were first hydrated for tempering (app. 2%), ground, sifted and separated (milling yield 70%). The wheat flour was sifted by 212-micron sieve because of that min 98% of it must pass from the sieve according to Turkish Food Codex, Wheat Flour communique. The hulled rice (paddy) and rice sample were supplied from a local rice producer (milling yield 68%) in Tosya district of Kastamonu. For preparing of rice flour, the dried rice grains were milled with a laboratory mill (EQM-402 Mixer Mill, Spain) in Kastamonu University Central research laboratory. The mill used was designed for the crushing, and milling of hard or semi-hard samples. The barley sample which was named "Aydan Hanım", local oat and the rye which were named "Black rye" were supplied from the grains which were harvested in 2017 from Gövdecili village of Yozgat (Turkey), and the samples were milled by the traditional stone mill in a local company (İhsangazi, Kastamonu). Before milling, the grains were hydrated by adding 200 g of water to 10 kg of the kernel to make the seed coats less brittle and prevent kernel breakage [13]. The milling processes of barley, oat, and rye performed in the stone mill. The grains were separated from the foreign material (straw and stones) by passing through the selector and then followed the stages of, hydrating/conditioning, grinding (for open the kernel and scrape off the endosperm), sizing and sifting (sieving) respectively. The stone mill made from chiseled emery stone was used in this study. The cleaned grains were fed through a hopper in to between the two plates and ground to flour. The flours were sifted through a 60-mesh sieve.

Physico-chemical properties

Ash and protein content were determined according to the AACC methods [14]. All the parameters were reported on dry weight basis. The protein content was performed by using Kjeldahl method. The amount of nitrogen determined for the expression of the results was multiplied by 5.70 for wheat, and flour and 5.83 for barley, rye, oats, rice and flour.

Table 1

Supplying properties of the raw materials

Sample	Space	Source (district/city)	Milling technique	Supplier/Miller
Wheat	Ekiz wheat (<i>Triticum aestivum</i>)	July 2017 Devrekani/ Kastamonu	Modern milling process (Cleaning, tempering, milling, sifting and separating)	Üçbaşak Milling Factory/ Devrekani/ Kastamonu
Paddy/rice	Tosya rice	August 2017 Tosya/ Kastamonu	Modern milling process (Pre-cleaning, husking, paddy separation, polishing)	Atılım Ticaret/ Tosya/ Kastamonu
Rice flour			Laboratory mill (EQM-402)	Kastamonu University Central research laboratory
Barley	Aydan hanım	July 2017 Gövdecili/ Yozgat	Traditional stone miller (Cleaning, hydrating/ conditioning, grinding, sizing and sifting)	Mergüze Organik Tarım/ İhsangazi/ Kastamonu
Oat	Local variety			
Rye	Black Rye (local variety)			

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

The sample (1 g) was incubated with 25 mL of pepsin solution (0.03 N 1 L HCl+2 g pepsin) at 37 °C for 3 h. Each sample was filtered by an ashless filter paper. The pellet and filter paper were burned together in the furnace at 900 °C and the ash value was calculated. The digestible mineral content was obtained with their differences. The MD value (%) was obtained [15] by using the following equation:

$$MD\% = \frac{\text{Digestible Mineral Content}}{\text{Total Mineral Content}} \times 100$$

The *in vitro* PD values determined by the method of Rizzello *et al.* [16]. PD values (%) were obtained by using the following equation:

$$PD\% = \frac{N \text{ in supernatant} - N \text{ in pepsin enzyme}}{N \text{ in sample}} \times 100$$

Phytic acid content

For determination of phytic acid content, the seed samples were grounded and the phytic acid extraction was carried out by adding 10mL of Hydrochloric acid (0.2 N) to 0.5g and placed on the horizontal shaker at room temperature for 24 h. The sodium salt of phytic acid ($C_6H_6O_{24}P_6Na_{12}$) obtained from Sigma (D216305) was used to preparing the phytate reference solution. The stock solution was prepared 0.15 g of sodium phytate in 100 ml of distilled water. The reference solution was prepared with diluting the stock solution with HCl concentration in the range from 3 to 30 $\mu\text{g/ml}$. The phytic acid was analyzed in the protocol as described by Haug and Lantzsch [17].

Mineral content

The mineral content was measured by using the microwave (Milestone MLS 1200, Italy) nitric acid digestion procedure and it was followed by inductively coupled plasma – optical emission spectrometry (ICP-OES) (Spectro Blue, Germany) method. The samples were grinded and then approximately 1g of them was weighed directly to PTFE flasks after adding 7 mL of HNO_3 (67% v/v) and 1 mL H_2O_2 (30% v/v) and subjected to following digestion program: the temperature was raised to 170 °C (15 min), and waited at 170 °C for 10 min. After cooling at room temperature, sample solutions were transferred into 50 mL polyethylene flasks. The digested samples were analyzed by ICP-OES [18].

Statistical analysis

The data were reported as the average \pm standard deviation. Variance of analysis (ANOVA) (IBM-SPSS 1.0.0.781) by Tukey test ($p<0.05$) was used for comparison of the results between all samples. *t*-test was used to determine the effects of the application (before and after milling) on the statistical significance level and to compare the varieties of grains among themselves.

Results and discussion

Physiochemical properties

The ash contents of the paddy (3.499^a), oat (2.294^b) and barley samples were significantly ($p<0.05$) higher than the other grains, respectively (Table 2). The ash contents of the cereals decreased at various ratios by the debranning and milling processes applied to the wheat, barley, oats, rye, and rice. Especially the ash content seriously reduced in the paddy and wheat via milling process ($p<0.05$). This case may be attributed to the difference in the bran and hull layer content and consequently in the grain morphology. Furthermore, the amount of ash in the cereals increases from the center of the grain to the outer layers depending on the distribution of the major and trace elements [3]. The paddy is a hulled grain and this layer is removed from the raw grain to reveal whole rice before rice flour production. During the rice milling process, this stage is named as dehusking [19]. The ash content of the dehusked rice was significantly lower ($p<0.05$) than the other grains.

Table 2

Ash and protein contents of the cereal grains and flour samples

Cereal		Ash (%)	<i>p</i> value*	Protein (%)	<i>p</i> value*
Wheat	Grain	1.261±0.168 ^d	0.010	10.619±0.339 ^a	0.065
	Flour	0.502±0.012 ^f		9.520±0.113 ^{ab}	
Barley	Grain	2.092±0.089 ^{bc}	0.197	10.168±0.058 ^{ab}	0.329
	Flour	1.948±0.046 ^c		10.567±0.227 ^a	
Rye	Grain	1.373±0.055 ^d	0.016	9.462±0.478 ^{ab}	0.174
	Flour	1.292±0.002 ^d		10.698±0.346 ^a	
Oat	Grain	2.294±0.016 ^b	0.479	9.217±0.132 ^b	0.063
	Flour	2.247±0.044 ^{bc}		10.328±0.111 ^{ab}	
Paddy	Grain	3.499±0.054 ^a	0.003	7.917±0.115 ^c	0.019
	Flour	0.930±0.014 ^e		5.439±0.074 ^d	

* ($p < 0.05$) means that the values statistically different.

** Different letters in the same column indicate significant differences ($p < 0.05$) between the samples.

In a study conducted by Oghbaei and Prakash [20], which was examined the effect of the milling process to the flour of the physicochemical properties, the ash content of the whole wheat flour was 1.89%, the ash content of the wheat flour obtained as a result of refining was 0.78%. The removing of the germ and bran layers during milling process reduces the ash content of the flour [21–23]. However, the final nutrient content of the wheat will depend on the extent to which the outer bran and aleurone layers are removed during debranning processing, as this is where the fiber and minerals tend to be concentrated.

Barley, oats and rye used in the study were made into flour by grinding in the stone mill. These cereals entered to milling system together with their husk, they were grinded together at the mill. At the end of the milling system, although some part of them removed by the coarse bran sieving system, a little bit of fine bran (especially aleurone layer) could mix to flour during the sifting. At the same time, the morphologically combined structure of the husk and endosperm section of the barley caused the bran layer especially aleurone part to be not completely separated from the endosperm. Therefore, no statistically significant difference ($p > 0.05$) was found in the ash contents of the cereal grains and their flours, especially in barley. This situation reduces the quality of the flour from the technological point of view, but it makes them rich in nutritional quality. The results of the study have shown that mineral content increases depending on bran/husk content of the grains.

The lowest protein content was determined in the paddy (7.917^c) and rice (5.439^d) samples and the highest rate was found in wheat (10.619^a) grain (Table 2). When the raw materials and flour were evaluated together, the amount of protein decreased in wheat and rice flour by milling process, the amount of protein increased in barley, oats and rye flour slightly. But the change between grains and flours was found statistically insignificant except paddy/rice and indicated with the same letters in Table 2. 70% of the cereal proteins found with starch granules in the endosperm, which forms the main structure of cereal flours, are in glycoprotein form. Especially oat proteins exhibit differences in terms of the structural and protein fractions and as different from the other cereals, most of the storage proteins in the oat are found in the endosperm [24]. It can be said that the presence of storage proteins predominantly in the endosperm leads to an increase in the protein content of oat flour after

separated the bran (Table 2). Therefore, the protein contents of the oat sample were found statistically insignificant ($p>0.05$) before and after the debranning process. In a study conducted about dephytinization of wheat and rice brans, the ash and protein content of the wheat and rice bran were found as 5.6%; 14.2% and 9.7%; 16.5%, respectively [10]. According to these results, the rice bran has more ash and protein content than the wheat bran. In the present study similarly, removing the bran layer has been more effective in the rice sample than the wheat sample ($p_{\text{rice}} < p_{\text{wheat}}$) (Table 2).

***In-vitro* digestibility and phytic acid content**

Table 3

Digestibility rate (%) and phytic acid content of the samples

Cereal		Mineral digestibility (%)	<i>p</i> value*	Protein digestibility (%)	<i>p</i> value*	Phytic acid (mg/100 g)	<i>p</i> value*
Wheat	Grain	45.38±3.60 ^{abc}	0.073	50.66±3.30 ^{cd}	0.032	2471.88±0.31 ^a	0.000
	Flour	49.61±0.49 ^{ab}		74.46±2.19 ^{ab}		1900.50±0.71 ^{bc}	
Barley	Grain	40.72±0.00 ^{bc}	0.097	36.49±0.97 ^{ef}	0.041	2328.13±2.08 ^{ab}	0.000
	Flour	44.61±0.60 ^{abc}		65.19±2.85 ^b		1940.63±0.66 ^{bc}	
Rye	Grain	45.70±0.42 ^{ab}	0.050	53.81±3.19 ^c	0.055	1715.63±1.10 ^c	0.186
	Flour	58.30±1.20 ^a		66.51±1.09 ^a		1709.38±1.28 ^c	
Oat	Grain	36.24±2.01 ^{bc}	0.067	40.54±0.10 ^{de}	0.036	2050.00±2.74 ^{abc}	0.001
	Flour	46.44±1.08 ^{ab}		65.63±1.41 ^b		1818.75±0.44 ^c	
Paddy (rice)	Grain	30.16±1.01 ^c	0.064	48.69±1.24 ^{cd}	0.016	1559.38±0.22 ^c	0.000
	Flour	58.35±2.86 ^a		66.13±0.43 ^{ab}		921.87±0.49 ^d	

* ($p<0.05$) means that the values statistically different.

** Different letters indicate significant differences ($p<0.05$) between the samples.

However, this rising was not found statistically significant ($p>0.05$) except for the rye sample ($p\leq 0.05$). While the sample which had the highest MD rate was the rice (58.35^a), the lowest one was the paddy (30.16^c). This was related to the high amount of ash content of the paddy hull which was completely separated from the grain, and to the low rate of phytic acid content in the rice. This case was supported by the decreased total phosphorus (P) content of the rice flour after dehulling and milling in Table 4. As shown in Table 3 the rice flour is the sample that has the lowest phytic acid content and one of the samples which has the highest PD rate. It has been found that the milling process resulted in a greater increase in PD compared to the MD of the cereal grains. It was determined that the milling process applied to cereals caused an increase in PD in all flour samples compared to grain (Table 3). The highest increase ($p<0.05$) was detected in paddy. The flour which had the highest protein digestibility was the wheat flour. Except for rye ($p>0.05$) the increase in other cereals was found statistically significant ($p<0.05$). It is known that the antinutrients such as phytic acid reduces mineral bioavailability and protein absorption of foods thanks to their chelating properties. They causes to micronutrient malnutrition and mineral deficiencies. (11).

In the present study, the highest phytic acid content was found 2471.88 mg/ 100g in the wheat sample. This result supported the view that wheat and bran were rich sources of phytic acid. It was stated that phytic acid was found mostly in the outer layers of the wheat grain, and that different wheat fractions obtained by milling process had different phytic acid content [25]. Moreover, it was indicated that the bran of wheat and rice contains a considerable amount of phytic acid (myo-inositol hexaphosphate) which acts as an antinutrient substance [10]. Fulcher and Duke [26] reported that most of the phytic acid phosphorous (87%) that caused the limitation of digestibility was present mostly in aleocon layer. Gupta et al. [27], also reported that the antinutrients such as phytic acid were also removed by debranning of the bran layer from the cereal grains during milling. In this study, the phytic acid content of the cereal grains decreased in the milling process. Except for rye ($p>0.05$), the decrease other cereals were found statistically significant ($p<0.05$). The milling process is not a process that activates the phytase enzyme that breaks down phytic acid. However, the debrannig/dehulling process is a common method to removing of phytic acid from cereals. Despite its change according to grain varieties, the bran and husk layers of the grain generally contain high phytic acid.

Table 3 shows the untreated cereal grains have a high content of phytic acid due to their bran and hull layer. However, it is seen that the amount of phytic acid decreases in the flour samples due to the removing of bran and hull layers via the milling process. Especially rice, wheat, and barley were provided in serious decline. This case may be attributed to the process of debranning/dehusking process of the wheat and paddy samples during milling. The situation in the other grains was caused by milling of the stone mill and subsequent sieving.

Minerals content in cereals

When the mineral contents of the grains and flours used in the study were evaluated together, the highest minerals in the cereals were determined as K, P, Mg, Ca and Na, respectively (Table 4). The grain with the highest K and Ca content was oats; with the highest P and Na content was barley; the grain with the highest Mg content was paddy. In a study, it was stated that oats were a bit richer than barley in terms of some minerals [1]. In this study, the oat sample was found richer than other grain samples in terms of "Ca, K, Fe, Ba, Al" content, was found to be equal in terms of Se amount. Although Al, Fe, Zn, Ba and Se were low in cereals, paddy sample due to the owned husk layer had very high values in terms of Al and Fe content compared to other grains. Although the wheat has high daily intake in the human diet, it contains insufficient level Fe and Zn. In a study conducted by Welch and Graham [28], it was indicated that the wheat had inadequately Zn content inherently.

Phosphorus (P) is an important element obtain growth and maturation of grain in many cereals. 80% of the phosphorus is the phytate phosphorus which is located in the bran (aleurone) layer. The amount of phosphorus increases with the growth of the cereal grain [29]. p level of the samples decreased due to the removal of the bran/husk by the peeling, polishing and grinding processes applied to the grains. Because a significant part of the bran layer was separated, the phosphorus level was significantly reduced after milling, especially in wheat ($p = 0.004$) and paddy ($p = 0.000$).

Table 4

Mineral content (ppm) of the cereal grain and flour samples

		Minerals									
	Milling	Na	Ca	K	Mg	Zn	Fe	Se	Ba	Al	P
Wheat	Before (ppm)	62.40 ±0.6	356.50 ±3.3	3528.70 ±17.7	765.10 ±2.90	11.30 ±0.10	11.90 ±0.10	2.90 ±0.00	3.01 ±0.01	14.00 ±0.03	1772.50 ±13.60
	After (ppm)	24.20 ±0.50	253.90 ±2.60	1772.00 ±3.40	251.80 ±2.90	1.50 ±0.10	2.60 ±0.10	3.20 ±0.00	1.28 ±0.02	19.99 ±0.13	826.20 ±6.10
<i>p</i> * value		0.001	0.001	0.001	0.001	0.006	0.003	0.000	0.007	0.013	0.004
Barley	Before (ppm)	157.80 ±1.00	325.70 ±3.50	4393.10 ±18.50	786.50 ±1.70	12.70 ±0.00	7.10 ±0.00	2.70 ±0.00	0.88 ±0.00	4.24 ±0.02	1819.50 ±4.60
	After (ppm)	84.90 ±0.50	279.00 ±4.30	4347.70 ±22.90	834.30 ±2.90	14.0 ±0.20	14.0 ±0.10	3.10 ±0.10	1.62 ±0.01	15.41 ±0.04	1997.70 ±7.80
<i>p</i> * value		0.001	0.045	0.041	0.039	0.097	0.009	0.156	0.009	0.001	0.028
Rye	Before (ppm)	13.90 ±0.30	251.70 ±2.70	4436.00 ±3.30	707.10 ±2.90	9.50 ±0.10	3.30 ±0.10	2.90 ±0.20	2.36 ±0.01	2.93 ±0.01	1609.30 ±1.40
	After (ppm)	26.30 ±0.50	374.00 ±5.20	4480.00 ±5.80	764.70 ±4.90	14.40 ±0.20	32.70 ±0.30	3.30 ±0.10	3.09 ±0.01	47.93 ±0.34	1722.90 ±2.90
<i>p</i> * value		0.026	0.027	0.083	0.054	0.026	0.006	0.156	0.009	0.000	0.016
Oat	Before (ppm)	21.40 ±0.20	532.70 ±4.90	4664.60 ±7.80	734.80 ±1.70	7.90 ±0.10	24.50 ±0.10	2.50 ±0.20	6.41 ±0.07	6.90 ±0.22	1187.50 ±3.30
	After (ppm)	46.90 ±0.50	497.90 ±2.50	4534.00 ±14.80	754.50 ±1.00	10.00 ±0.00	26.00 ±0.20	3.20 ±0.10	5.31 ±0.01	29.06 ±0.07	1369.10 ±2.80
<i>p</i> * value		0.000	0.046	0.072	0.032	0.006	0.084	0.090	0.006	0.000	0.010
Paddy	Before (ppm)	48.70 ±0.20	355.10 ±6.10	4243.60 ±12.40	903.90 ±2.50	6.10 ±0.10	139.60 ±1.30	3.50 ±0.10	1.29 ±0.04	172.28 ±6.13	1687.80 ±2.50
	After (ppm)	5.90 ±0.10	20.50 ±0.70	1232.40 ±4.20	224.10 ±0.90	2.70 ±0.00	nd	2.40 ±0.00	0.07 ±0.00	1.87 ±0.27	651.40 ±4.60
<i>p</i> * value		0.000	0.000	0.000	0.000	0.002	-	0.006	0.000	0.000	0.000

* ($p < 0.05$) means that the values statistically different.

In the study, the decrease in the level of all minerals as a result of milling of paddy and wheat grain was evaluated statistically significant ($p < 0.05$). However, the decreases in mineral contents were at different levels, this decrease could be due to the distribution of minerals in the different layers and in the fractions of the wheat. From the outside to the inside, the wheat bran comprises the pericarp, the intermediate layers (the seed coat, the nucellar epidermis), and the aleurone layer. The removing the outer layers of the wheat grain towards to aleurone layer leads to a significant decrease in the amount of some minerals. In a study investigated to effects of different degree of pearling treatment onto concentrations of different elements, it was reported that as the pearling degree increased, the rates of K, Mg, Fe, and Zn decreased. Because the aleurone layer is especially rich in terms of K, P, Mg, Zn, and Cu elements compared to other wheat layers [4]. In a study conducted by Lorenz et al. [30], as the amount of ash in wheat increased, the amount of Fe and Zn increased relatively, but the same relationship was not observed in the Ca. The amount of K, Mg, Cu, Zn, and Fe minerals increased as the milling yield of the wheat increased and there was no change in Ca mineral level in accordance with the data reported by Ekinici and Unal [31]. It was associated with the different distribution of Ca in wheat grain by them.

The opposite results were obtained for the content of the Fe and Zn of barley, oat and rye, their rate increased with the milling process. In agreement with earlier findings [4, 32], it can be evaluated as that Zn and Fe are more evenly distributed in these grains.

The rye flour had the highest Zn (14.40 ppm) and Fe (32.70 ppm) content compared to the other samples. It was stated that in a study conducted by Kutman et al. [33], the nitrogen application to soil affected Fe and Zn concentrations in the whole grain (50%) and endosperm (80%) significantly. It was found also a close relationship between Zn and protein in biological systems and that Zn required protein to form structural integrity in the grain [34,35]. Moreover, Ekinci and Unal [30] reported that there was a positive correlation between the amount of protein in the flours and the amount of K and Mg. These findings specify that there is a positive relationship between the protein content and accumulation of some minerals to plant and also their concentration. In this study, it was determined that rye flour contained the highest rates of Zn and Fe and the highest protein content. Furthermore, by milling rye flour, there was provided an increase in the amount of Mg and K, together with increasing protein. Weaver et al. [36] stated that some minerals increased due to the removal of the outer layer by grinding of oat. As the similar finding was observed in oat sample. The amounts of Na, Mg, Zn, Fe, Se, Al, Ba, and P increased with the milling.

Selenium concentrations in the barley, oat and rye were not influenced by the milling process statistically ($p>0.05$). Although the changing of its' level was found statistically significant in wheat and rice samples, it's concentration exhibited less change relatively compared to other minerals. In a study, it was reported that Se was located to be uniform throughout the aleurone layer and the protein matrix which surrounded the starch granules in wheat endosperm [37]. Moreover, it was stated similar to our findings that Selenium was the only mineral distributed almost homogeneously in all grain types and fractions of the grain [4].

Conclusion

The result of the study has shown that the level of the minerals changed depending on the bran/hull content of the grains. Whereas the bran and ash contents were decreased by dehulling/debranning process, the mineral rates were differently affected. Although the low bran and ash content is desirable with respect to regulations and technological usage, the low level of mineral content means undesirable nutritional quality for the cereal products. The rate of some minerals increased due to their distribution and location in the grain layer. Moreover, the mineral composition in cereals was also affected by the type of grain. The dehulling/debranning process generally increased the digestibility rates and decreased phytic acid content significantly. Because the different treatments such as soaking, fermentation or cooking process increased the digestibility and decreased the phytic acid level via they activate the phytase enzyme, the effects of the combination of the debranning/dehulling treatments with these processes should be researched for improving the nutritional quality of cereal products. It can be suggested that performing technologically appropriate level debranning/dehulling process, and combination with some process such as soaking, fermentation which affected digestibility and dephytinization.

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Effect of the complex improver on consumer properties of bakery products

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Abstract

Keywords:

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Freshness

Introduction. In order to improve the consumer properties of bakery products made using accelerated technologies, a study was conducted on the possibility of using a directional baking improver "Freshness +".

Materials and methods The consumer properties of the bakery product "Freshness" (from premium wheat flour, yeast, salt, margarine, white sugar) were investigated in the work. The technological process was carried out with the duration of the dough keeping – 20 min and the use of the "Freshness +" improver, which was dosed in the amount of 2.0% by weight of flour. The quality of the finished products was evaluated by physicochemical and organoleptic parameters.

Results and discussion The use of improver in amount of 2.0% by weight of flour increases the specific volume of products by 11%, improves form resistance, porosity and reduces the fermentation time by three times, namely up to 20 minutes. This is due to the use of amylolytic enzyme improver in the composition, which contributes to the increase of gas-forming and sugar-forming capacity. Product with improver preserves freshness, as evidenced by an increase in total crumb deformation by 26.0%, a smaller subcutaneous layer and fewer air layers in the baking bundle for 72 hours without packaging. There is more accumulation of dextrins and bisulfite binders in the products when using the improver, which indicates the inhibition of the staling processes of the products and improvement of consumer properties. This is due to the fact that there are moisture-retaining additives in the products, namely maltodextrin and p gelatinized starch, which retain osmotic and adsorption-bound moisture during storage. Maltodextrin is also a water-soluble hydrocolloid that increases moisture retention and forms a three-dimensional network that inhibits the interaction of gluten and starch, resulting in retrograde of starch.

Conclusions. The use of the improver slows down the starch retrograde due to the formation of coagulated proteins from its constituents with flour during baking, in the middle of which there are swollen, often gelatinized starch grains, and increasing the amount of dextrose.

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Introduction

As a result of the staling of the baked goods, they lose the luster of the crust, the intensity of the taste and aroma, the softness and springiness of the crumb, and acquire considerable fragility. The deterioration of the consumer properties of bakery products is due to the transformation of the biopolymers of the products during storage, namely, the transition of starch from the amorphous state to the crystalline and loss of some water by protein [1].

For the simultaneous adjustment of the quality of flour, technological process and ensuring the lengthening of freshness of bakery products, it is advisable for manufacturers to use complex baking improvers [2, 3].

The range of complex baking improvers (CBI) is very diverse, depending on the direction of their action [3]. In general, complex baking improvers consist of different ingredients, namely gluten oxidizers and reducing agents, enzymes, emulsifiers and various food additives or ingredients with specific effects [4, 5, 6]. All components of complex baking improver are carefully selected due to their activity and synergistic effect [7]. Complex baking improvers act throughout the technological process and are designed for solving specific challenges.

The complex baking improver "Freshness +" is known for prolonging the freshness of bakery products made using accelerated technologies [8].

To extend the freshness of the bakery products, the Austrian company "Backaldrin" offers a comprehensive baking improver, "Winer Note", consisting of vegetable oil, sugar, skimmed milk powder, emulsifier, dextrose, salt, lecithin, ascorbic acid. The recommended dosage is from 1.0 to 10.0% by weight of flour. CBI "Winer Note" extends the shelf life several times [3]. This improver prevents moisture from condensing to preserve softness and freshness, but does not affect the intensification of the process.

A Dutch company "Zeelandia" offers a comprehensive bakery improver "Gamma Soft", which consists of soy flour, emulsifier, ascorbic acid, enzymes, to extend the freshness of bakery products [9]. A significant disadvantage of this improver is the use of soy flour, so it is advisable to study the use of improvers made from other non-traditional raw materials, namely dry mashed potatoes.

The French company "Lessaffre" recommends to use the complex baking improver "Mazhimix" with a white label for long-term storage products such as bread from wheat, bakery products and muffins in order to extend their freshness up to 2 months. It contains specially selected monoglycerides, which slow down the starch retrogradation process. The enzyme complex of this improver allows to obtain an additional amount of dextrins, whereby the pulp will retain its properties over time [10]. The disadvantage is that manufacturers keep a secret about the composition of a complex baking improver, in particular a complex of enzymes, so it is unclear whether its use will accelerate the technological process.

The authors of [11] recommend to use the composition of dry wheat gluten, an enzyme preparation of amyolytic action and a mixture of xanthan and guar gums in complex baking enhancers to prolong the freshness of bakery products. The developed improver extends the freshness of bakery products up to 72 hours of storage unpackaged. But the paper does not provide recommendations for its optimal dosage in the case of processing flour with different baking properties.

It is advisable to investigate the effect of the developed improver on the consumer properties of bakery products made using accelerated technology, where the fermentation stage is replaced by keeping.

The purpose of this work was to substantiate the feasibility of using the complex baking improver "Freshness +" to improve consumer properties of bakery products.

To achieve this goal, the following tasks were formulated:

- To investigate the influence of the improver on the technological process and quality of bakery products;
- To study the effect of improver on the processes of staling of bakery products";
- To investigate the influence of the improver on the aroma of bakery products.

Materials and methods

Objects and materials

The complex baking improver "Freshness +" includes: dry potato powder, enzyme preparation "Alphamalt VC 5000", maltodextrin, ascorbic acid.

The bakery product "Freshness" was made from premium wheat flour by an accelerated method:

- Premium wheat flour – 100 kg;
- Pressed bakery yeast – 3.0 kg;
- Salt – 1.5 kg;
- Margarine – 2.5 kg;
- White crystalline sugar – 2.5 kg.

For development of the complex baking improver "Freshness +", dry potato powder, was used as the main filler. It is made according to the technology [19]. In terms of physico-chemical parameters, dry potato powder complies with the requirements shown in Table 1.

Table 1

Physico-chemical parameters of dry potato powder, n = 3, p<0.95

Indicator	Value
Mass fraction of moisture,%	12.0
Mass fraction of protein,%	8.3
Mass fraction of mono-and dissacharides,%	3.3
Mass fraction of starch,%	74.5
Mass fraction of fiber,%	6.6
Mass fraction of fat,%	0.4
Mass fraction of insoluble in hydrochloric acid ash,%	3.5
Minerals, mg/100g:	
Magnesium	24.0
Potassium	572.0
Calcium	11.0
Phosphorus	60.0
Sodium	29.0
Water absorption capacity,%	71.3
Autolytic activity,% of dry matter	37.9
The degree of caking,%, for the pore size of the sieve – 250 nm	18.4
Whiteness, units	90.5

The dry potato powder according to whiteness corresponds to whiteness of wheat flour of the premium grade, has a low tendency to formation of lumps (the degree of caking did not exceed 3.0%), therefore it can be used as a functional basis for complex baking improvers.

For the production of the complex baking improver the enzyme preparation “Alphamalt VC 5000” (5000 SKB/g) of the German company “Muhlenchemie” is used, maltodextrin of the Polish company, defatted lecithin from sunflower produced by the Ukrainian company “BIOLER” and ascorbic acid produced in China are used.

Methods of research of the quality of bakery products with a complex baking improver

Method of laboratory baking for research of the quality of bakery products.

Laboratory baking was carried out to investigate the indicators of the technological process, biochemical, physico-chemical changes in the dough and qualitative indicators of bread. The dough was prepared in an accelerated manner with a moisture content of 43.5%. The dough was kneaded in a two-speed dough mixing machine. Keeping time was 30 min. The dough was processed manually, perseverance of the dough pieces was carried out in a thermostat at a temperature of $(38 \pm 2)^\circ\text{C}$ and relative humidity $(78 \pm 2)\%$ until readiness. The products were baked in an oven at $220\text{--}240^\circ\text{C}$.

Bread quality was evaluated by physical and chemical (specific volume, shape resistance, structural and mechanical properties of crumb) and organoleptic parameters (appearance, crust surface condition, porosity structure, taste, smell).

Method of research of total deformation for investigation of the duration of storage of freshness of products. The duration of storage of freshness of products was investigated by changes in the structural and mechanical properties of the crumb. Its total deformation after 48 h of storage was determined with the use of penetrometer AP 4/1 [12].

The area of the subcutaneous layer was determined organoleptically by changing its stiffness and crumb. The transition between the subcutaneous layer and the crumb was circled by a marker [13].

Method of research of microscopy of bakery products. Microscopy of bakery products was carried out after 4 h of baking and at the end of storage, ie after 72 h of storage. The samples were stored unpackaged at $(20 \pm 0)^\circ\text{C}$. Samples were prepared by freezing, lyophilic drying and spraying in a vacuum chamber of carbon on a piece of dried sample. The samples were examined using the electron scanning microscope “TEOLJSM – 200” at a magnification of 1000 times and the photos of the most visible sections were taken.

Method of research of content of dextrans. The content of dextrans was determined by the method of their mass fraction, which is based on the ability of dextrans to precipitate at different concentrations of ethyl alcohol in solution. Samples were inactivated by enzymes to release them from water-soluble carbohydrates, digestion of sugars for their extraction, precipitation of dextrans with solutions of alcohol of different concentration, dissolution of recovered dextrans with water and hydrolysis with 2% hydrochloric acid, determination of the amount of glucose in the hydrolyzate of dextrans of different molecular weight by the method of Wiltter and Schudl. On the basis of the determined content of dextrans the mass fraction of dextrans was determined by fractions, depending on the mass fraction of dextrans at different concentrations of ethyl alcohol [14].

Method of research of moisture bond in the dough. Determination of the moisture bond in the dough was performed by thermogravimetry using a “Q – 1000” derivatograph in the temperature range of 20–200 °C at a rate of heating of samples weighing 1.00 g – 1.25 °C/min [15].

Method of research of the content of flavoring substances in bread. The content of flavoring substances in bread was inferred by the amount of bisulfite binding compounds determined by the method [12, 20].

Results and discussion

Investigation of the effect of the improver on the process and the quality of bread

The obtained results due to laboratory baking were compared to those obtained by straight dough process. The dosage of the improver was 2.0% to weight of flour, the duration of dough keeping was 20 minutes. The obtained results were compared to those obtained by straight dough process. A sample prepared according to the recipe above without improver was as a control sample. The results of the study are given in Table 2.

The analysis of the results of the research shows that the time spent on fermentation of semi-finished products with the addition of the improver is three times less, compared to their production by traditional technologies. The use of the improver increases the specific volume by 11%, the porosity and improves the form resistance of finished products. The researcher improves the freshness of bakery products by 26.0%.

It was established that the use of the improver helps to reduce the duration of the technological process and increase the specific volume of bakery products. This is due to the use of amylolytic enzyme which is in the improver, which increases the gas-forming and sugar-forming capacity.

Investigation of the effect of the improver on the process of staling of bread

The difference in organoleptic characteristics between the crumb and the crust is the result of baking processes when the surface is exposed to higher temperatures than the crumb. Baking creates a relative moisture gradient and moisture content between the crust and the crumb, which causes the redistribution of moisture in the product. This causes the softness of crust, staling of crumb, form a thicker subcutaneous layer during storage. Therefore, it was advisable to investigate the effect of the improver on the area of formation of the subcutaneous layer (Figure 1).

Table 2
Influence of the improver on technological process and product quality, n = 3, p≤0.95

Indicator	Bakery product	
	Control sample	Sample with the improver
<i>Dough</i>		
Mass fraction of moisture,%	43.5	
Duration of fermentation, min	210	–
Duration of keeping, min	–	20
Duration of proofing, min	40	35
Acidity, degrees	1.0	1.0
initial	1.8	1.4
<i>Finished products</i>		
Organoleptic characteristics:		
Correctness of form	Oval, slightly vague, the cuts are clear	Oval, not vague, the cuts are clear
Color of the crust	Light	From golden to brown
State of the surface of the crust	Smooth enough, single small bubbles, barely noticeable small short cracks and blasts, glossy	Impeccably smooth, free from bubbles, cracks, blasts, glossy
Porosity structure	The pores are small, thin-walled and medium-sized, evenly distributed	
Flavor	Intensely pronounced, relative for bread	
Taste	Intensely pronounced, relative for bread	
Specific volume, cm ³ /100 g	330	367
Form resistance, h/d	0.39	0.44
Porosity,%	80	86
Acidity, degrees	1.8	1.4
Total crumb deformation, units of penetrometer		
in: 4 h	82	102
in: 72 h	46	84
Preserving of freshness,%	56	82



Control sample

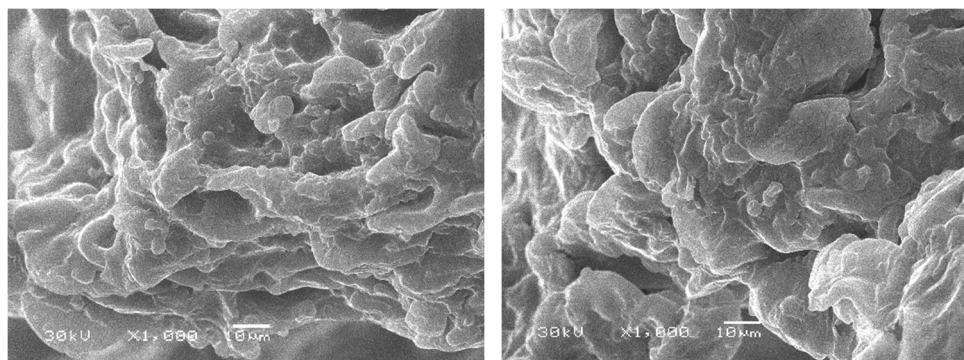


Sample with the improver

Figure 1. Formation of a subcutaneous layer during the baking process after 72 h of storage

The results of the studies show that during the storage process, using developed improver the subcutaneous layer of the bakery product is smaller after 72 h of storage, compared to the subcutaneous layer of the control sample.

Further studies were concerned with the microscopic examination of the bakery product during storage. The results of the studies are shown in Figure 2.



Control sample

Sample with the improver

Figure 2. Microstructure of the crumb of bakery products after 72 hours of storage

The bread crumb structure is characterized by the presence of pores that are wrapped around by the interstitial walls that create a spongy frame. The control sample has layers of air in the interstitial walls under the microscope, indicating a decrease in the volume of starch grains due to the formation of the crystalline structure of starch. The crumb of the product with the improver consists of a whole mass of coagulated proteins during the baking process, in the middle of which partially glistened starch grains are swollen, and only in some places there are visible layers of air.

The lengthening of freshness is caused by the introduction of moisture-retaining additives, namely maltodextrin and gelatinized starch, which contain osmotically and adsorbed bound moisture during the storage of the products with the improver. Maltodextrin is also a water-soluble hydrocolloid that increases the degree of moisture retention and forms a three-dimensional net that inhibits the interaction of gluten and starch, resulting in retrograde of starch [16, 17].

It has been found that the use of the improver reduces the formation of a subcutaneous layer, improves the crumb structure in the case of keeping the loaf for 72 hours without packing.

During the baking process, the destruction of starch occurs, and amylolytic enzyme and maltodextrin are introduced into the dough with the improver, so it was expedient to investigate the change in the amount of dextrans in bakery products. Dextrans were determined after 4 h of cooling. The results of the studies are presented in Table. 3.

Table 3

Content of dextrins in bakery products, n = 3, p≤0.95

Samples of bakery products	Content of dextrins by fractions,% to dry matter			Total content of dextrins
	Amylodextrins	Erythodextrins	Malto- and achrodextrins	
Control sample	0.968	0.402	0.908	2.278
Sample with the improver	1.387	0.428	1.678	3.493

In the case of the use of the improver, there is a significant increase in dextrins – by 55.3% compared to the control sample due to the presence of α -amylase in the improver, the introduction of additional oligosaccharides with dry potato powder and directly maltodextrin. In this regard, the process of staling of bakery products is slowed down by the formation of a three-dimensional net by low molecular weight dextrins, which impedes the interaction of gluten and starch and the yield of moisture by starch [13, 18].

Literature sources show that the ability of bakery products to maintain freshness is related to the content of bound water [15]. Therefore, it was necessary to determine the content of bound and free water in the crumb. The determination was performed using derivatograph. The analysis of thermogravimetric curves made it possible to obtain quantitative characteristics of the distribution of moisture in the crumb of products with additives and to change its state during storage (Figure 3).

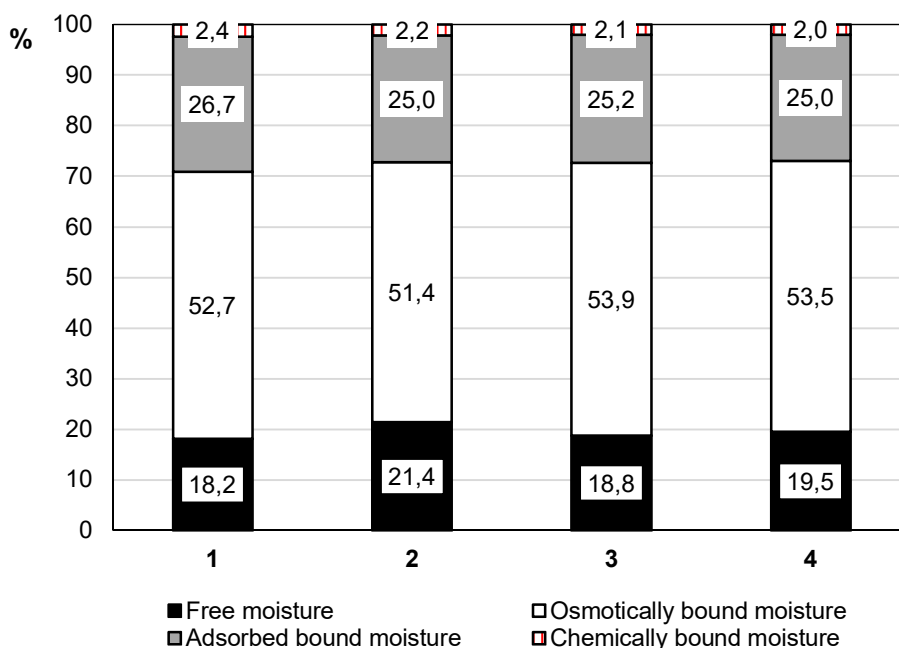


Figure 3. Changes in moisture bounds in the samples of products during storage:
 1 – control sample after 24 hours; 2 – control sample after 72 hours;
 3 – sample with the improver after 24 hours; 4 – sample with the improver after 72 hours.

At the first stage of removing moisture from the samples of bread, there is its significant loss. In this temperature range, free moisture, moisture contained in macro- and microcapillaries, and immobilized water is removed. The total amount of this moisture in the samples of products is 18.2% of the total weight of water for the control sample during the first day of determination, and 18.8% of the total weight of water in the product for the sample with the improver. On the fourth day of storage of products, the moisture content of these forms of bond for the control sample increases by 3.2% of the total weight of water in the product, and for the product with the improver – by 0.7% of the total weight of water in the product.

The second and third temperature intervals correspond to the endothermic peak, that is, in these ranges endothermic processes occur, which may be related to the removal of moisture with significant binding energy, obviously, osmotically and adsorbed bound water. As it can be seen from Figure 3 the amount of osmotically bound moisture is higher than in the control sample and is 53.86% of the total weight of water in the product. After four days of storage, the amount of osmotically bound moisture in bread with the improver is slightly reduced, but remains greater than in the control sample. The amount of adsorbed bound water during the first day of storage and after four days of storage is almost the same.

The fourth temperature interval corresponds to the removal of chemically bound water. As it can be seen from the analysis, the content of this water in the samples is increasing, but it is very small compared to other forms of bonding moisture.

During the study at the end of storage, it was found that samples of products tended to decrease osmotically and adsorbed bound moisture and to increase the free moisture and moisture of microcapillaries, but this reduction is less with the improver.

Therefore, the results of the analysis of the moisture bond in the samples of the products with the improver suggest that the slowing of staling of these samples is associated with a lower content of free moisture, macro- and microcapillaries at the beginning of storage and an increase in the amount of osmotically bound moisture [13]. This correlates with the obtained data to determine the total deformation of the crumb.

Investigation of the effect of the improver on the aroma of bread

Consumer and nutritional value of products depend on organoleptic quality indicators, namely aroma.

The effect of the improver on the content of carbonyl compounds in the finished products are shown in Table 4.

Thus, the introduction of the improver to the products increases the content of bisulfite binding compounds, compared to the control sample, by 1.3–4.1 times, despite the duration of storage. Increasing the content of carbonyl compounds in finished products with additives correlates with improved crust color and bread aroma.

When adding the improver to the dough, the content of carbonyl compounds in the crumb and crust of the bakery products increases. This is because of the fact that the improver contains an enzyme preparation of amylolytic action, which accelerates the process of persistence and separation of more carbonyl compounds. The increase in the content of bisulfite binding compounds is further explained by the fact that the improver includes maltodextrin, which, along with the slowing of the staling, accelerates the fermentation process.

Table 4

Content of bisulfite binders, eq. mg/100 g of bread, n = 3, p≤0.95

Selection area	Control sample	Sample with the improver
After 4 hours		
Crumb	6.8	27.9
Crust	7.6	31.1
After 24 hours		
Crumb	5.5	22.6
Crust	6.3	26.1
After 48 hours		
Crumb	4.6	5.6
Crust	18.7	24.4
After 72 hours		
Crumb	3.3	4.6
Crust	13.2	18.8

Therefore, the use of the improver helps to extend the freshness of the bakery products up to 72 hours of storage unpackaged.

However, the impact of the improver on the mechanism of loss of moisture during storage remains unclear.

Further studies will be aimed on the impact of the improver on biological activity, the degree of digestion of proteins of bakery products and on selection of packaging materials for their storage.

Conclusions

1. It was found that the use of the improver in the amount of 2.0% by weight of flour using the accelerated technology of bakery products leads to an increase in the volume, improve the form resistance and porosity and reduce the fermentation process to 20 minutes.
2. The use of optimum dosing of the developed improver prolongs the storage time up to 72 hours without packaging. This is evidenced by an increase in the total deformation of the crumb, a decrease in the subcutaneous layer and a smaller number of layers of air in the crumb, as well as a greater accumulation of dextrans in the products.
3. It was established that in case of the use of the improver, the number of bisulfite binding compounds increases, which has a positive effect on the aroma of bakery products, which is less removed during storage compared to the control sample.

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Effect of extruded corn flour on the stabilization of biscuit dough for the production of gluten-free biscuit

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Abstract

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Introduction. Studies have been carried out to determine the effect of extruded corn flour on the stabilization of the biscuit dough, the indices of the moisture-holding capacity of the flour, foam stability and density of the biscuit dough to create a biscuit semi-finished product for gluten-free food.

Materials and methods. Studies of moisture-holding capacity of flour raw materials were carried out by the method of centrifugation. Foam stability was defined as the ratio of the height to the foam column after holding for 24 hours at a temperature of 18–20 °C to the total height of the foam column of the sample. Microscopic examination of the structure of the product was performed using a digital binocular microscope series "MicroMed".

Results and discussion. The optimum value of the density of biscuit dough is 0.444–0.446 kg/m³. Increasing the concentration of extruded corn flour above 20 mass% leads to a high density of dough, which is undesirable in the production of biscuit semi-finished products, because it makes them denser and less porous. However, the presence of biscuit dough density, at the absence of premium wheat flour, indicates the possibility of creating a gluten-free biscuit semi-finished product using solely corn flour. In the sample of the biscuit dough prepared with corn flour extruded 100 mass% (mass percent), the foam bubbles are of almost the same size, moreover, we can see the formed channels between them that promotes leveling of air pressure inside the foamed biscuit dough. It helps to stabilize the foam. The results of the optimization of the biscuit semi-finished product using extruded corn flour have the following intervals of optimization parameters: replacement of 100 mass% of wheat flour with extruded corn flour with a quantitative ratio of recipe components "eggs:sugar:flour" 2.1:1:1.02.

Conclusions. Extruded corn flour helps to stabilize the foam biscuit dough system, which allows to create a dietary gluten-free biscuit semi-finished product.

Introduction

The effect of extruded corn flour on the stabilization of biscuit dough and the creation of a biscuit semi-finished product for gluten-free food has not previously been described.

The possibility of considering biscuit as a promising product for the development of gluten-free semi-finished product for dietary nutrition was considered. As biscuit dough is a thermodynamically unstable foamy food system, therefore, one of the important technological problems is the stabilization of the system during product formation [1].

A promising way to create gluten-free food is to make deliberate use of ingredients that have a wide range of technological properties. These properties make it possible to improve the structural-mechanical and organoleptic characteristics of the biscuit semi-finished product [2] to correct the nutritional value and, at the same time, to exclude gluten. These types of raw materials include gluten-free cereals and their products [3]. Promising in this aspect is the use of extrusion processing of such raw materials [4].

Literature analysis

Recent studies have shown that the use of extrudates from soybeans, wheat, rye, corn improves the quality and elastic properties, increases the viscosity of the dough, which increases the volume output of finished products [5; 6]. Considering this, it is necessary to study the possibility of using corn extrudate in flour technology in order to stabilize the foam structure of biscuit dough.

There are studies [7–9] based on finding the optimal ratio of structure-forming components for gluten-free flour confectionery. These technologies are based on rice flour and gluten-free starch-protein mixtures. It has been proved [9] that different ratios of protein and starch can be used to adjust the specified quality parameters, such as sugar cookies. However, the possibility of using extruded corn flour, in particular as a source of extruded starch, is warranted.

In the technology of gluten-free bakery products in order to solve the technological problem of the absence of gluten, the use of hydrocolloids and concentrates of animal proteins is proposed to form a foamy structure of gluten-free yeast dough. It was established that the proposed additives in quantities of 0.5–1.0% of Helios-11 and 0.5% of solution of CCSS (carboxymethylcellulose sodium salt) cause 100% stability of the foam from eggwhite. In this case, the foaming capacity increases only with the addition of Helios-11 in the amount up to 1.0% and decreases with the higher amount of Helios-11 or in the presence of CCSS. This can be explained by the increase in the density of the whipping mass and the ability of both additives to thicken the solutions [10].

For the production of biscuit semi-finished products, food industry has traditionally used baked wheat flour of the highest grade. Its high technological potential in biscuit dough is not rationally used because it is intended to reduce the "strength" of flour by adding potato starch. A possible solution is the use of extruded corn flour, which is a source of modified starch extrusion and contains gluten-free proteins and can be used in technology of biscuit semi-finished products [11].

The presence of fundamental developments in the production and use of various types of extruded flour in the production of food indicates the possibility of its use in technology of biscuit semi-finished products [12–14].

The purpose of the study is to determine the effect of extruded corn flour on the stabilization of biscuit dough to create a dietary gluten-free biscuit semi-finished product.

Materials and methods

Materials under study—extruded corn flour, flour mixtures of premium wheat flour and extruded corn flour, biscuit dough and biscuit semi-finished flour mixtures of wheat flour and extruded corn flour 90:85, 85:15, 80:20, 50:50, 0:100 mass% [15].

Extruded corn flour is a dry mixture of homogeneous consistency in the form of powder and fine grains with the taste, smell and color inherent in the raw material, made by grinding parts of the grain (endosperm, aleurone layer, fruiting membranes) with pre-removed gastric. The chemical composition of the used extruded corn flour and premium wheat flour are shown in Table 1.

Table 1

Chemical composition of extruded corn flour and premium wheat flour, %

Product	Moisture content	Protein content	Fat content	Starch content	Ash content	Fiber content
Extruded corn flour	9.0±0.01	6.1±0.02	8.1±0.02	70.9±0.03	4.8±0.03	1.0±0.02
Premium wheat flour	14.5±0.03	11.4±0.05	1.08±0.04	67.7±0.05	0.5±0.03	0.1±0.01

Preparation of prototypes

A biscuit semi-finished product according to the recipe "Basic" was chosen as a control sample.

The prototyping technology includes the following operations: the egg-sugar mass is beaten to a volume of 2.5–3 times, or about 30–40 minutes. Then the premium wheat flour is gradually added, mixed with starch or extruded corn flour. If the kneading is carried out in a whisking machine, then it should last no more than 15 s.

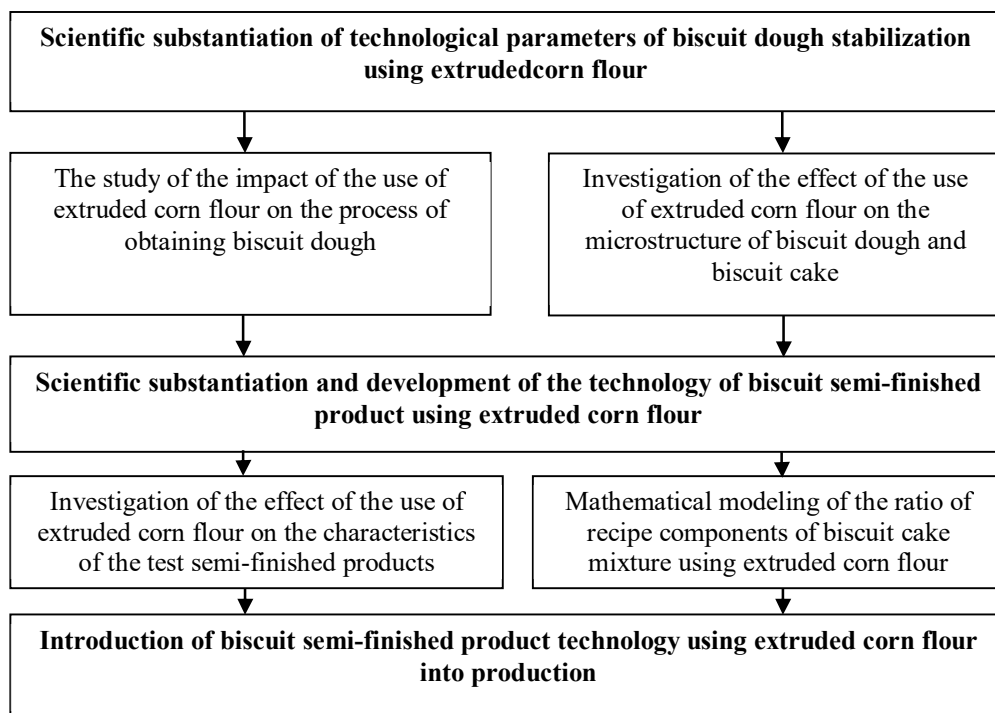
Ready-made biscuit dough is baked immediately in capsules or cake forms and on sheets, as the biscuit dough settles when stored. Capsules and forms are covered with paper or greased with margarine. Biscuit dough is shaped into $\frac{3}{4}$ of their height, as it increases when baking. Biscuit dough is baked at 200–210 °C. Baking time depends on the thickness and volume of the dough. So, in capsules, biscuit is baked 50–60 minutes, in cake forms – 35–40 minutes, on sheets – 10–15 minutes. The baked biscuit cake is cooled during 20–30 minutes and removed from the molds. The biscuit semi-finished product is left at 8–10 hours for proofing, after which it is possible to cut it and carry out the following technological operations.

Premium wheat flour is used with a crude gluten content of 23.0±0.4% and a crude gluten deformation value of 60±1.1%, potato starch, chicken eggs, white crystalline sugar (Table 2).

Table 2
Formulation of experimental samples of biscuit cake (control sample and gluten free product)

Raw materials	The solids content (SC),%	Raw material consumption per 100 kg of semi-finished product, kg			
		Biscuit semi-finished product (Control sample)		Gluten-free biscuit cake	
		real	SC	real	SC
Premium wheat flour	85.50	28.12	24.04	-	-
Corn flour extruded	91.0	8.07	7.34	30.58	27.82
Potato starch	80.00	6.94	5.55		
Chicken eggs	27.0	57.85	15.62	63.44	17.12
White sugar	99.7	34.71	34.65	30.26	30.16
Total		135.69	87.2	124.28	75.1
Output		100.0	87.0	100.0	75.00

Procedure for conducting research



Description of methods

Studies of moisture retention capacity were carried out by centrifugation. 1 g of the test sample was weighed into the centrifuge tube, 30 cm³ of distilled water was added, the mixture was stirred for 1 minutes using an electric mixer with a speed of 1000 rpm for 1 minutes. The formed flaking fluid was drained. To remove the residue of water, the tube was tilted onto filter paper, and the tube was weighed after 10 minutes. Water retention capacity (WRC) was calculated by the formula, %

$$WRC = (m_1 - m_2 / m) \times 100,$$

where: m – weight of sample, kg;

m_1 – the weight of the test tube with a dry sample, kg;

m_2 – wet tube weight, kg.

The foam stability of the egg-sugar mixture with the addition of the flour mixture was determined as the ratio of the height of the foam after ageing for 24 hours at a temperature of 18–20°C to the total height of the foam column (FC) of the sample, expressed as a percentage, calculated by the formula:

$$FS = \frac{Hf_{24 \times 60^2}}{Hf} \times 100\%$$

where FS – foam stability, %;

$Hf_{24 \times 60^2}$ – height of foam 24 hours after cessation of whipping, m;

Hf – initial foam height, m.

Microscopic examination of the structure of the product was performed using a digital binocular microscope series "MicroMed" equipped with a built-in lighting system. The micrographs of the samples were taken at the following magnifications: 40, 100, 400 and 1000 times [17].

The porosity of the samples of the biscuit semi-finished product (control sample) and with extruded corn flour were measured by the effective pore diameters on the biscuit cross sections by visualizing the biscuit microstructure, processing digital images, and based on measurement results [18].

Processing of research results

Mathematical and statistical analysis. To determine the effect of the ingredients (independent factors x_i) on the dough whipping with the appropriate density (optimization parameter G), two-factor experiment was performed with variation of the egg mélange content (x_1 , %), sugar (x_2 , %) at fixed flour values (x_3 , %), that is, determining the magnitude of the density from changes in three main factors: the content of egg mélange (E , %), sugar (C , %) by fixed values of flour (B %), $G = f(x_1, x_2, x_3)$. Properties of the samples are shown in Table 3.

Table3
Measurements and statistical characteristics of the main indicators of biscuit products during the experiments

Indicators	Sample 1	Sample 2	Sample 3	Sample 4
Moisture-proofing ability of flour mixtures,%	92.0±5.0	98.0±3.0	96.0±3.0	94.0±4.0
Density, kg /m ³	5.9±0.6	6.5±0.5	6.2±0.6	6.0±0.6
Porosity,%	80±8.0	81.5±5.0	78.0±7.0	79.0±7.0
Weight loss during baking,%	12.0±1.2	10.0±0.8	12.3±1.2	11.5±1.0

The processing of the obtained data of the results of the experimental are performed according to known methods and methods of statistical processing and regression analysis of experimental results. It is used the differential operators of the program MathCAD-14 to obtain empirical regression equations, which allows to get qualitative and quantitative evaluation of characteristics.

Results and discussion

Investigation of the moisture-holding capacity of flour mixtures

The study envisages obtaining a biscuit semi-finished product with complete replacement of premium wheat flour by extruded corn flour. Its technological characteristics and moisture holding capacity in the foaming system were determined.

Table 4 shows the results of the study of the moisture-holding capacity of flour mixtures.

Table4
Comparison of the value of the moisture-holding capacity of flour mixtures

№	Samples	Water retention rate,% at 20±2°C
1	Control sample – 100 mass% of wheat flour	29±1.5
2	Wheat flour:extruded corn flour 95:5 mass%	38±1.0
3	Wheat flour:extruded corn flour 90:10mass%	49±2.5
4	Wheat flour:extruded corn flour 85:15 mass%	58±3.0
5	Wheat flour:extruded corn flour 80:20 mass%	70±2.0
6	Wheat flour:corn extruded flour 50:50 mass%	81±1.5
7	Sample with 100 mass% of extruded corn flour	93±4.0

Table 4 shows that with the increase in the proportion of extrudedcorn flour in the mixture moisture retention capacity increases:for the sample containing 20 mass% of extruded corn flour by two and a half times, and three times for the sample with extruded corn flour – 100 mass%. This tendency of change, in our opinion, is explained, in particular, by the swelling of whole grains of starch due to the absorption and retention of moisture. The corn starch of extruded corn consists of a part of its whole granules, damaged granules,

gelatinized starch, polymers of starch [21]. All these components have different effects on the moisture-holding capacity of the flour mixture system. Swollen starch grains are in the middle of the void where water gets. It breaks and weakens some of the hydrogen bonds between the chains, which causes the starch grains to expand, with dissolving the free starch polymers to form a dispersed system [22].

In general, the above-described studies on the moisture-holding capacity of extruded corn flour indicate the feasibility of its use in technology of biscuit semi-finished product. This requires studying the mechanism of the effect of extruded corn flour on biscuit dough components.

Effect of adding extruded corn flour on the density of biscuit dough

To substantiate the use of extruded corn flour in the production of biscuit semi-finished product and the possibility of complete replacement of premium wheat flour with extruded corn flour, the effect of adding extruded corn flour on the density of biscuit dough was investigated. The average experimental values of the biscuit dough density using extruded corn flour are presented in Figure 1.

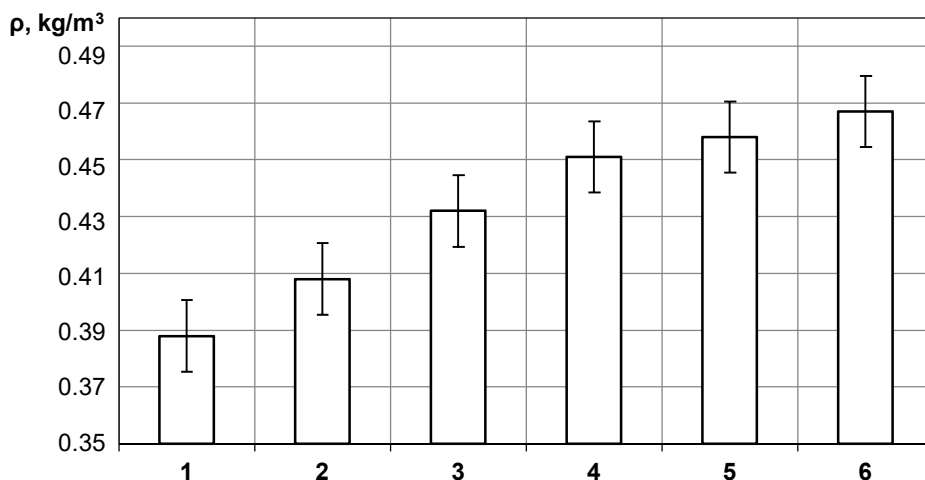


Figure 1. Dependence of biscuit dough density (ρ) on ECF content in samples:
 1 – (control); 2 – WF:ECF – 95:5 mass%; 3 – WF:ECF – 90:10 mass%;
 4 – WF:ECF – 85:15 mass%; 5 – WF:ECF – 80:20 mass%;
 6 – WF:ECF – 0:100 mass%.

Figure 1 shows that with the increase in the proportion of extruded corn flour in the flour mixture, the dough density increases. Optimal value of biscuit dough density is 0.444–0.446 kg/m³. Increasing the amount of extruded corn flour above 20 mass% leads to a significant dough density, which is undesirable in the production of biscuit semi-finished products, as it makes them less porous [23]. However, the presence of biscuit dough density, in the absence of premium wheat flour, indicates the possibility of creating a gluten-free biscuit semi-finished product solely using corn flour.

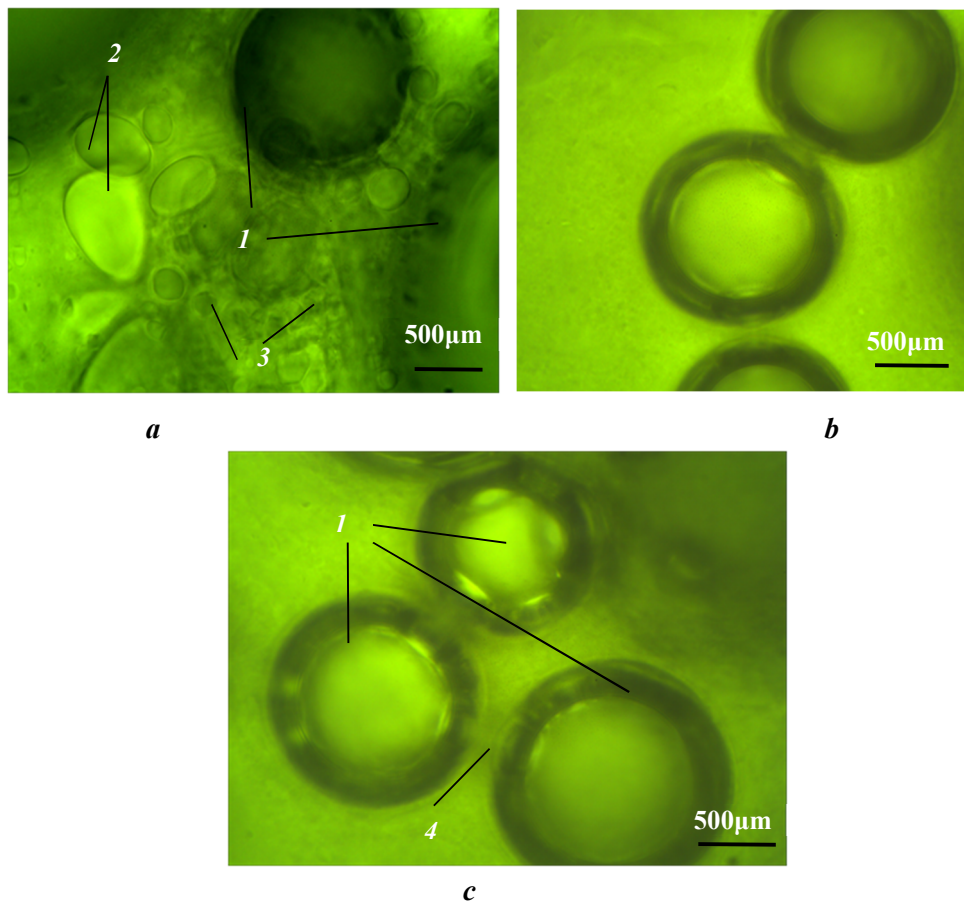
Study of microstructure of biscuit dough

In the process of production of biscuit semi-finished product, the recipe components undergo physical-chemical transformations and interact with each other to form interconnections, which leads to a change in the microstructure of the semi-finished product. In order to determine the mechanism of stabilization of the foam structure of the biscuit dough with the use of extruded corn flour, the changes in the microstructure of the biscuit dough and the biscuit semi-finished product were studied. The process of making biscuit dough is essentially whipping of the egg-sugar mixture and the dispersion of gas in the liquid. Considering foam as a system that holds air bubbles that are separated only by a thin film of liquid. Schematically, the foam structure can be represented as the packing of gas bubbles with thin films of the basic fine particulate filler [24]. This filler is film-coated with surfactants [25].

Broken mass in the production of biscuit semi-finished product refers to a plastic-viscous structured system [23]. Its whipping is accompanied by complex physico-chemical, colloidal and mechanical processes. All of them are aimed at the formation of stable dispersion systems [25]. In order to demonstrate the described structures forming the foam of the biscuit dough (Figure 2), there a comparative characteristic of the microstructure of the following samples is given: wheat flour (WF) and extruded corn flour (ECF) in the ratios: *a*– WF – 100 mass%; *b* –WF:ECF – 80:20 mass%; *c* –ECF – 100 mass%.

With the introduction of potato starch there is an increase in the plasticity of the dough due to its increased ability to swell and lower retrogradation of starch compared to starch cereals. In control sample (*a*) (Figure 2) it can be seen particles of potato starch, characterized by a large size and oval shape, as well as particles of wheat starch, fractional composition of which is finely dispersed. Sample (*b*) with a ECF content of 20 mass% is characterized by the presence of only granules of wheat flour starch. We have proposed a complete replacement of starch and part of premium wheat flour by 20 mass% of ECF, as well as a complete replacement of wheat flour by ECF for the manufacture of gluten-free biscuit semi-finished product.

According to the microstructure of biscuit dough, Figure 4 shows that the structure of the prototype looks like foam. Its structural feature is the presence and uniform distribution of air bubbles, which later forms the porous structure of the biscuit cake. The control sample (*a*) containing wheat flour and starch shows that the size of the formed air bubbles has a large difference in diameters, that is, some bubbles are almost twice the size of the others. One of the reasons of foam destruction is the diffusion of gas between the bubbles and it is determined by the pressure in the middle of the bubbles [24]. The destruction of the air bubble film is directed towards the larger bubble because its pressure is lower than that of the small bubble [23]. For comparison, in the sample (*c*) of biscuit dough using ECF of 100 mass% foam bubbles are almost the same size, in addition, it is noticeable that between them channels of size are formed, which promote the equalization of air pressure in the middle of the foam system of biscuit dough, which promotes the stabilization of the foam system. For epy sample (*b*) with a content of 20 mass% of ECF, the same size of gas bubbles is also characteristic, which leads to an improvement in the structural and mechanical characteristics of ready biscuit products.



**Figure 2. Microstructure (1:1000) of samples of biscuit dough, containing:
WF and ECF in the ratios:
a – WF – 100 mass%; b – WF:ECF – 80:20 mass%; c – ECF – 100 mass%;
1 – air bubbles; 2 – potato starch grains;
3 – wheat flour starch grains; 4 – channels between air bubbles.**

Investigation of the stability of foam biscuit dough

To determine the role of the liquid phase of the dough during its short storage before baking, the stability of the formed foam was investigated. This value is characterized by the foam settling rate [24]. The results of the study are shown in Figure 3.

Experiments have shown that the stability of the foam depends on the used flour. The introduction of extruded corn flour in the biscuit semi-finished technology helps to reduce the movement of hydrophobic particles, which minimizes their negative impact on the foam stability. High foam stability corresponds to large volume and fine uniform porosity of biscuit cake.

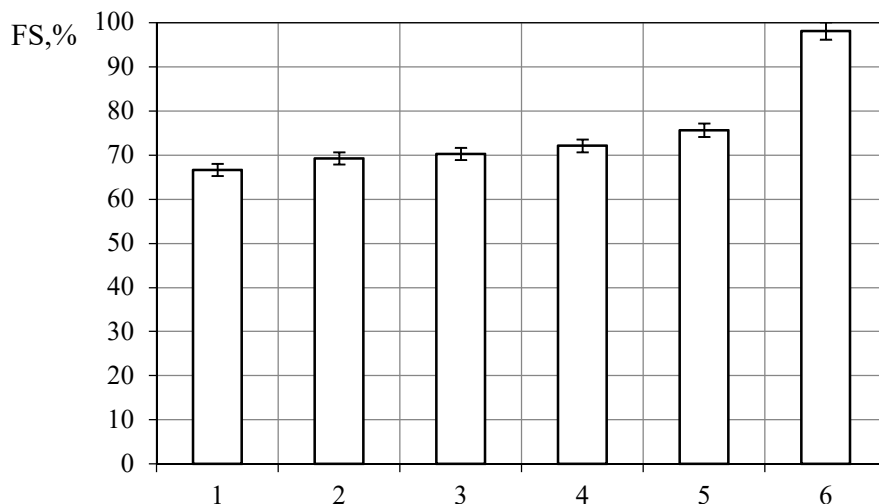


Figure 3. Dependence of biscuit dough foam stability on ECF content:
1 – WF:ECF – 100:0 (control); 2 – WF:ECF – 95:5 mass%;
3 – WF: ECF – 90:10 mass%; 4 – WF:ECF – 85:15 mass%;
5 – WF:ECF – 80:20 mass%; 6 – WF:ECF – 0:100 mass%

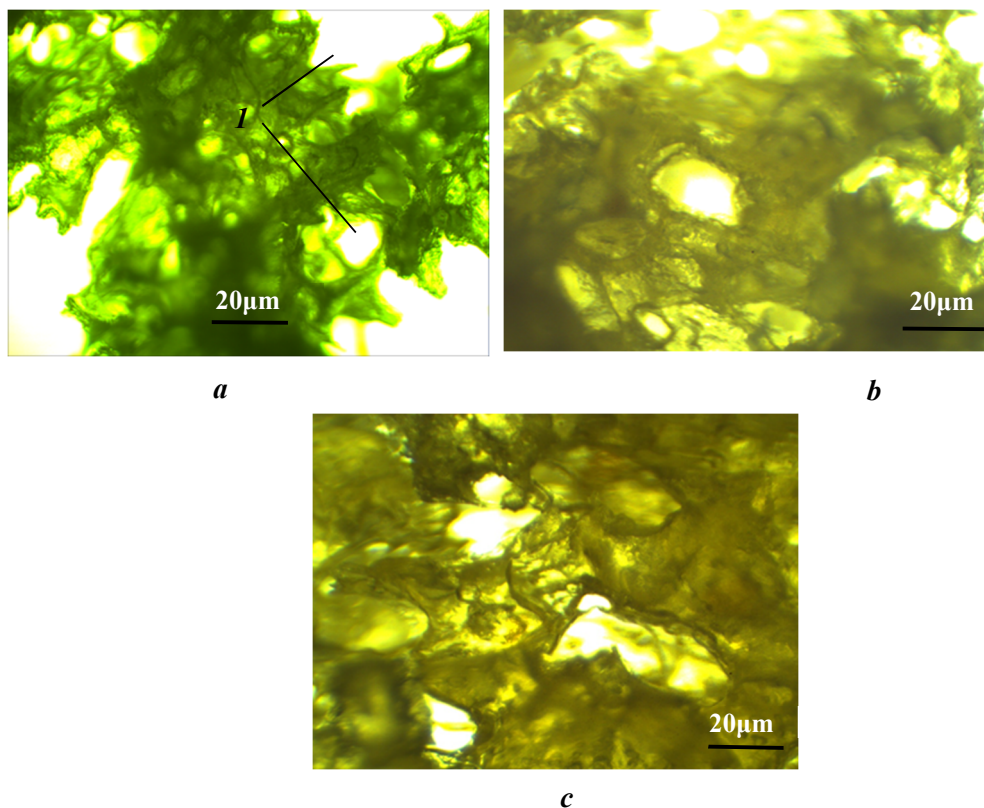
Figure 3 shows that the use of extruded corn flour instead of wheat flour significantly affects the stability of the biscuit dough foam, this indicator monotonically increases almost 1.5 times with the complete replacement of wheat flour. This ability will help to stabilize the foam of the biscuit dough and increase its resistance to mechanical action during its molding.

The use of whipped egg white with sugar forms a stable system that settles rather slowly and gives a small volume. Thus, studies have shown that settling time for the sample 1 is 120 minutes. This phenomenon can be explained by the flocculation caused by the formation of large aggregates and large volume sediment [24].

Study of microstructure of biscuit cake

After baking (b), a semi-finished biscuit with a volume at the level of the control sample with fine uniform porosity was obtained. The use of extruded corn flour promotes change in the properties of a thick starch paste that becomes gelatinized to produce a viscous dough. Changing the properties of starch when interacting with the emulsifier has a significant impact on the dough system. We see that the formation of a fine-porous structure of biscuit dough using 20 mass% and 100 mass% of extruded corn flour is due to the properties of the starch of extruded flour.

In Figure 4 it can be seen that the structure of the biscuit semi-finished product looks like a spatial grid. The results of the study indicate the presence and uniformity of pore distribution in samples *b* and *c* using extruded corn flour, which is an integral part of the porous structure of the biscuit cake.



**Figure 4. Microstructure (1:40) of samples of biscuit semi-finished product containing: WF and ECF in the ratios:
A – WF – 100 mass%; b – WF:ECF – 80:20 mass%; c – ECF – 100 mass%;
1 – pores.**

Therefore, the effect of extruded corn flour on the properties of biscuit dough and biscuit cake is caused first of all by the properties of corn flour starch that has undergone extrusion treatment. Using the obtained results allows to recommend extruded corn flour for the production of gluten-free biscuits for dietetic nutrition.

The results of the studies showed that it is advisable to have 100 mass% substitution of wheat flour for corn flour extruded in biscuit semi-finished technology. During the experimental research, measurements of the basic characteristics of the biscuit were performed and their statistical analysis was performed.

Comparison of the porosity of the obtained samples of biscuit cake

The porosity of the samples of the biscuit semi-finished product (control sample) and sample with extruded corn flour were carried out by measuring the effective pore diameters on the biscuit cross sections (Figure 5), by visualizing the biscuit microstructure, processing the digital images and measuring them.

The processing of the research results allowed to obtain color gradient matrices on fragments of biscuit images with extruded corn flour. All selected fragments observed the presence of areas of high gradient values of the digital image brightness matrix around the inhomogeneities (inclusions, pores, areas with variable humidity). They also observed the indicated areas with slightly smaller maximum values of the structural characteristics gradient compared to the trivial materials in the matrix around the air inclusions or included particles. This has shown that structural changes occur to a less extent and are explained by the formation of super molecular structures in the form of globular aggregates of macromolecules, both around the air inclusions and in the volume of the matrix, indicating the formation of a heterogeneous structure [25]. Within the two-factor model, the method of modeling moisture content, density, porosity and sintering from the content of egg melange, sugar and extruded corn was used.

A program for calculating the parameters of a quadratic biscuit density dependence model G (kg/m^3) from the content (%) of ingredients: E (egg product), C (sugar) and B (flour) according to the results of tests for 4 samples, characterized by the sets of content of ingredients is are given in. Accordingly, the density dependence and its possible absolute and relative error were evaluated.

$$g_1=5.9 \quad g_2=6.5 \quad g_3=6.2 \quad g_4=6$$

$$sg_1=0.6 \quad sg_2=0.5 \quad sg_3=0.6 \quad sg_4=0.6$$

$$G = \begin{pmatrix} g_1 \\ g_2 \\ g_3 \\ g_4 \end{pmatrix} \quad E = \begin{pmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \end{pmatrix} \quad C = \begin{pmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \end{pmatrix} \quad B = \begin{pmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \end{pmatrix}$$

$$y_1=0.5 \quad y_2=0.5 \quad y_3=0.5 \quad \text{Given}$$

$$G - g_2 = y_1(E - e_2)^2 + y_2(C - c_2)^2 + y_3(B - b_2)^2$$

The last relation gives an opportunity to obtain a polynomial formula for further studies:

$$G(E, C, B) = 102.25 \cdot C - 87.66 \cdot C \cdot B - 165.12 \cdot C^2 - 143.25 \cdot B^2 + 145.88 \cdot B - 37.08.$$

The calculation of the absolute dG and relative δG the error of the density G of the biscuit product makes it possible to state that the maximum density of the biscuit product is reached for the parameters $(E, C, B) = (0.5; 0.23; 0.23)$ with a high level of accuracy – 1.4%

The MathCAD operator of the extremum estimate for the function $G(E, C, B)$ gives the maximum density result: $\max(g) = 6.47 (\text{kg/m}^3)$ Figure 5. The third parameter B – the content of the flour is not clearly affected, based on the condition: $E + C + B = 1$.

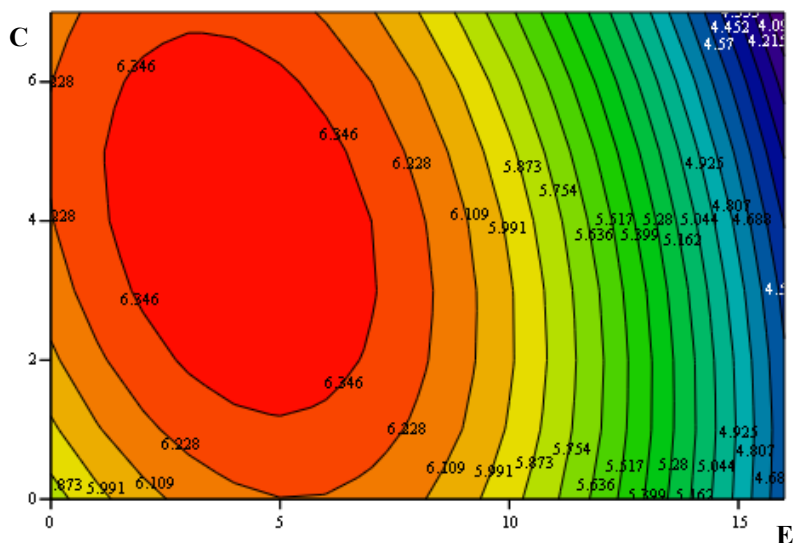


Figure 5. Density levels of biscuit G with respect to parameters E and C

Similarly, to the results of the program of calculating the parameters of the quadratic model of density dependence on the content of ingredients using MathCAD-14, analytical expressions were obtained representing the dependence of indices of absorption (U), porosity (P) and moisture content (V) on the parameters of egg content (E), sugars (C) and flour (B).

$$V(E,C,B) = 13.06 \cdot E + 0.61 \cdot C + 2.31 \cdot B - 13.11 \cdot E^2 - 1.31 \cdot C^2 - 4.97 \cdot B^2 - 2.61;$$

$$U(E,C,B) = -3.38 \cdot E - 2.88 \cdot C + 3.38 \cdot E^2 + 6.27 \cdot C^2 + 11.18;$$

$$P(E,C,B) = 625 \cdot E - 625 \cdot E^2 - 2393 \cdot C^2 + 1100.78 \cdot C - 2339.0 \cdot B^2 + 1263.06 \cdot B - 371.85;$$

Analytical expressions make it possible to state that the optimum density of biscuit semi-finished product is achieved for the parameters (E, C, B) = (0.5; 0.24; 0.24) with a high level of accuracy – 1.4% (Figure6).

The operator of the MathCAD program of extremum estimation for the function G (E, C, B) gives the optimality result: max (V) = 98%, min (U) = 10%, max (P) = 81.1%. Note that the analytical expression of the dependence of the biscuit packing does not contain parameter B (flour content).

Analysis of diagrams (Figure 6) of biscuit product parameters gives reason to claim that the found optimum corresponds to the values of the content of ingredients: egg – 50%, sugar – 23%, flour – 27% with accuracy: 0.4, 0.14 and 0.12% respectively.

According to the results of the optimization of biscuit cake with the use of extruded corn flour has the following optimization intervals: 100 mass%. That is, at x1(E) = 51%, x2(C) = 24.4%, and ECF content of 24.6% with accuracy: 0.4, 0.14 and 0.12% respectively, with the best performance of sintering, porosity, dough density and moisture content.

The mathematical processing of the results of the study allowed to optimize the main recipe components of the biscuit semi-finished product, called "Gluten-free" and to determine their optimal ratio – eggs:whitesugar:ECF – 2.1:1:1.02.

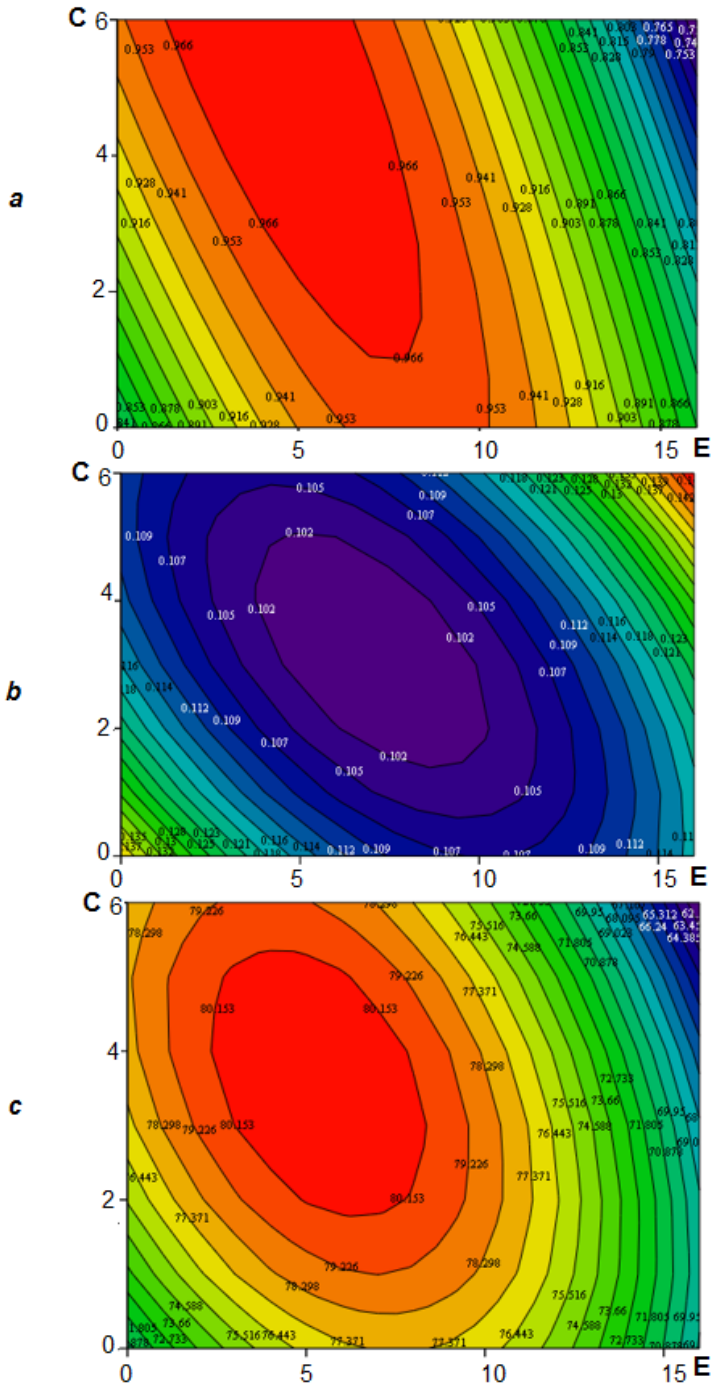


Figure 6. Effect of content of egg E and sugar C on moisture content V (figure 6a), indices of absorption U (figure 6b) and porosity P (figure 6c) of the biscuit

Conclusions

As a result of solving the research problems, new scientific results were obtained, which are as follows:

1. The stabilization of the rheological properties of the foam system of biscuit gluten-free dough is achieved due to the properties of partially gelatinized starch of extruded flour.
2. Optimized formulation of gluten-free biscuit based on 100% replacement of wheat flour with extruded corn flour with a quantitative ratio of recipe components "eggs: sugar: ECF" 2.1:1:1.02. The ratio of the main recipe components is: egg content – 51%; sugar content – 24.4% and content of extruded corn flour- 24.6%.
3. The conducted research is the technological basis for the development of a wide range of food products with improved organoleptic characteristics for special dietary nutrition.

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Influence of tryptophan on auxin-synthesizing ability of surfactant producer *Acinetobacter calcoaceticus* IMV B-7241

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Abstract

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Introduction. The aim of this work is to determine the optimal tryptophan concentration and the time of its addition into the culture medium of the surfactant producer *Acinetobacter calcoaceticus* IMV B-7241 to achieve maximum auxin synthesis.

Materials and methods. Cultivation was carried out on a liquid nutrient mineral medium using as a substrate of ethanol and waste of biodiesel production (crude glycerol). Tryptophan was added into the medium as a 1% solution in an amount of 100, 200 or 300 mg/L at the beginning of the process or at the end of the exponential growth phase (48 h of cultivation). Phytohormones were isolated by three times extraction with organic solvents from the supernatant of the culture liquid after extraction of surfactants. The qualitative and quantitative determination of gibberellins was carried out by high performance liquid chromatography.

Results and discussion. The results show that regardless of the time of addition of tryptophan in the culture medium of the strain IMV B-7241 with crude glycerol a significant increase in the synthesis of auxins compared with those on the medium without this precursor was observed. The highest concentration of auxins was achieved by adding 300 mg/L of tryptophan into the medium with both substrates. Thus, *A. calcoaceticus* IMV B-7241 synthesized 1404.73, 1295.04 and 4850.98 µg/L of auxins on the crude glycerol medium with 100, 200 and 300 mg/L tryptophan added at the end of exponential phase respectively (and without precursor the concentration of auxins was 175,4 µg/L). Increased synthesis of auxins by strain IMV B-7241 correlated with the activity of tryptophan transaminase (key enzyme of biosynthesis): under cultivation on crude glycerol without precursor it was 163 nmol·min⁻¹·mg⁻¹ of protein, while in the presence of 300 mg/L of tryptophan added at the end of the exponential growth phase the activity was in 3.2 times higher – 526 nmol·min⁻¹·mg⁻¹ of protein. The highest concentration of auxins under cultivation on ethanol was achieved when 300 mg/L of tryptophan were added at the beginning of cultivation – 2261.66 µg/L.

Conclusion. The result of the work established the possibility of increasing by one or two orders of magnitude of synthesized auxins in the case of addition into the culture medium of *A. calcoaceticus* IMV B-7241 low concentrations of the precursor of their biosynthesis.

Introduction

Previous studies have established the ability of the producer of surfactants *Acinetobacter calcoaceticus* IMV B-7241 to synthesize stimulatory phytohormones – auxins, cytokinins and gibberellins [1]. Such results are the basis for the development of complex preparations with growth-stimulating and antimicrobial activities against phytopathogenic bacteria properties for use in plant production.

The following studies [2, 3] conducted at the greenhouses of the D.K. Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine using as test cultures of tomatoes and barley, confirmed that the supernatant of the culture broth *A. calcoaceticus* IMV B-7241 has a positive effect on plant growth and development. It was found that during the treatment of barley seeds with diluted supernatant of *A. calcoaceticus* IMV B-7241 culture broth, the yield increased up to +69.23% compared with water treatment of seeds. In the case of treatment of the root system of tomatoes with a solution of phytohormones *A. calcoaceticus* IMV B-7241, the number of fruits exceeded the control by 2.4 times and the total weight by 145.0%.

However, it should be noted that the concentration of extracellular phytohormones synthesized by the strain was low, which significantly reduces the efficiency of the use of such preparations in plant production.

In our published review [4], we focused on the fact that most soil microorganisms, both associated and non-associated with plants, synthesize phytohormones of the auxin nature in the presence of exogenous tryptophan in the culture medium, which is the precursor of indol-3-acetic acid (IAA) synthesis. Moreover, the researchers added tryptophan into the medium at the beginning of the cultivation process and usually at a sufficiently high concentration (up to 10 g/L). We note that phytohormones are secondary metabolites, the formation of which begins in the stationary phase of growth, so it seems more logical to add a precursor at this stage of the process. In addition, the concentration of precursors used for the intensification of synthesis in microbial biotechnology, as a rule, is 0.1–0.2% of the carbon source content in the culture medium [5].

It should be noted that in [1] we found that *A. calcoaceticus* IMV B-7241 synthesizes auxins under growth conditions on medium with different substrates without a precursor, and therefore there are potential opportunities for enhancing its synthesis.

In connection with the above, the aim of this work is to determine the optimal concentration of tryptophan and the time of its addition into the culture medium of the surfactant producer *A. calcoaceticus* IMV B-7241 to ensure maximum auxin synthesis.

Materials and methods

Object of research

The object of research is *Acinetobacter calcoaceticus* K-4 strain, registered in Microorganisms Depository of D.K. Zabolotny Institute of Microbiology and Virology, the National Academy of Sciences of Ukraine under the number IMV B-7241.

Medium composition and conditions of cultivation

Strain *A. calcoaceticus* IMV B-7241 was cultivated in the liquid medium (g/L distilled water): $(\text{NH}_2)_2\text{CO}$ – 0.35, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.1, NaCl – 1.0, Na_2HPO_4 – 0.6, KH_2PO_4 – 0.14, pH 6.8–7.0. Yeast autolysate – 0.5% v/v and solution of trace elements – 0.1% v/v were also

added to the medium. Trace elements solution contained (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. Crude glycerol (Komsomolsk biofuel plant, Poltava region, Ukraine) and ethanol were used as the carbon and energy sources in concentration of 2.0% v/v.

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

The culture in the exponential phase was used as the inoculum and added in concentration of 5–10% of nutritive medium volume. The concentration of the corresponding carbon source in the medium for the inoculum obtainment was 1.0% v/v. The cultivation was carried out in 750 mL flasks, containing 100 mL of medium, on the shaker (320 rpm) at 28–30 °C during 7 days.

Obtaining of auxin extracts

After cultivation of the strain *A. calcoaceticus* IMV B-7241, the biomass was separated by centrifugation (5000 g) for 25 min. Residuals of sunflower oil were extracted from the cultural liquid using petroleum ether (ratio 1:1).

The extracellular phytohormones auxins were isolated by the method of redistribution of phytohormones in two immiscible phases of solvents [6]. Ethyl acetate, pH 3.0 was used as the organic solvent. The obtained extracts were evaporated under vacuum at 40–45 °C. The dry residue was redissolved in 80% ethanol and transferred into microtubes. The obtained extracts were stored at -24 °C.

Qualitative and quantitative determination of auxins

Purification and concentration of phytohormone extracts were carried out on silicagel plates of the mark Silufol UV254 (*Chemapol*, Czech Republic) in a mixture of solvents used sequentially: chloroform, 12.5% aqueous ammonia, ethyl acetate:acetic acid (20:1).

The qualitative and quantitative composition of auxins was analyzed by high performance liquid chromatography (HPLC), using an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) and an Agilent G1956B mass spectrometry (MS) detector. HPLC/MS analysis of auxin extracts of *A. calcoaceticus* IMV B-7241 was performed at the Center for Collective Use at the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine.

Standard synthetic phytohormones Sigma (Germany) and Acros Organic (Belgium) were used for comparison:

- IAA – Indole-3-acetic acid;
- ICal – Indole-3-carboxaldehyde;
- IC – Indole-3-carbinol;
- ICA – Indole-3-carboxylic acid;
- IAA-hydr. – Indole-3-acetic acid hydrazide;
- IBut – Indole-3-butyric acid;

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

Compound detection was performed using a diode array detector with signal recording at 254 and 280 nm and fixation of absorption spectra in the 191-700 nm range. Agilent G1956B mass spectrometric detector (Agilent Technologies, USA) was used to determine the molecular weights of the tested compounds. Ionization was performed in ESI and APCI mode with positive ion fixation in SCAN mode in the range of 100-1200 m/z. The calibration was performed using standard auxin solutions.

Enzymatic analyzes

Preparation of acellular extracts. To obtain acellular extracts, the culture broth obtained after cultivation of *A. calcoaceticus* IMV B-7241 in a liquid mineral medium with crude glycerol was centrifuged (4000 g, 15 min, 4 °C). The cell pellet was washed twice from the residual medium with 0.05 M K⁺ phosphate buffer (pH 7.0) while centrifuging (4000 g, 15 min, 4 °C). The washed cells were resuspended in 0.05 M K⁺ phosphate buffer (pH 7.0) and destroyed by ultrasound (22 kHz) 3 times for 60 s at 4 °C on an UZDN-1 apparatus. The disintegrated cells were centrifuged (12000 g, 30 min, 4 °C), the precipitate was discarded and the supernatant was used as an acellular extract.

Analysis of tryptophan transaminase activity. The activity of tryptophan transaminase (EC 2.6.1.27, other names: L-phenylalanine-2-oxoglutarate aminotransferase; tryptophan aminotransferase; 5-hydroxytryptophan-ketoglutaric transaminase; hydroxytryptophan aminotransferase; tryptophan aminotransferase; L-tryptophan transaminase) was determined by the formation of indole-3-pyruvate from L-tryptophan and 2-oxoglutarate, which was analyzed spectrophotometrically at 330 nm [7].

Statistical analysis

All the experiments were repeated three times, and the number of parallel measurements in each experiment made up 3–5. The statistical processing of the experimental data was carried out in accordance with the algorithm described in [1]. Differences of mean indicators were deemed as reliable at the significance level $p < 0.05$.

Results and discussion

Previous studies have shown that the synthesis of auxin metabolites was dependent on the nature of the carbon source in the culture medium of *A. calcoaceticus* IMV B-7241 [1].

In this work, the choice of substrates (ethanol and crude glycerol) for the cultivation of *A. calcoaceticus* IMV B-7241 with the aim of intensifying auxin synthesis was due to the following reasons. First, in conditions of growth on biodiesel production waste, the strain *A. calcoaceticus* IMV B-7241 synthesized the highest amount of auxins (122.0 µg/L) compared to that on other substrates [1]. Secondly, there is the problem of crude glycerol disposal due to the increase of biodiesel production in the world. 10 kg of crude glycerol is formed from every 100 kg of biodiesel [8]. Third, the complex microbial preparation should be characterized by high antimicrobial activity against phytopathogenic bacteria, and previously [9] it was found that such properties are inherent to the surfactants synthesized during the cultivation of *A. calcoaceticus* IMV B-7241 on ethanol. The intensification of IAA synthesis in the presence of tryptophan is due to the fact that in microorganisms this amino acid is a precursor of 3-indolylacetic acid biosynthesis [10].

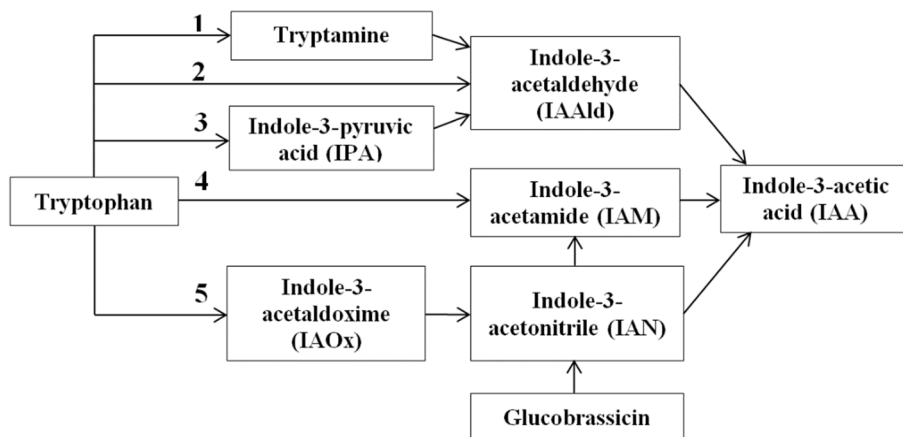


Figure 1. Ways of synthesis of indolyl-3-acetic acid from tryptophan in bacteria:
 1 – through tryptamine; 2 – bypass tryptophan pathway; 3 – through indole-3-pyruvate;
 4 – through indole-3-acetamide; 5 – through indole-3-acetonitrile.

Conversion of tryptophan to IAA can be accomplished in three ways (Fig. 1):

- Synthesis through indole-3-pyruvic acid and indole-3-acetic aldehyde. This is the main pathway characteristic of fungi and bacteria;
- Conversion of tryptophan to indole-3-acetic aldehyde may involve an alternative synthesis pathway in which tryptamine is formed. This pathway is found in mycorrhizal fungi and cyanobacteria.
- IAA formation via indole-3-acetamide. This is characteristic of phytopathogenic bacteria and fungi.

The data presented in the Table 1, indicate that regardless of the time of introduction of tryptophan in the culture medium of strain IMV B-7241 with crude glycerol a significant increase in the auxin synthesis compared with the indicators obtained on the medium without this precursor was observed. Among the auxins indole-3-acetic acid, indole-3-carboxylic acid, indole-3-butyric acid, indole-3-acetic acid hydrazide were identified, but the highest content was IAA, whose precursor is tryptophan.

A significant increase in auxin concentration during cultivation of *A. calcoaceticus* IMV B-7241 on ethanol (Table 2) was observed only when the maximum amount of tryptophan (300 mg/l) was introduced. With the introduction of 100 mg/L tryptophan, the level of auxin synthesis was virtually indistinguishable from that obtained during cultivation without the precursor. And with the addition of 200 mg/L, in particular, at the end of the exponential growth phase, there was a slight increase (in 1.6 times).

It is known [5] that most precursors are involved in the processes of secondary metabolite biosynthesis at the end of exponential or early stationary growth phases. This was clearly observed under the cultivation of *A. calcoaceticus* IMV B-7241 on biodiesel production waste: the introduction of 100, 200, and 300 mg/L tryptophan at the end of the exponential growth phase was accompanied by an increase in the concentration of synthesized auxins in 8, 7.4, and 27.7 times, respectively (see Table 1). At the same time, other patterns were observed during the cultivation of *A. calcoaceticus* IMV B-7241 on ethanol medium: for most variants, the highest auxin concentration was observed when

tryptophan was added at the beginning of the cultivation process (Table 2). Our further research will be devoted to clarifying these issues. However, it should be noted that from the point of view of the organization of technological production introduction of the precursor at the beginning of the process is much easier.

Table 1
Effect of tryptophan on the auxin synthesis under cultivation of *A. calcoaceticus* IMV B-7241 on raw glycerol

The amount of tryptophan, mg/L	Growth phase	The concentration of auxins, µg/L				The amount of auxins, µg/L
		IAA	ICA	IBut	IAA-hydr	
0	Lag phase	150.8	24.6	–	–	175.4
100	Lag phase	207.65	171.15	–	–	378.80
	The end of the exponential phase	359.85	309.22	–	735.66	1404.73
200	Lag phase	242.46	184.38	–	–	426.84
	The end of the exponential phase	694.74	600.3	–	–	1295.04
300	Lag phase	1123.0	401.70	47.74	–	1572.44
	The end of the exponential phase	4091.0	717.89	42.09	–	4850.98

Note. IAA – Indole-3-acetic acid; ICA – Indole-3-carboxylic acid; IBut – Indole-3-butyric acid; IAA-hydr – Indole-3-acetic acid hydrazide. «–» – not found.

Table 2
Synthesis of auxins under cultivation of *A. calcoaceticus* IMV B-7241 on tryptophan-containing ethanol medium

The amount of tryptophan, mg/L	Growth phase	The concentration of auxins, µg/L		The amount of auxins, µg/L
		IAA	ICA	
0	Lag phase	173.32	47.10	220.42
100	Lag phase	126.98	113.89	240.87
	The end of the exponential phase	130.08	95.67	225.75
200	Lag phase	135.67	97.30	232.97
	The end of the exponential phase	136.04	216.84	352.88
300	Lag phase	995.47	1242.0	2261.66
	The end of the exponential phase	710.13	396.74	1106.87

Note. IAA – Indole-3-acetic acid; ICA – Indole-3-carboxylic acid.

The data presented in the Tables 1 and 2 indicate that the concentration of synthesized auxins increased with increasing of precursor concentration in the culture medium of *A. calcoaceticus* IMV B-7241. It is possible that further increase of tryptophan will be accompanied by intensification of auxin synthesis. However, at this stage, for the creation of an effective microbial preparation with growth-stimulating properties it is unnecessary, because with the achieved auxin concentration (2000-5000 $\mu\text{g/L}$, see Tables 1 and 2) the culture broth of *A. calcoaceticus* IMV B-7241 with the purpose of seed or roots treatment of plants seedlings must be diluted at least in 400-500 times.

To confirm that exogenous tryptophan is involved in auxin biosynthesis, the activity of one of the key enzymes for the synthesis of indole-3-acetic acid (tryptophan transaminase) was analyzed. Tryptophan transaminase catalyzes the reaction of the formation of indole-3-pyruvic acid from L-tryptophan and 2-oxoglutarate. According to the data shown in Figure 2, when cultivating *A. calcoaceticus* IMV B-7241 on the crude glycerol medium with 300 mg/L tryptophan the activity of this enzyme was higher than on the medium without this precursor. In addition, it should be noted that when introducing tryptophan at the end of the exponential growth phase, the activity of tryptophan transaminase was in 3.2 times higher than when tryptophan was introduced in the lag phase, which is consistent with the data given in Table 1 regarding the concentration of formed auxins. These data suggest that IAA biosynthesis in *A. calcoaceticus* IMV B-7241 going through the formation of indole-3-pyruvate (see Figure 1).

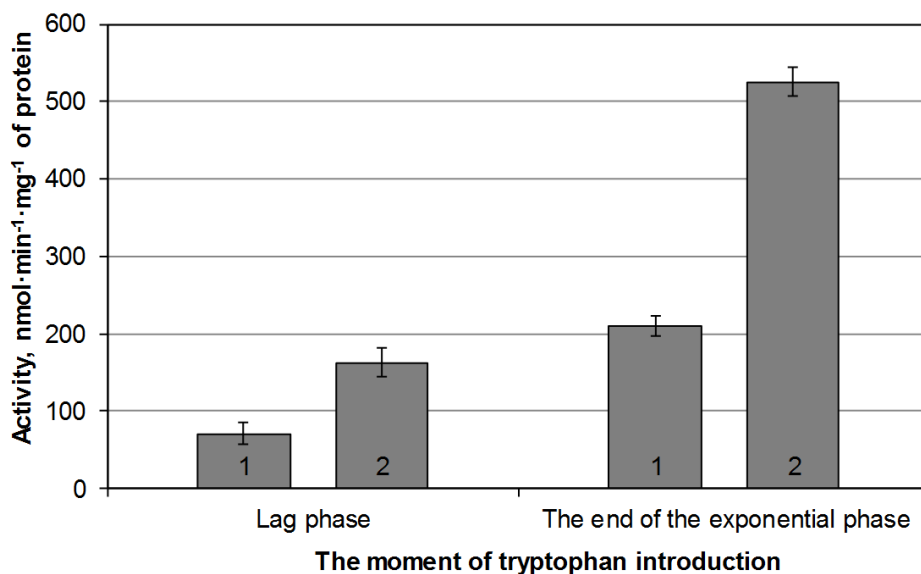


Figure 2. The effect of tryptophan on the activity of tryptophan transaminase under different conditions of cultivation of *A. calcoaceticus* IMV B-7241
1 – without tryptophan; 2 – tryptophan, 300 mg/L

In 2015–2016, we published two papers [12, 13] in which we first reported the ability of surfactant producers *Rhodococcus erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *Nocardia vaccinii* IMV B-7405 to synthesize phytohormones of auxin nature. Only after the publication of this work it was reported that indolyl-3-acetic acid was formed by bacteria (mainly *Rhodococcus* species) isolated from contaminated hydrocarbons and heavy metals [60]. However, the authors have established the ability to synthesize surfactants by the index of emulsification and the reduction of surface tension, which appeared to be insignificant – up to 60–65 mN/m (against 30–35 mN/m in the surfactant producers).

In 2018, papers [14–16] were published in which the ability of producers of surface-active lipopeptides and rhamnolipids to synthesize phytohormones of auxin nature was established. Thus, endophytic strain *Bacillus* sp. Fc11 [14] synthesized iturin A and surfactin, which had antimicrobial effects on the phytopathogenic fungi of the genera *Fusarium*, *Phytophthora*, *Sclerotium*, *Corynespora*, as well as indole-3-acetic acid, presence of which in the culture broth was determined by a qualitative reaction with a Salkowski reagent. The authors did not analyze the concentration of synthesized lipopeptides and indolyl-3-acetic acid.

Bacillus sp. B19, *Bacillus* sp. P12 and *B. amyloliquefaciens* B14, isolated from the soil, synthesize a complex of antimicrobial compounds (surface-active lipopeptides kurstakin, surfactin, iturin, fengycin and antibiotic polymyxin), as well as auxins [15]. The concentration of auxins synthesized by strains B19 and P12 was 5.71 and 4.90 mg/L, respectively. Tryptophan was not added to the culture medium. However, tryptone which contains tryptophan was used as a carbon source for cultivation of producers.

The endophytic strain of *Pseudomonas aeruginosa* L10 [16] under the cultivation on diesel fuel (5 g/l) synthesized rhamnolipids, which reduced the surface tension to 29.5 mN/m, and indolyl-3-acetic acid at a concentration of 27 µg/l. It should be noted that in this work the authors did not try to increase the synthesis of IAA.

It is worth noting that after the publication of our review [4], in which the existing literature on the synthesis of phytohormones by different microorganisms was analyzed, there were papers in which the researchers compared the synthesis of auxins in the absence and presence of precursors.

The causative agent of tomato disease *P. syringae* DC3000 synthesizes IAA through the formation of indole-3-acetaldehyde from indole-3-pyruvate (see Fig. 2) [17]. When 0.25 mM tryptophan was added to the culture medium, the IAA concentration was 2.7 µg/ml, which is several orders of magnitude higher than without the introduction of the biosynthesis precursor (0.03 mg/L). At the same time, the researchers tested the effects of other precursors on the formation of IAA, in particular, the effects of indole-3-acetaldehyde and indole-3-acetonitrile. Thus, with introduction into the culture medium of these precursors, the concentration of auxin at 48 h of cultivation was 11.7 µg/ml and 14.1 µg/ml, respectively.

Analysis of the genome and metabolic pathways of *Variovorax boronicumulans* strain CGMCC 4969 shows that IAA biosynthesis in these bacteria begins with indole-3-acetonitrile, which enters the cell exogenously. Therefore, with addition to the culture medium of acetonitrile and cobalt, the concentration of phytohormone after 60 hours of cultivation was 1.88 mmol/L (the concentration of IAA synthesized without a precursor was not determined).

Scientists from Thailand and Japan [18] have established the ability of the endophytic fungi *Colletotrichum fructicola* CMU-A109 to synthesize IAA in high concentrations in the presence of tryptophan. After 26 days of CMU-A109 cultivation, the IAA concentration was 1.2 mg/L.

When introducing tryptophan into the culture medium of the strains of *Mortierella* sp. MA DEM7 and MA DEM32 [19], an intensification of IAA synthesis was observed. The highest concentration (32 mg/L) was reached after 9 days of cultivation of the MA DEM32 strain on the medium with 1.5 mM tryptophan. In the case of growing of MA DEM7 strain in the presence of 3.0 mM tryptophan, the amount of synthesized IAA was twice lower.

Thus, analysis of the literature has shown that introduction of biosynthesis precursors is effective for enhancing the synthesis of phytohormones. However, the authors of works [17–19] added tryptophan in fairly high concentrations. The use of large quantities of tryptophan as a component of the nutrient medium is not economically feasible. Our studies have shown that at significantly lower concentrations of tryptophan, the intensification of the auxin synthesis is in hundreds of times.

In addition, most scientists are studying the synthesis of phytohormones on rich nutrient medium that contain glucose, sucrose, dextrose, glucuronic acid, peptone, tryptone, mannitol as carbon source [10, 11, 15, 16]. Such mediums for growing phytohormone producers are expensive, so there is a need to reduce their cost, in particular by finding cheaper carbon substrates. We have for the first time established the possibility of the auxin formation on a cheap medium using waste biodiesel production as a substrate. There is no such information in the literature.

Conclusion

Thus, the result of the work established the possibility of increasing by one or two orders of magnitude of the synthesized auxins in the case of introduction low concentrations of their biosynthesis precursor in the culture medium of strain *A. calcoaceticus* IMV B-7241 with biodiesel production waste. The obtained results are the basis for increasing the efficiency of the use of the complex preparation in plant production.

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Vacuum-caps membranes' equilibrium state forms based on the energy criterion

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Abstract

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Introduction. In order to describe the energy transformation that conditions the membranes' stable operation under a given mode of product processing, the studies of glass containers' vacuum caps membranes equilibrium state forms have been carried out.

Materials and methods. The glassware containers represent the study object hereunder, the research subject being their caps' flexible membranes. Instrumental methods have been used to assess the membrane thickness and deflection. The forms of equilibrium state and membrane equilibrium energy levels were investigated through mathematical simulation by energy modeling.

Results obtained and discussion. After loss of stability, the membrane shifts into a different position of stable equilibrium. The measurement results showed that at stability loss state, the membrane center additional deflection is $f = 0,07$ mm, that is significantly less than the membrane center initial deflection value, $f_{init} = 0,25$ mm. Therefore, as a result of the stability loss the membrane working cone is not subjected to the mirror deformation. These deflection parameters ensure that the membranes are operable over a wide range of drop between the system backpressure (at an autoclave) and the container internal pressure throughout the product thermal processing.

The circular plate total energy is represented as the sum of the zero-torque stress state energy, bending energy and the external pressure work values. Calculated is the derived membrane total energy equation, obtained are the membrane energy levels for different stable equilibrium states.

Substituting the experimentally obtained value f in the calculated equation of stability loss state membrane total energy we get the cap membranes stability loss pressure value calculated for the considered case, $P_l = 0,0326 \cdot 10^6$ Pa. The calculated value P_l is close to the cap membranes stability loss pressure value $P_l = 0,03$ MPa, specified by the caps manufacturer. The P_l pressure calculated value deviation from the real one can be influenced by the hardness of tin used to produce the cap bearing such membrane.

The obtained energy levels of membrane's different equilibrium states at two different loads do correspond to the critical pressures of stability loss P_1^* and load releasing P_2^* . Both pressures energy levels diagrams built using the calculated equation, include the potential energy minimums. This form of the energy criterion curves for critical pressures is completely consistent with the Lagrange-Dirichlet theorem, since the pressure P_{lmin}^* corresponds to the energy level minimum relative to other adjacent states.

Conclusions The membrane total energy score equation and the energy levels for the equilibrium states corresponding to the critical pressures have been obtained.

Introduction

Modern capping elements for glass containers of various capping systems contain membranes specific with a small initial deflection and, depending on their positively or negatively convex state indicate the vacuum presence or absence in the packed container, and, consequently, its tightness [1].

Depending on the glass container size and the mode of heat treatment, the caps are made of different thickness and hardness tin, i.e. tin of different energy levels [2]. In modern science, there are several methods for calculating thin plates. They are presented in [3,4,5]. The authors calculate mainly thin flat plates under different types of load. However, thin plates with an initial deflection due to the load applied, making this study subject, have not been considered in those sources.

The thin plates calculation energy method exposed by numerous authors, is widely known and effectively used [6, 7, 8]. The article [9] uses the energy method to solve the local bending problem for a thin-walled cylindrical steel tube with elastic filling under the concentric axial load applied. The problems of calculating thin plates that bear an initial deflection to counteract the load are not reflected in those works.

The study of Volmir A. [10] describes the operation of initially deflected round flexible plates, fixed in various ways, at different, relative to the initial deflection, directions of applied load action. However, it is presented in general form and is not supported with a complete scientific calculation that would describe and explain the membranes operation including the consideration of basic material energy component.

In the study by Timoshenko S. et al. [11], approximate formulas for calculating an uniformly loaded round plate with significant deflections are given. In [12], the general Lagrangian formulation for flexible plates is considered accordingly to the finite element method for plates under large displacement and rotation applied. The round plates with small displacement studied in our research were not considered therein.

Regarding the metal packaging containers, the vacuum cap membranes operation studies have been carried out. In [1], an equation for the relationship between pressure, geometric parameters, and membrane thickness has been obtained. In [13], described is a simulation as to the deformation behavior of the canned glass containers metal caps membranes during storage and processing of packaged products. On the basis of the obtained scope equation, a computer program has been developed for building the "pressure – deflection" relationship model. Obtained are the membranes deformation characteristics depending on changes in the tin sheet initial deflection and thickness [13]. However, the membrane total energy change during the deformation process, and primarily in the equilibrium states, has not been studied thus implying such study necessity. As a result, certain difficulties arise regarding the elastic plate functional reliability and its design.

This study is purposed to describe the initially deflected round plate's energy change that causes the membranes' stable operation in a given mode.

This study objectives:

1. To solve the equations of local energy effects on the membrane during its operation and to get the membrane system total energy scope equation.
2. To assess experimentally the membrane additional deflection in the working position.
3. To check the obtained equation adequacy for the operation of glass containers metal caps real membranes.
4. To build graphs of the membrane energy levels according to its characteristic equilibrium states.

Materials and methods

Studied materials

For experimental studies, we used caps with a "safety button" according to the "twist-off" system [2] with a diameter of 82 mm, produced by the German company Silgan White cap, and used by the EU and Ukrainian canning enterprises.

Structurally, the membranes are designed to operate in a controlled stability loss mode, Figure 1a. The membranes include a support cone of D_3 outer diameter, a working cone of D_2 outer diameter, and a flat section of D_1 diameter. They lose their stability due to the vacuum occurring in the container that causes a pressure drop on the cap field. Further we refer to this pressure drop as to the pressure (applied).

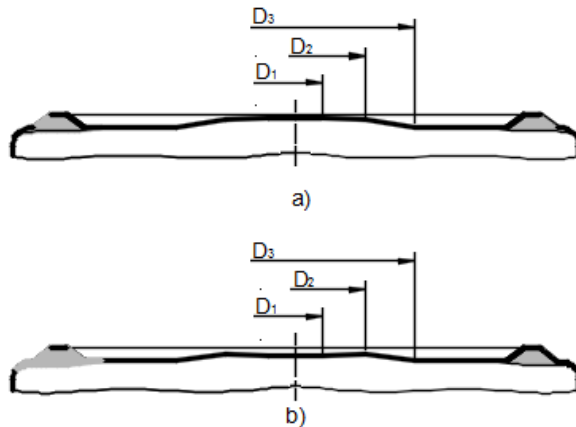


Figure 1. Cap field:
a – membrane non-loaded state; b – membrane stability loss state.

Next to the stability loss, the membrane moves to the stable equilibrium state, Figure 1b. The values of stability loss and shape restoring critical pressure which determine the existing membranes operation interval are generally known and some companies-manufacturers of glassware caps do specify those values

Accordingly, this research object is a glass packaging container with vacuum cap. The research subject represents the flexible membranes of metal caps.

The membrane center additional deflection in the stability loss state was determined experimentally.

Preparing the experimental samples

The packaging samples have been prepared in production enterprise conditions. The caps were fed to a steam-vacuum capping machine, where they were used to seal glass jars with products in the operating mode. Then the product was cooled reaching the ambient temperature to create a vacuum in the empty container volume, after that the tested sample was ready for experiment.

Procedure for research conducting

The round plate potential energy depends on the geometry of its cross-section (configuration). In the analytical study of the membrane equilibrium state kinds we will adhere to the design scheme, when the membrane working cone is pinched along the contour with a free radial displacement of the contour points, since it is closest to reality (Figure 2). The membrane has an initial deflection f_{in} .

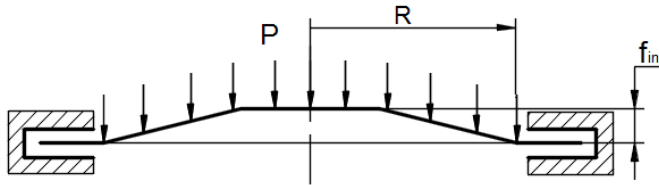


Figure 2. Position fixing diagram: membrane non-loaded state.

The measurement of membrane center additional deflection in the stability loss state followed such sequential steps.

1. After cooling the product, the capped jar was placed on the indicator device control plate that instrument being adjusted ready for measurements (see paragraph 1-3 below).

2. Using a sharp awl, a hole was pierced in the cap field (not on the membrane). The pressure in the container was compared with atmospheric pressure and the membrane did stepwise return to its original state of equilibrium. At that the indicator arrow deviated from the zero position. The instrumental readings were recorded.

3. Additional deflection of the membrane center in the stability loss state f , measured from the fixing contour plane, Figure 3, was found as the difference between the indicator reading and the membrane center initial deflection.

The membrane fixing contour possible deflection due to the action of vacuum in the container was neglected with respect to its insignificant value in comparison with the functional travel of the membrane center.

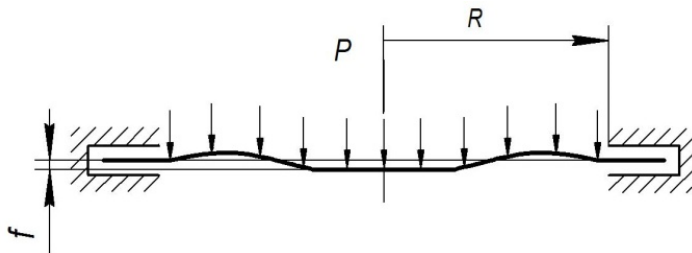


Figure 3. Position fixing diagram: membrane stability loss state

Description of methods and equipment

Additional deflection and thickness of the membrane sheet were determined by instrumental methods on real glassware with a cap.

The deflection was measured using an indicator device Figure 4. The metering device consists of a tripod for measuring heads 1, fixed on a horizontal control plate 2 bearing holes for arresters, a clock-type indicator 3, (measurement error 0,01 mm), and two locking arresters 4.

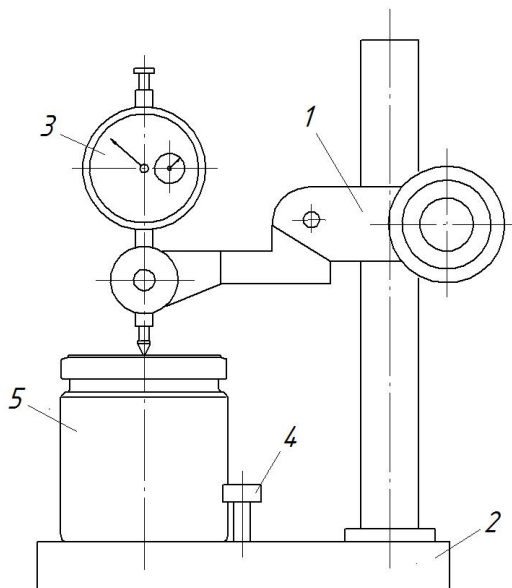


Figure 4. Indicator device.

1 – tripod; 2 control plate; 3 – dial gauge; 4 – locking stops; 5 – tested sample.

Preparing the device for measurements followed such sequential steps.

1. The tested sample placed its bottom on the control plate, the indicator measuring rod was raised thus the sample was wound up under the measuring head.
2. The indicator tip being brought into contact with the membrane flat section center after that the container position was fixed using two locking arresters, previously installed in the control plate holes suitable for the given container body dimensions.
3. The indicator clip terminal on the tripod released, the indicator measuring tip was adjusted to contact the cap membrane center so that the arrow on its small scale deviated from zero by an integer number of millimeters, but not less than the membrane's initial deflection twice value. Next the indicator was set to zero with fixing its position on a tripod.

Since the tested sample consists of several parts (jar, cap, sealing gasket of the cap) that have their own height tolerance, each sample will have its own height. Suffice to say is that only the glass containers height tolerance can vary between 1-3 mm. Therefore, for each tested sample measurement required is an individual presetting of the device according to procedure preparing the experimental sample.

The tin caps thickness was measured by a micrometer with an error of 0,01 mm.

The membrane equilibrium state kinds' mathematical simulation was performed using the plates and shells theory energy method described by numerous authors [6, 7, 8]. The membranes' operation in the mode of controlled stability loss is closely related to their material energy component, so it is most appropriate to use namely this method. According to the method above, the round plate total energy is represented as the sum of the zero-torque stress state energy, the bending energy, and the work of external pressure.

The membrane energy levels diagrams were built by mathematical modeling using computational methods in the Scilab environment [14, 15].

Study of research results

The experiment used a membrane with parameters $f_{in}=0,25$ mm; tin thickness $\delta=0.18$ mm; membrane fixing contour radius $R=12$ mm, made of low-carbon steel. The measurements results showed that in the stability loss state, the membrane center additional deflection was $f=0,07$ mm, that is significantly less than the membrane center initial deflection $f_{in}=0,25$ mm, Figure 3. That is, as a result of stability loss membrane working cone mirror deformation does not occur.

For a visual interpretation of the membrane operation, the membrane energy levels diagrams were built for its characteristic equilibrium states: loss of stability and load releasing. The energy levels diagrams allowed us to check the research results compliance with the main provisions of theoretical mechanics, in particular the Lagrange-Dirichlet theorem [16].

Results and discussions

Derivation of the membrane system total energy scope equation.

For calculating the system energy we use the initial equations from the plates and shells theory. The membrane system total energy is found similarly to the axi-symmetric bend energy for a circular plate [10]

$$E_t = E_m + E_b - W, \quad (1)$$

where E_m is the stress energy in the membrane middle surface,

$$E_m = \frac{E\delta}{2} \int_0^R \left[\left(\frac{d^2\Phi}{dr^2} \right)^2 + \left(\frac{1}{r} \frac{d\Phi}{dr} \right)^2 - \frac{2\mu}{r} \frac{d\Phi}{dr} \frac{d^2\Phi}{dr^2} \right] 2\pi r dr; \quad (2)$$

E_b is the bending energy,

$$E_b = \frac{D}{2} \int_0^R \left[\left(\frac{d^2\omega}{dr^2} \right)^2 + \left(\frac{1}{r} \frac{d\omega}{dr} \right)^2 - \frac{2\mu}{r} \frac{d\omega}{dr} \frac{d^2\omega}{dr^2} \right] 2\pi r dr; \quad (3)$$

W is the external load work,

$$W = \int_0^R P\omega 2\pi r dr = 2\pi P \int_0^R \omega r dr, \quad (4)$$

where E is the modulus of membrane material normal elasticity;

δ is the membrane (tinplate) thickness;

R is the membrane fixing contour radius;

Φ is the stress function;

r is the membrane current radius;

μ is the membrane material Poisson's ratio;

$D = \frac{E\delta^3}{12(1-\mu^2)}$ is the cylindrical stiffness of the membrane;

ω is the membrane additional current deflection;

P is the pressure (load) applied to the membrane.

Write an expression for $\frac{d\Phi}{dr}$ [10]

$$\frac{d\Phi}{dr} = \frac{Ef^2}{6R} \left(3\frac{r}{R} - 6\frac{r^3}{R^3} + 4\frac{r^5}{R^5} - \frac{r^7}{R^7} \right), \quad (5)$$

where f is the membrane center additional deflection.

We introduce the dimensionless current radius $r_1 = \frac{r}{R}$, then $dr_1 = \frac{dr}{R}$; $\frac{d\Phi}{dr} = \frac{1}{R} \frac{d\Phi}{dr_1}$;

$$\frac{d^2\Phi}{dr^2} = \frac{1}{R^2} \frac{d^2\Phi}{dr_1^2}.$$

After substituting these references in (5), we get

$$\frac{d\Phi}{dr_1} = \frac{Ef^2}{6} (3r_1 - 6r_1^3 + 4r_1^5 - r_1^7). \quad (6)$$

Respectively

$$\frac{d^2\Phi}{dr_1^2} = \frac{Ef^2}{6} (3 - 18r_1^2 + 20r_1^4 - 7r_1^6). \quad (7)$$

Entering the $R(r_1) = 3r_1 - 6r_1^3 + 4r_1^5 - r_1^7$, we get (6) and (7) respectively in the form

$$\frac{d\Phi}{dr_1} = \frac{Ef^2}{6} R(r_1) \quad \text{and} \quad \frac{d^2\Phi}{dr_1^2} = \frac{Ef^2}{6} \frac{dR}{dr_1}. \quad (8)$$

Write (2) for the dimensionless current radius r_l , given that for $r=0 \rightarrow r_l \rightarrow 0$, and for $r=R \rightarrow r_l=1$

$$E_m = \frac{2\pi E\delta}{2} \int_0^1 \left[\frac{1}{R^4} \left(\frac{d^2\Phi}{dr_1^2} \right)^2 + \frac{1}{R^4} \left(\frac{1}{r_1} \frac{d\Phi}{dr_1} \right)^2 - \frac{1}{R^4} \frac{2\mu}{r_1} \frac{d\Phi}{dr_1} \frac{d^2\Phi}{dr_1^2} \right] R^2 r_1 dr_1. \quad (9)$$

After transformations and substitutions of expressions (8), the equation (9) takes the form

$$E_m = \frac{\pi E^3 f^4 \delta}{36R^2} \int_0^1 \left[r_1 \left(\frac{dR}{dr_1} \right)^2 + \frac{1}{r_1} R^2(r_1) - 2\mu R(r_1) \frac{dR}{dr_1} \right] dr_1. \quad (10)$$

Denote the integral part of the equation (10)

$$I_1 = \int_0^1 \left[r_1 \left(\frac{dR}{dr_1} \right)^2 + \frac{1}{r_1} R^2(r_1) - 2\mu R(r_1) \frac{dR}{dr_1} \right] dr_1.$$

Then the equation of membrane middle surface stress energy is

$$E_m = \frac{\pi E^3 f^4 \delta}{36R^2} I_1. \quad (11)$$

Now we consider the bending energy E_b equation (3).

In the case of plate elastic deformation, the approximate expression for additional deflections corresponds to the similar problem solution in the case of a small deflection plate [11],

$$\omega = f \left(1 - \frac{r^2}{R^2} \right)^2. \quad (12)$$

Write (12) for the dimensionless current radius r_l

$$\omega = f(1 - r_l^2)^2 = f(1 - 2r_l^2 + r_l^4), \quad (13)$$

$$\frac{d\omega}{dr_l} = 4fr_l(r_l^2 - 1). \quad (14)$$

Entering the $W_1(r_l) = r_l(r_l^2 - 1)$, we get (14) taking the form

$$\frac{d\omega}{dr_l} = 4fW_1(r_l), \quad (15)$$

respectively

$$\frac{d^2\omega}{dr_l^2} = 4f \frac{dW_1}{dr_l}. \quad (16)$$

We write (3) for, the dimensionless current radius r_l , given that for $r=0 \rightarrow r_l \rightarrow 0$, and for $r=R \rightarrow r_l=l$

$$E_b = \pi D \int_0^l \left[\frac{1}{R^4} \left(\frac{d^2\omega}{dr_l^2} \right)^2 + \frac{1}{R^4} \left(\frac{1}{r_l} \frac{d\omega}{dr_l} \right)^2 + \frac{1}{R^4} \frac{2\mu}{r_l} \frac{d\omega}{dr_l} \frac{d^2\omega}{dr_l^2} \right] R^2 r_l dr_l. \quad (17)$$

After transformations and substitutions (15) and (16), the equation (17) takes the form

$$E_b = \frac{16f^2\pi D}{R^2} \int_0^l \left[r_l \left(\frac{dW_1}{dr_l} \right)^2 + \frac{1}{r_l} W_1^2(r_l) + 2\mu W_1(r_l) \frac{dW_1}{dr_l} \right] dr_l. \quad (18)$$

We denote the integral part of the equation (18)

$$I_2 = \int_0^l \left[r_l \left(\frac{dW_1}{dr_l} \right)^2 + \frac{1}{r_l} W_1^2(r_l) + 2\mu W_1(r_l) \frac{dW_1}{dr_l} \right] dr_l.$$

Then the bending energy equation is

$$E_b = \frac{16f^2\pi D}{R^2} I_2. \quad (19)$$

Now we consider the external load work W equation (4).

We write (4) for, the dimensionless current radius r_l , given that for $r=0 \rightarrow r_l \rightarrow 0$, and for $r=R \rightarrow r_l=l$, so after substituting (13) and transformations

$$W = 2\pi PfR^2 \int_0^l (r_l - 2r_l^3 + r_l^5) dr_l. \quad (20)$$

Then, integrating performed, the external load work equation is

$$W = \frac{\pi PfR^2}{3}. \quad (21)$$

After substituting (11), (19) and (21) into the equation (1)

$$E_t = \frac{\pi E^3 f^4 \delta}{36R^2} I_1 + \frac{16f^2\pi D}{R^2} I_2 - \frac{\pi PfR^2}{3}. \quad (22)$$

We introduce a dimensionless additional deflection of the membrane center $\zeta = \frac{f}{\delta}$ and a dimensionless pressure (load) on the membrane $P^* = \frac{PR^4}{\delta^4 E}$.

After substituting them in (22) and transformations, we get

$$E_t = \frac{\pi}{R^2} \left[E\delta^5 \frac{\zeta^4}{36} I_1 + 16\delta^2 D\zeta^2 I_2 - E\delta^5 \frac{\zeta P^*}{3} \right]. \quad (23)$$

Now we find integrals I_1 and I_2 . Poisson's ratio for steel $\mu=0.35$.

Integrating I_1 we get

$$I_1 = 48,8167 + 1,7047 + 1,4 = 51,9214.$$

Integrating I_2 we get

$$I_2 = 0,5 + 0,1667 + 0 = 0,6667.$$

To graphically compare the membrane different equilibrium states energy levels we introduce the dimensionless total energy

$$E_t^* = \frac{E_n R^2}{E\delta^5}. \quad (24)$$

After substituting (23) in (24) and calculation carried out, we get

$$E_t^* = 1,4423\zeta^4 + 1,013\zeta^2 - 0,3333P^*\zeta. \quad (25)$$

We reduce equation (25) to the equilibrium equation form. We differentiate equation (25) by $d\zeta$ and equate it to 0. As a result, we get

$$\zeta^3 + 0,3512\zeta - 0,0578P^* = 0. \quad (26)$$

Analysis and verification of the obtained equilibrium equation correctness

Substituting the experimentally obtained value of f after stability loss in the equilibrium equation (26), we can obtain the corresponding critical pressure value.

If $f=0,07$ mm, the corresponding dimensionless deflection is $\zeta=0,39$. Expressing from (26) P^* we get

$$P^* = \frac{\zeta^3 + 0,3512\zeta}{0,0578},$$

where, for the case under consideration, we get $P_1^*=3,3958$. Accordingly we find P_1 from

$$P = \frac{P^* \delta^4 E}{R^4},$$

where from, for the case under consideration, we get $P_1=0.0326 \cdot 10^6$ Pa.

The calculated value of P_1 is close to the value of cap membranes pressure stability loss $P_1=0,03$ MPa, specified by the jar caps manufacturer [17]. The calculated pressure value P_1 deviation from the experimental one may be due to the hardness class of the tin plate used to produce the membrane-equipped cap.

After the stability loss, the membrane shifts to the stable equilibrium state. The measurement results showed that in the stability loss state, the additional deflection of the membrane center $f=0,07$ mm, significantly less than the initial deflection of the membrane center $f_{in}=0,25$ mm, Figure 3. That is, as a result of stability loss membrane working cone mirror deformation does not occur.

These additional deflection parameters guarantee the membrane's performance over a wide range of drop between the system's back pressure (in the autoclave) and the container's internal pressure throughout the entire product heat treatment process. As P_I hazardous value usually considered is this one greater than 0,07 MPa. Then during sterilization (thermal exposure or cooling start stages), there may be a malfunction of the control button due to the metal elastic deformation transition into plastic one.

The degree of tin hardness is increased to improve its rigidity. Through hardness increasing compensated is the decrease in tin strength which inevitably occurs in the case of the rolled steel thickness decrease. In the range of tin thickness used for caps, the range of hardness changes is constant and narrow.

Analysis and explanation of energy levels at membrane equilibrium states

We shall consider the energy levels of different membrane equilibrium states under two different loads that correspond to critical pressures: stability loss P_1^* and load release P_2^* . They are shown in Figure 5, and are built using the graphically interpreted equation (26) for pressures P_1^* and P_2^* . At values $P_1^* = 3,12$ and $P_2^* = 0,52$, corresponding to $P_I = 0,03 \cdot 10^6$ Pa and $P_2 = 0,005 \cdot 10^6$ Pa, for the membrane center initial dimensionless deflection $\zeta_{in} = 1,39$, which is defined as $\zeta_{in} = \frac{f_{in}}{\delta}$, and the membrane parameters given above. Other design parameters $\mu = 0,35$; $E = 190 \cdot 10^9$ Pa. Here E and μ correspond to low-carbon steel.

The diagrams show that there is a minimum energy value for both pressures. The pressure P_{1min}^* corresponds to the moment when it causes the center's certain additional deflection, which reached the membrane abruptly loses stability, passing into another, opposite, state of equilibrium (Figure 3). This state corresponds to the center's additional deflection which value was measured using an indicator device.

The pressure P_{2min}^* corresponds to the moment when the membrane, being unloaded, abruptly resumes its shape under its internal energy action, but it does not yet reach the initial state of equilibrium, since $P_{2min}^* \neq 0$.

This type of energy criterion curves for critical pressures fully corresponds to the well-known Lagrange-Dirichlet theorem [16], according to which the main stable equilibrium state is the plate state corresponding to the pressure P_1^* , since the pressure P_{1min}^* corresponds to the energy level, minimum relative to other adjacent states.

The membrane initial state when no external load applied, i.e. $P^* = 0$, and it is in a state of equilibrium, is taken as zero energy value level. This state is the same for both pressures, since after load full release, the membrane returns to the initial state of equilibrium when the controlled stability loss mode maintained.

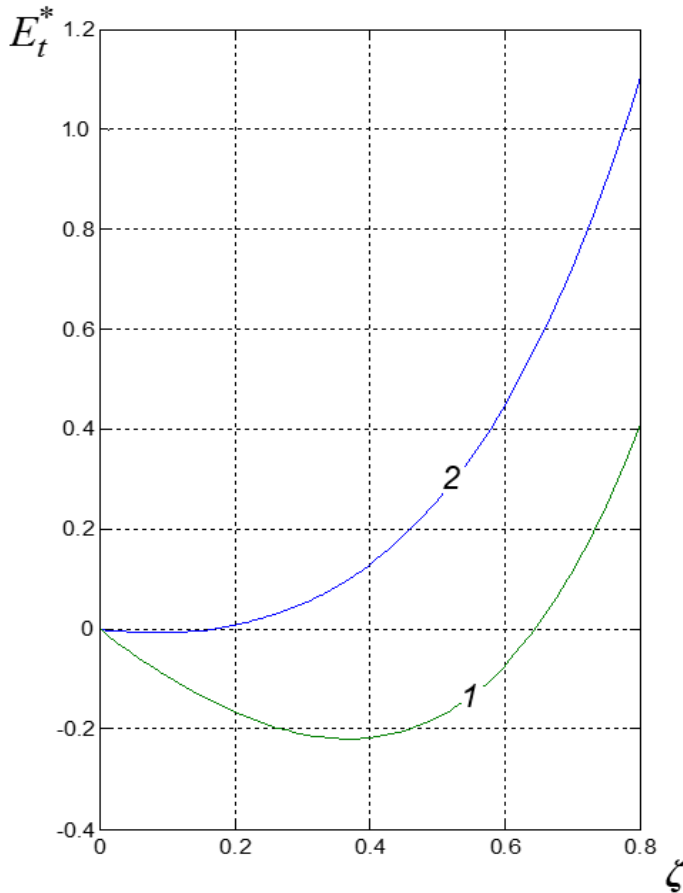


Figure 5. Diagrams of the membrane energy levels:
1 – for the stability loss pressure P_1^* ; 2 – for the load release pressure P_2^* .

Conclusions

1. The equation (26), obtained by solving the local energy effects equations, is a scope equation that characterizes and represents the energy transformations in the membrane body.
2. It is experimentally found that the membrane additional deflection when operational state is significantly less than its initial deflection.
3. The quoted calculation demonstrates the resulting scope equation's adequacy to the initially bent real metal plates in case of load counteracting the initial deflection, in particular for glass canning containers' metal caps membranes
4. The membrane energy levels diagrams obtained using equation (26) for characteristic equilibrium states are correct and agree with the Lagrange-Dirichlet theorem. Therefore, the equation (26) can be used in the industry manufacturing the canned food products metal packaging materials, as well as in the instrument building industry.
5. The round elastic plate controlled stability loss computational mode has been build, checked and developed.

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Influence of geometric and dynamic parameters of a water-polymer jet on characteristics of food products hydro-cutting process

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Abstract

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Introduction. The process of water-polymer jet cutting of food products in order to increase the efficiency of the method of hydro-jet cutting by modifying the working fluid is investigated.

Materials and methods. The process of cutting chicken fillet, hake fish, beef and pork meat is researched. The experiments were carried out at temperatures of -7 °C and -25 °C, pressure changes from 50 MPa to 150 MPa, nozzle diameter $0.35 \cdot 10^{-3}$, $0.6 \cdot 10^{-3}$ m and velocity of the water-polymer jet movement relative to the sample of food product of 0.015, 0.025, 0.050 and 0.100 m/s. The PEO concentration with molecular mass of $6 \cdot 10^6$, $4 \cdot 10^6$ and $3 \cdot 10^6$ varied from 0 till 0.05%.

Results and discussions. The depth of the cut in a frozen food product increases quite rapidly with the increasing of concentration and PEO molecular mass and reaches its maximum at some optimal concentrations (C_{opt}). For PEO with a molecular mass of $3 \cdot 10^6$, the C_{opt} equal to 0.015-0.020%, and for molecular mass of $4 \cdot 10^6$ and $6 \cdot 10^6$ – 0.007-0.010% and 0.0015-0.0020%, respectively. Increasing the diameter of the nozzle hole under the constant pressure of PEO water solution leads to increasing of the cutting depth in a food product. Noted character of influence of nozzle hole diameter of hydro-cutting jet-shaping head on the cutting depth is due to the fact that the nozzles usage with a relatively big nozzle outlet diameter under the constant velocity of water-polymer jet movement in relation to the food sample and pressure of PEO water solution in front of the nozzle should lead to increasing of energy per unit of the cutting food product surface. To achieve the same cutting depth of frozen food products (in the range of -7 and -25 °C) it's enough to have water-polymer jet pressure of only 45-65% from the water jet, and vice versa, under the same initial pressure, 1.5–2.5 times increase in the depth and cutting velocity with water-polymer jet is observed, what tells about the special mechanism of its interaction with a food product. The dependence of the dimensionless cutting depth by water-polymer jet in frozen food products from the dimensionless distance to the nozzle section is obtained. The usage of high-velocity jet of PEO water solution for cutting frozen food products considerably increases the efficiency of the cutting process and the quality of the cutting surface.

Conclusions. In order to significantly improve the efficiency of food products water-cutting process, it is advisable to use jet of PEO water solution as the working fluid.

Introduction

Scientific research in the field of cutting of different materials by high-speed water-jet [1, 2] show that the efficiency of the cutting process (depth of cut) is significantly increased under water pressures above 400 MPa. The results of research carried out in [1, 3] show that the destruction efficiency is nonlinear (depth of cut is increasing more intense than the hydraulic power supplied). In works [4, 5] is shown the results of the research of cutting process of thin polymeric materials by high-pressure water jets. It was found that with an increase of the nozzle diameter from $0.1 \cdot 10^{-3}$ till $0.4 \cdot 10^{-3}$ m, the cutting depth in the material decreases. For example, for vinyl plastics in 2.85 times, for getinax in 5 times and for fiberglass in 8 times. It should be noted, that there is a critical pressure below which the destruction process does not occur, and temperature reduction of the food product to -25 °C and below, makes impossible to use water-jet cutting under pressures less than 200–300 MPa [6–8].

Cutting with using water-jet mixed with abrasive (sand, etc.) allows to cut virtually any material [9–11]. During contact with the material being cut, the kinetic energy of the water-jet with the abrasive is converted into mechanical energy of the material micro-destruction, and the cutting process takes place [12, 13]. Obviously, the use of the traditional abrasive water-jet cutting method in the food industry is not possible at all, due to the inevitable contamination of the product with abrasive substances.

In hydro-jet cutting of food products, a high-velocity thin jet of fluid is used as the cutting unit [6, 7]. The ability to obtain the necessary hydrodynamic characteristics of the hydro-jet, that providing maximum productivity depends on the properties of the working fluid. Minimization of energy costs, first of all, should be provided by reducing of the fluid working pressure before the nozzle to the lowest [7]. Therefore, the choice of type and content of the working fluid is one of the main issues that need to be addressed when developing the technological process of food products hydro-jet cutting. Solution this question can be approached using the observed features in the hydrodynamic behavior of polymer solutions under longitudinal flow [14–17]. Longitudinal Velocity Gradient is realized in the inlet section of the nozzle [18–20]. The polymer should be a safe substance and allowed for use in the food industry. Such polymer can be polyethylene oxide (PEO), which is a safe substance and used in the food industry as a thickener, flocculant, etc. [21, 22]. Patent No. 74609. 2012, Ukraine (Water-polymer Method of Cutting Frozen Food Products and Materials) indicates the perspective of cutting food products by water-polymer jet.

The aim of the work is to establish regularities of the influence of geometric and dynamic parameters of water-polymer jet on the frozen food products cutting efficiency needed for supporting of the design and processing works of the food products hydro-cutting equipment.

Materials and methods

Materials: chicken fillet, hake fish, beef [7,19] and pork meat, polyethylene oxide solutions of molecular mass $3 \cdot 10^6$, $4 \cdot 10^6$ and $6 \cdot 10^6$ [20–22].

Experimental methods of study with the use age of: the created hydro-stand with a working pressure up to 500 MPa and the possibility to change and control both integral and differential parameters of food products cutting process by water-polymer jet; developed

special stands with temperature control and temperature stabilization within a wide range (up +20 °C to -40 °C); capillary and rotary viscometers for rheological measurements [7, 20]. The profilograms of the cutting surfaces in the food product were obtained by the stylus method of measurement. The profilograms obtained by this method allow not only to record the profile of the lateral cutting surface, but also to measure the parameters of roughness, waviness and deviation from the form. The quality of cutting surfaces in frozen food products was determined by using profilograph-profilometer block design with digital display of measurement results. This device allows to measure parameters of the roughness R_a from 0.02 till 100 mkm and H_{max} and H_{min} from 0.1 till 1000 mkm. The device error does not exceed 10%. The proposed three-grade scale for assessing the quality of the lateral cutting surface is given in the work [7].

Results and discussion

The criteria for assessing the efficiency of the food products cutting process by the water-polymer jet is the cutting depth h and the growth rate of the lateral cutting surface S_0 [7, 20]. The growth rate of the lateral surface characterizes the productivity of this process [7]. The productivity of the process of hydro-jet water-polymer food products cutting depends, firstly, on power and geometric properties of the high-speed water-polymer jet, the mutual arrangement of the water-polymer jet and food product, hardness, cutting marginal stress and tensile strength under uniaxial compression of food products, sizes of cutting food product, and also of the molecular mass of PEO and its concentration in the water-polymer jet [20].

First, let's consider the influence of PEO concentration, and then the geometric and dynamic parameters of the hydro-jet on the characteristics of the process of hydro-jet water-polymer food products cutting with different *subzero* temperatures.

Influence of PEO concentration and distance from the nozzle section to surface of food product on the process of hydro-jet water-polymer cutting

Experimental studies of the influence of distance from the nozzle section to the frozen food product cutting surface l_0 on the cutting depth h were carried out on samples of beef and pork, and also of the hake fish and chicken fillet at temperatures t from room temperature till -25 °C and the pressure change ΔP_0 from 50 MPa till 150 MPa, nozzle diameter $d_{noz.}$ of $0.35 \cdot 10^{-3}$, $0.6 \cdot 10^{-3}$ m and the speed of water-polymer jet movement relative to the food product sample $V_{m.j.}$ of 0.015, 0.025, 0.050 and 0.100 m/s. The PEO concentration of molecular mass M_{PEO} of $3 \cdot 10^6$, $4 \cdot 10^6$ and $6 \cdot 10^6$ varied from 0 till 0.05%. The distance from the nozzle to the food product cutting surface l_0 varied from $2 \cdot 10^{-3}$ to $100 \cdot 10^{-3}$ m.

Figure 1 shows the dependence of the cutting depth in fillet of the hake fish, with a temperature of minus 25 °C and the distance between its surface and the nozzle section under various PEO concentrations in water-polymer jet.

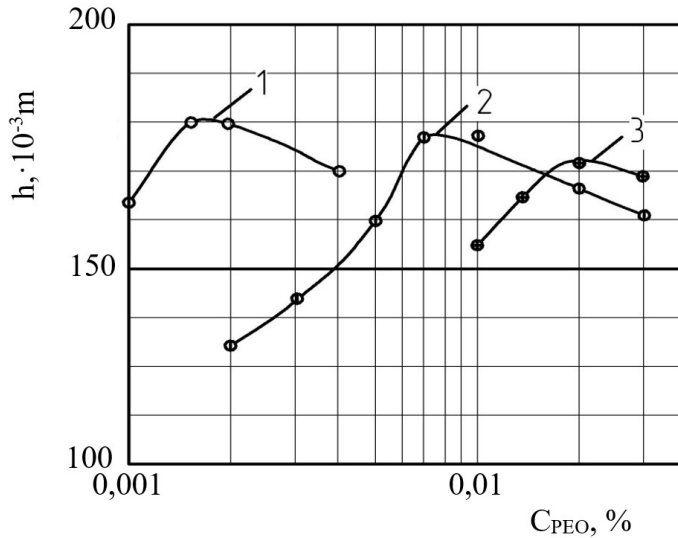


Figure 1. Dependence of the cutting depth in the fillet of hake fish on PEO concentration in water-polymer jet
 ($t = -25 \text{ }^\circ\text{C}$, $\Delta P_0 = 100 \text{ MPa}$, $d_{noz.} = 0.6 \cdot 10^{-3} \text{ m}$, $V_{m.j.} = 25 \cdot 10^{-3} \text{ m/s}$, $l_0 = l_{opt}$;
 M_{PEO} : 1 – $6 \cdot 10^6$, 2 – $4 \cdot 10^6$, 3 – $3 \cdot 10^6$)

The provided material *shows that* the cutting depth in fillet of the hake fish increases quite *greatly* with increasing PEO concentration and *molecular mass* and reaches its maximum at some optimal concentrations. For PEO with a molecular mass of $3 \cdot 10^6$, the optimal concentration equals 0.015–0.020%, and for molecular mass of $4 \cdot 10^6$ and $6 \cdot 10^6$ – 0.007–0.010% and 0.0015–0.0020%, respectively.

Data describing the influence of distance from the nozzle section to the food products surface, which are cutting by water-polymer jet, on the cutting depth, are given in Table 1 (for chicken fillet), in Figure 2 (for pork meat) and in Table 2 (for beef meat). The water-polymer jet had the PEO concentration, which corresponds to the optimum.

The provided material *shows that* the dependence of the cutting depth h on the distance between nozzle section and frozen food surface l_0 passes through the maximum. Such character of the dependence h on l_0 is maintained under different experiment conditions, that is, for all researched by us pressures of water PEO solution ΔP_0 , nozzle diameters $d_{noz.}$ and speeds of water-polymer jet movement relative to the sample of the frozen food product $V_{m.j.}$. At relatively small distances from 0 to some l_{opt} , an increase in the cutting depth with increasing distance from the nozzle section to the frozen food product surface is probably due to the fact that the process of jet formation does not stopped immediately at the nozzle section, but at some distance from it, equal l_{opt} . In this case, it should be noted that the distance l_{opt} from this point of view is rational.

Table 1
An influence of distance from the nozzle section to the chicken fillet surface, which is cut by the water-polymer jet, speed of jet movement and nozzle diameter on the cutting depth
 ($C_{PEO}=0,007\%$, $M_{PEO}=4 \cdot 10^6$, $\Delta P_o=150$ MPa)

№ of experiment	temperature of	$d_{noz}, 10^{-3}$ m	$V_{m.j.}, 10^{-3}$ m/s	Cutting depth $h, 10^{-3}$ m								
				$l_o, 10^{-3}$ m								
				2	2.5	3.5	4	6	10	20	60	90
1	-7	0.35	50	110	117	133	135	141	188	136	126	122
2			100	66	71	80	81	82	85	81	75	72
4		0.6	100	111	120	135	138	140	143	138	126	122
5			25	105	123	145	142	149	151	142	136	126
6	-25	0.35	50	63	74	83	84	89	89	84	75	72
7			100	38	44	50	52	55	53	44	38	37
8		0.6	50	108	126	142	143	149	152	143	128	124
9			100	65	74	84	87	92	92	75	62	56

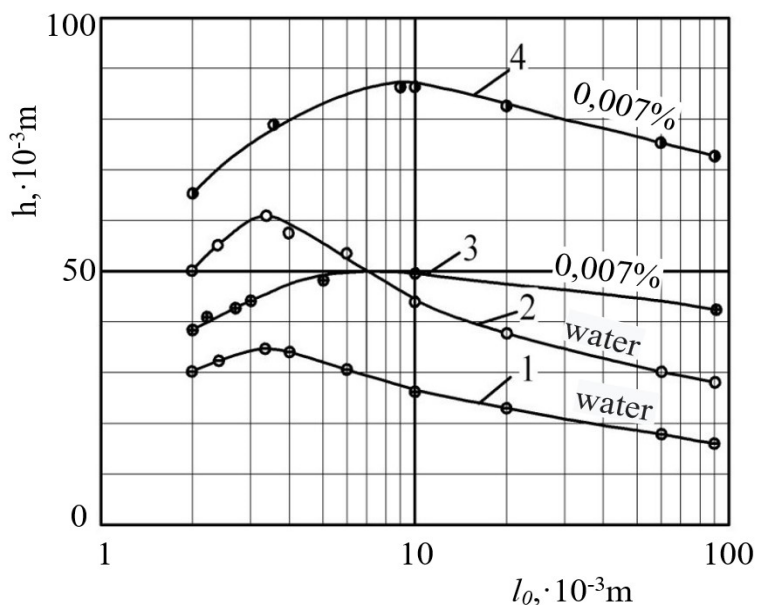


Figure 2. Dependence of the cutting depth by a water-polymer jet in the meat of pork on the distance between its surface and the nozzle section
 ($t = -7$ °C; $\Delta P_o = 100$ MPa; $d_{noz} = 0.35 \cdot 10^{-3}$ m; $M_{PEO} = 4 \cdot 10^6$;
 $V_{m.j.}$: 1 i 3 – $100 \cdot 10^{-3}$ m/s, 2 i 4 – $50 \cdot 10^{-3}$ m/s)

Table 2

Influence of the distance from the nozzle section to the beef meat surface, which is cut by the water-polymer jet, speed of jet movement and nozzle diameter on the cutting depth ($C_{PEO}=0,007\%$, $M_{PEO}=4 \cdot 10^6$, $\Delta P_0=50$ MPa)

№ of experiment	Temperature of meat t, °C	$d_{noz}, 10^{-3}$ m	$V_{m.j.}, 10^{-3}$ m/s	Cutting depth h, 10^{-3} m								
				$l_0, 10^{-3}$ m								
				2	2.5	3.5	4	6	10	20	60	90
1	-7°C	0.35	15	76	85	96	98	103	103	98	90	86
2			25	51	58	65	67	70	71	68	61	59
3			50	32	34	39	39	41	41	40	37	36
4			100	19	20	23	23	24	24	23	22	21
5		0.6	50	54	58	65	66	70	71	67	62	60
6			100	32	35	39	40	40	41	40	37	36
7	-25°C	0.35	15	50	58	66	68	71	72	68	62	59
8			25	34	40	45	46	49	49	46	42	41
9			50	21	24	27	27	29	29	27	25	24
10			100	12	14	16	17	18	17	14	12	12
11		0.6	25	54	68	77	79	82	83	79	72	69
12			50	35	41	46	47	49	50	47	42	40
13			100	21	24	27	28	30	30	25	20	18

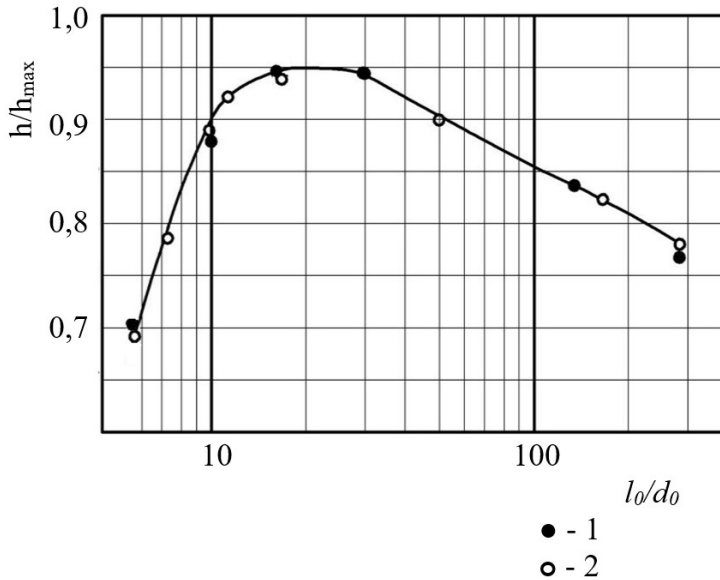
Experimental results, which showing how changes the cutting depth by water-polymer jets in the frozen food products with the distance to the nozzle section is presented in Figure 3. The gained experimental data are described by a function having a maximum in the area 15-20 of dimensionless distance from the nozzle.

Dependence of the $l_{opt.}$ that corresponds to the maximum on the dependence curves $h/h_{max} = f(l_0/d_{noz.})$, on the nozzle diameter $d_{noz.}$ and the length of the *initial section* $l_{i.p.w.j.}$ of water-polymer jet with a variation coefficient of 10-15% in a dimensionless form has the following form:

$$\frac{l_{opt.}}{d_{noz.}} = \frac{\varepsilon \cdot \theta \cdot l_{i.p.w.j.}}{5.4 \cdot d_{noz.}}$$

where ε – longitudinal velocity gradient in the entrance region of the nozzle, s^{-1} ; θ – relaxation time of water PEO solution, s.

When $\varepsilon \theta < 1$, the *initial section* of the water-polymer jet $l_{i.w.p.j.}$ is equal to the *initial section* of the water jet $l_{i.w.j.}$. This formula has the following usage limits: the water PEO solutions shall satisfy the Debay concentration criterion $-[\eta]_0 \cdot C < 1$ [23], and the value of the product of the longitudinal velocity gradient ε in the entrance region of the nozzle on the relaxation time θ of the water PEO solution, shall satisfy the condition $1 \leq \varepsilon \theta < 10$ [7].



**Figure 3. Dependence of the dimensionless cutting depth by a water-polymer jet in frozen food products on a dimensionless distance to the nozzle section:
1 – beef meat, 2 – fillet of hake fish**

The increase in the distance l_0 more than the l_{opt} , as a result of the interaction of the water-polymer jet with air, leads to a gradual loss of the kinetic energy of the jet, its diameter increases, and the value of the axial dynamic effect on the sample of the food product decreases, leading to a decrease the cutting depth. Having reached a certain limit value of the distance l_{lim} , from the nozzle section to the food product cutting surface, the cutting process is stopped.

Influence of pressure and nozzle diameter on the process of hydro-jet water-polymer food product cutting

The analysis of experimental data on the food products cutting by water-polymer jet, gained during the study of the influence of geometric and dynamic parameters of jets of water PEO solutions on the cutting depth in food products is shown (see Table 1 and 2, Figures 2 and 3), increases with increasing pressure of water PEO solution in front of the nozzle in the whole range of its values. This result is explained by the fact that an increase in the pressure of the water PEO solution in front of the nozzle leads to an increase in the hydraulic power of water-polymer jet, and, consequently, to increase its cutting ability.

The increase in diameter of nozzle hole under constant pressure of water PEO solution leads to an increase in the cutting depth in the food product. It is noted a character of influence of nozzle outlet diameter of hydro-cutting jet-shaping head on the cutting depth can be related to the fact, that the use of nozzles with a relatively large outlet diameter under a constant speed of water-polymer jet movement relative to the food product sample and pressure of water PEO solution in front of the nozzle, should lead to increase the amount of energy per

unit of the food product cutting surface. A similar character of pressure influence ΔP_0 and nozzle hole diameter $d_{noz.}$ on the cutting depth h has been observed also when cut samples of beef and pork meat at a temperature of liquid nitrogen $-195.8\text{ }^\circ\text{C}$ [7], as well as cutting of frozen minced fish, fillet of hake fish and chicken. However, in the works [4,5], there are other data indicating inverse relation between of the influence $d_{noz.}$ on the cutting depth, especially when thin polymeric materials cut by water jet. Thus, there is a reason to assert, that nowadays it is not fully found out the character of change of the cutting depth in the various materials from the nozzle hole diameter, even when cut by a water jet [7], and moreover – by water-polymer jet.

Considering the question on finding the rational nozzle outlet diameter of hydro-cutting jet-shaping head, it is also necessary to take into account that high-pressure water-polymer [7], as with water jets [6], of small diameter, are subject to faster decomposition and with $d_{noz.} < 0.05 \cdot 10^{-3}$ m the process of hydro-cutting practically stops. An increase of the nozzle diameter leads to increase of flow rate of water PEO solution, and, consequently, increases energy costs for the formation of a water-polymer jet.

At the same time, we gained the experimental data from the hydro-jet water-polymer frozen food products cutting, which is given above and the results of the water-cutting [6,7], convincingly show that the cutting depth of the relatively thick food products increases with the increase in the hydraulic parameters of water-polymer jets and increase nozzle outlet diameter of hydro-cutting jet-shaping head from $0.2 \cdot 10^{-3}$ till $0.8 \cdot 10^{-3}$ m. These contradictions can be related to the structural and dynamic characteristics of high-speed water-polymer jet.

The gained experimental data also show the following: achievement of the same food products cutting depth (in the range from -7°C to $-25\text{ }^\circ\text{C}$), enough the pressure of the water-polymer jet is only 45–65% of the water jet, and vice versa, under the same initial pressure there is an increase in depth and cutting speed by water-polymer jet in 1.5–2.5 times, indicating a special mechanism of its interaction with the food product.

Speed effect of high-speed water-polymer jet movement on the hydro-cutting process

To determine the optimal conditions for the realization of the process of hydro-jet water-polymer food products cutting that provide high productivity of the process [7, 20], the surface quality and shape of the cutting, it is necessary to establish the rational speed of water-polymer jet movement relative to a food product that is cut, which depends on the parameters of the leak of the water-polymer jet from the nozzle, and provides the correct shape and high quality cuts.

Typical results of experimental studies of the process of hydro-jet water-polymer food products cutting are given on Figure 2, Tables 1 and 2. It is seen that the character of the change of dependence of the cutting depth in pork meat, fillet of broiler chicken and beef meat h from the speed of jet movement of water PEO solution $V_{m.j.}$, relative to food samples, is qualitatively the same under different temperatures. An increase in the speed of the water-polymer jet movement $V_{m.j.}$ for water pressure of 50–150 MPa in front of the nozzle leads to a decrease in the cutting depth in the food product. The decrease in h with increase in $V_{m.j.}$, can be explained by the fact that under low speeds of the water-polymer jet movement, the density of energy spreading of the jet per unit of the cutting length is large. As a result, a greater cutting depth is formed in the food product than at high speeds of movement. For

large $V_{m.j.}$, the density of energy spreading of the water-polymer jet decreases, and thus, decreases the cutting depth.

It is worth noting that the decrease in the food product temperature, that is, an increase in the tensile strength for uniaxial compression, in other conditions is equal, take place corresponding decrease in the food product cutting depth [7]. This is due to the fact, that the cutting of stronger food products samples requires higher specific energy consumption.

Table 3 shows the width of cuts b by high-speed jet of water PEO solution in frozen beef depending on the distance between the nozzle section and the food product surface for different water pressures and nozzle outlet diameters. The gained experimental data show that with the increase in the distance between the nozzle and the food product surface, the cutting width varies. It is seen that with increasing of nozzle diameter and distance between nozzle and food product surface, the cutting width b increases. The dependence of the cutting width on the distance between the nozzle cut and the food product surface, that corresponds to the data given in Table 3, with *reasonable accuracy*, coincides with the dependence, which describes the change in the diameter of the water-polymer jet from the distance to the nozzle section. Experimental results given in Table 3 show that the cutting width b in the investigated range l_0 practically does not depend on the pressure of the water PEO solution in front of the nozzle ΔP_0 .

Table 3
Cutting width during hydro-jet water-polymer cutting of frozen beef meat, depending on the distance between the nozzle section and the cutting surface

№ of experiment	Temperature of meat $t, ^\circ\text{C}$	$\Delta P_0, \text{MPa}$	$d_{\text{noz.}}, 10^{-3} \text{ m}$	$V_{m.j.}, 10^{-3} \text{ m/s}$	Cutting width $b, 10^3 \text{ m}$				
					$l_0 \cdot 10^3, \text{ m}$				
					5	10	20	50	90
1	-7	100	0.35	15	0.35	0.36	0.37	0.40	0.47
2		100		25	0.36	0.37	0.38	0.42	0.48
3		50		15	0.35	0.36	0.37	0.41	0.48
4	-25	100	0.35	15	0.36	0.37	0.37	0.42	0.47
5				25	0.36	0.37	0.38	0.42	0.47
6		100	0.60	15	0.61	0.63	0.65	0.71	0.80
7		50	0.60	15	0.61	0.65	0.67	0.73	0.84

Experimentally confirmed independence or very weak dependence of the width b on the pressure ΔP_0 and speed of the water-polymer jet movement relative to the food product sample $V_{m.j.}$, may indicate a self-similar mode of leakage of water PEO solution from the nozzle.

For food products there are no objective methods for assessing the quality of lateral surfaces of cuts. In our experiments, the estimation of the quality of the lateral surfaces of the cuts during the hydro-jet water-polymer food products cutting was made visually, as well as using the profilograph-profilometer. It was established experimentally that the quality of cutting surface (in accordance to the evaluation criteria [7]) in the frozen food products

improves with the increasing in speed of the water-polymer jet, cutting speed up to the rational value $V_{rat.m.j.}$, and, deteriorates with increasing in the nozzle diameter. A forming cut in the process of water-polymer frozen food products cutting under a very low cutting speed ($V_{m.j.} \ll V_{rat.m.j.}$) has the profile of A-shaped form, whereas under a high cutting speed ($V_{m.j.} > V_{rat.m.j.}$) the profile of the cut has a weakly pronounced V-shaped form. Cutting food products by a water-polymer jet under a speed close to its rational value leads to the formation of a cut of the Π -shaped form.

When increasing distance between the nozzle and the surface of food product sample, which is cut, cutting width increasing due to the widening of the water-polymer jet, thus, the contact area of the jet with the food product also increasing, what is, in addition to other constant parameters of the water-polymer jet leak, reduce specific cutting pressure and increase the food product volume, which is removed per time unit, thus reducing productivity of the process of hydro-jet water-polymer cutting.

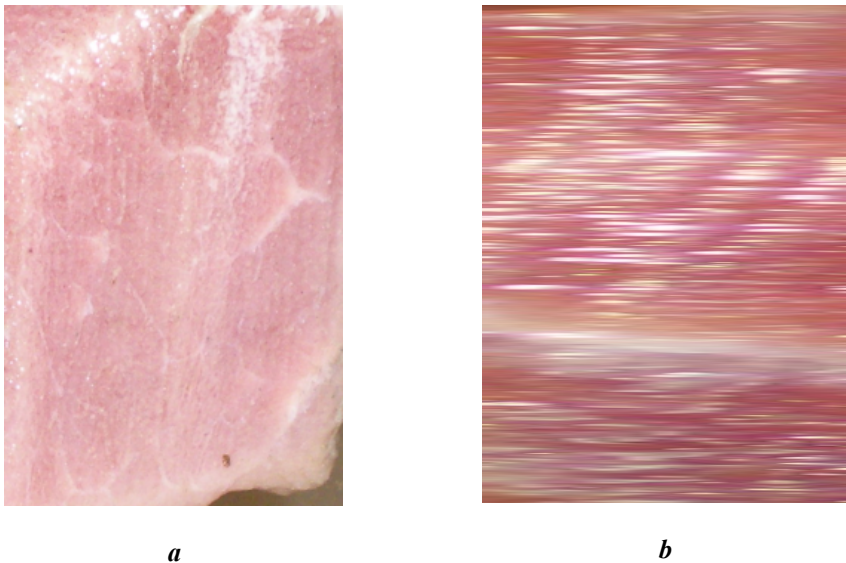


Figure 4. Photos of the cutting surfaces by the hydro-jets in a frozen pork meat:
a – the water-polymer jet, b – the water jet
($C_{PEO}=0.007\%$, $M_{PEO}=4 \cdot 10^6$; $l_0 = l_{opt}$; $\Delta P=100$ MPa, $V_{m.j.}=25 \cdot 10^{-3}$ m/s, $t=-25$ °C)

The Figure 4 shows the pictures of cutting surfaces in frozen pork meat related to "high-quality" cutting (a) and "equal-quality" cutting (b). Experimental data show, that the quality of the frozen food product cutting surfaces when it is cut by a water-polymer jet is significantly higher than when it is cut by a water-jet.

Conclusions

1. It was determined that the cutting depth in the food product increases quite greatly with increasing PEO concentration and molecular mass and reaches its maximum at some optimal concentrations. For PEO with a molecular mass of $3 \cdot 10^6$, the optimal concentration equal to 0.015–0.020%, and for molecular mass of $4 \cdot 10^6$ and $6 \cdot 10^6$ – 0.007–0.010% and 0.0015–0.0020%, respectively.
2. It has been found that the intensification of the frozen food products water-cutting process due to the PEO impurity in the water-jet allows to reduce the rational working pressure in 3–4 times, that allows to considerably reduce the cost of the water-cutting equipment.
3. It is experimentally proved that the quality of surfaces of cuts in food products improves with the increasing speed of the water-polymer jet, cutting speed up to the rational value and gets worse with increasing of the nozzle diameter.
4. The interpretation of the experimental data, gained from the influence of water-jet polymeric impurities concentration in water jet, geometric and dynamic parameters of water-jet on the characteristics of the food products water-cutting process, requires study of structural changes occurring in the water-polymer jet under different modes of its leak from a nozzle, as well, as the degree of force of the water-polymer jet on barrier.

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Limitation of dynamic power parameters in transitional processes

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Abstract

Introduction. The article is devoted to the analysis of the prospects of the complex use of the accumulated potential energy on the example of a mechanism for lifting, horizontal movement and loading of cargoes, for example, in transport container with limitation of dynamic loads.

Materials and methods. Synthesis of technological systems is aimed at overcoming the contradictions between the kinematic parameters of a highly productive machine, energy costs, and dynamic load. The study is theoretical, based on the laws and principles of mechanics, with the creation and analysis of appropriate mathematical formalizations that relate to energy and mechanical transformations with the final result of the combination of elevated kinematic parameters and limited force actions.

Results and discussion. On the basis of the analysis of the peculiarities of the shock loads in two-mass systems, the possibility of using the rigidity of the springy pendant as a variable factor to achieve the given relations between static and dynamic loads during their course in the gravitational field was determined. The idea of a sharp increase and fixation of the rigidity of the pendant system under maximum deformations and loads was used. Mechanical transformation of the springy system in the oscillatory process from low-frequency to high-frequency is carried out by parallel connection to springy elements of high rigidity in the form of a two-link hinge.

The fixed position of the deformed springy element allows to perform the role of "holder" of the potential energy of the field of springy forces, the use of which is carried out at the stage of inserting of loads to limit the speed of their contact with the supporting receiving planes. Exclusion of low-frequency oscillations from the system means limitation of energy losses on internal friction in springy elements.

The developed mathematical formalizations confirmed the validity of the idea of using a variable rigidity system, which, in addition to the positive result of limiting extreme dynamic loads, achieves positive energy and dynamic effects in the final stages.

Conclusions. The idea of using a system with variable rigidity of springy elements allows to limit the contradiction between the values of kinematic and dynamic parameters.

Introduction

Solving the problems of synthesis of technological machines is traditionally associated with the choice of structure, analysis of kinematics and dynamics with the need to limit the irregularities of the course of the leading links, loads of transition processes and energy costs. Such a set of characteristics of the parameters of systems has an inherent contradiction [1]. On the one hand, it is desirable to limit the mass characteristics of the equipment, dynamic loads and energy costs, on the other hand, solving the task of a given performance requires increasing kinematic parameters and inertial loads with them. Obviously, the latter requirements are necessarily related to transition processes in overclocking and running modes [3]. The dynamics of their implementation undergoes energy transformations and costs, since the work of the driving forces to overcome the forces of inertia corresponds to the increase of the kinetic energy of the moving masses of the system [1, 2].

Since the transition processes occur in the gravitational field, the number of force parameters in the system includes the forces of gravity, driving force and resistance factors (forces and moments of forces), forces and moments of forces of inertia and springy force factors [1]. The latter belong to the field of forces of springiness. So for the linear force of springiness which obeys the Hooke's law, due to which force action $\bar{F} = -c\bar{\Delta}$, where c is the stiffness coefficient; $\bar{\Delta}$ is the distance from the center of mass at which the force \bar{F} acts to the position of its static equilibrium at $\bar{F} = 0$. In the case of linear deformation, the springy force is reflected by a partial derivative of the energy potential [3]:

$$\frac{\partial u}{\partial \Delta} = F = -c\Delta. \quad (1)$$

Integrating the latter condition allows to write:

$$u = -\frac{c\Delta^2}{2} + const. \quad (2)$$

Given that $\Delta^2 = x^2 + y^2 + z^2$, we finally have:

$$u = -\frac{c\Delta^2}{2} + const = -\frac{c}{2}(x^2 + y^2 + z^2) + const. \quad (3)$$

The characteristic of the potential force field is expediently supplemented by a function that determines the energy reserve at a given point of the field, that is, the potential energy of the system at a given position, which is equal to the work that the potential forces of the field can accomplish when moving the material point from the flowing point to the starting point of the system []. From this position it follows that the potential energy P depends on the coordinates of the material point:

$$P = A; \quad P = P(x, y, z). \quad (4)$$

The above considerations allow to conclude that the potential energy of deformation of the springy system can be determined by the dependence:

$$\begin{aligned} P &= -u = Pz + const; \\ P &= -u = \frac{c}{2}(x^2 + y^2 + z^2) + const; \end{aligned} \quad (5)$$

The presence of springy elements in the drives of the working bodies of technological machines is one of the directions of limiting the dynamic components of the loads, each of which is accompanied by the corresponding deformations [4, 5]. In a considerable number of cases, systems with springy elements are modeled by two-mass, and the most difficult

conditions correspond to shock interactions between the leading and the driven masses [6, 7].

It is known that in such cases, the velocity of the driving mass remains stable, and the load of the springy element is reflected by the sum of static and dynamic components (Figure 1) [1, 2]:

$$P_{sp} = V \sqrt{m_2 c} \sin \sqrt{\frac{c}{m_2}} t + P_{re} \quad (6)$$

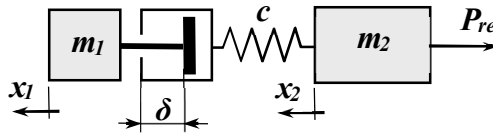


Figure 1. Diagram of a two-mass model

The presence of such dependence indicates the possibility to determine the maximum springy load and the corresponding maximum deformation of the springy element with rigidity c , as well as the potential energy of the deformed element [8–10].

The purpose of the study is to develop mathematical formalizations in search of opportunities to improve the performance of systems and machines with dynamic load limitations based on the use of the proposed springy pendant.

Materials and methods

The **object** of research is the synthesis of technological machines and features of transition kinematic and dynamic processes.

The **subject** of research are mechanisms for lifting, horizontal movement and stacking of goods, in particular a device for the insertion and removal of bottles from a transport container (patent of UA65929).

The research is theoretical, performed on the basis of the laws of physics, principles and methods of mechanics [3, 11] with the creation and analysis of analytical models [1].

Results and discussion

Henceforth we turn to the problem of the prospects for the integrated use of accumulated potential energy at the example of a mechanism for lifting, horizontal movement and stacking of goods, for example, in transport containers. In its composition there is a leading element, which moves the programmable route with vertical and horizontal sections with a definite sequence [3].

At a given productivity of the system, the velocity V cannot be considered as variable as the driven mass [1]. This means that the limitation of the dynamic component is associated with the rigidity of the system in the direction of its reduction. However, under this condition,

the frequency of natural oscillations $\sqrt{c/m_2}$ also decreases and the system turns into a slow decay. The latter can be considered as a disadvantage, although it is counteracted by the addition of a parallel springy damper of oscillatory processes.

An alternative to such a partial solution of the problem is the proposal reflected in the patent of Ukraine 65929, which combines the possibility of limiting shock dynamic loads in systems of fixed movement of loads in vertical and horizontal combined movements. The invention relates to a device for the insertion and removal of bottles from a transport container, consisting of mounted with the possibility of horizontal reciprocating movement of the carriage mounted on it with the possibility of vertical movement from the drive gripper head with clamps, characterized in that the gripping head united with the drive of vertical movement by traverses 1 and 2, interconnected by springs 3 and two-hinged hinge 4, one of the traverses is fitted with a retainer 5 for two-hinged hinge, and the other is fitted by lever 6 and connected with it rod 7 for fixing a double-joint hinge (Figure 2).

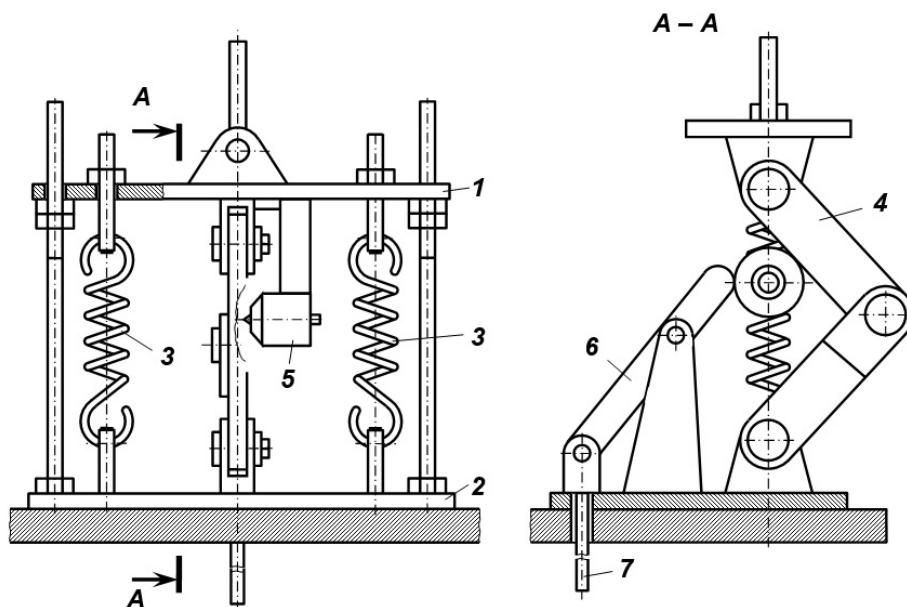


Figure 2. Scheme of springy pendant of cargo
[patent UA 65929]

Moving loads by the gripping head vertically takes place in two stages. At the first stage from the beginning of the movement of the traverse 1 stretching and loading of the springs 3 takes place to a value corresponding to the weight of the gripping head with the load and the opening of the hinge 4.

At the second stage, the accelerated movement of the traverse 2 begins together with the driven mass with the subsequent stretching of the springs. At the moment of reaching the maximum load on the springs 3, which comes at the equality of speeds of the traverses 1 and 2, full disclosure of the two-link hinge 6 and fixing in this position by the retainer 5 take place.

The introduction of the springs 3 to the pendant sharply reduces the dynamic load of the shock interaction, and their stretching is accompanied by the accumulation of potential energy, which in the absence of fixation would cause low-frequency oscillations and would disrupt the normal operation of the device. The actuation of the retainer 5 leads to the fact that in the future, the traverses 1 and 2 with the gripping head and load moved as one. The two-link hinge remains open and compressed force, which is equal to the difference of maximum load and gravity of the gripping head.

Loading of goods on the receiving plane corresponds to the third stage. In this case, the rod 7 rests on the support plane and, interacting with the lever 6, leads to the hinge 4 from the dead point, releasing the springs 3. Since the force in the springs exceeds the weight of the gripping head, the traverse 2 with the latter receives a movement towards the traverse 1, reducing their absolute speed of the lowering movement by triggering the potential energy of the springs. Depending on the kinematic parameters of the system and the choice of the moment of unlocking the hinge, it is possible to limit the speed of contacting the loads with the supporting receiving plane.

Such a combination of conditions makes it possible to limit the contradiction stated above between the set of kinematic and dynamic parameters.

In accordance with these stages of moving goods, let's turn to the tasks of drawing up their analytical models. The movement of the leading mass of the system together with the flexible pendant and the traverse 1 with a constant speed V corresponds to the first of them. At this stage, the driven mass m_2 remains stationary until the moment when the springy load P_{sp} is not equal the resistance to movement m_2g (Figure 3). Equations $x_1 = Vt$ and $x_2 = 0$ and the initial load of the springelement $P_{sp(i)} = 0$ correspond to this condition.

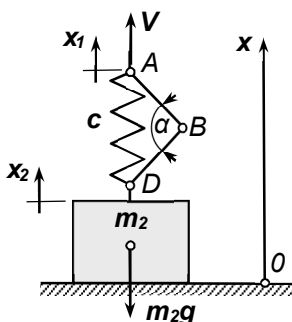


Figure 3. Calculation scheme of the first stage

The final value of the moving coordinate corresponds to the completion of the first stage:

$$x_{(f)}^I = \frac{m_2g}{c} \quad (7)$$

where c is spring stiffness, N/m.

The second stage is displayed by a system of two equations:

$$\begin{aligned} x_1 &= Vt; \\ m_2 \ddot{x}_2 &= c(x_1 - x_2) - m_2 g, \end{aligned} \quad (8)$$

the transformation of which leads to a condition:

$$\ddot{x}_2 + \frac{c}{m_2} x_2 = \frac{Vc}{m_2} t - g, \quad (9)$$

which matches the original data:

$$t_{(i)} = 0; \quad x_{2(i)} = -\frac{m_2 g}{c}; \quad \dot{x}_{2(i)} = 0 \quad (10)$$

The solution of equation (9) taking into account (10) is:

$$x_2 = Vt - V \sqrt{\frac{m_2}{c}} \sin \sqrt{\frac{c}{m_2}} t - \frac{m_2 g}{c}; \quad (11)$$

$$\dot{x}_2 = V - V \cos \sqrt{\frac{c}{m_2}} t; \quad (12)$$

$$\ddot{x}_2 = V \sqrt{\frac{c}{m_2}} \sin \sqrt{\frac{c}{m_2}} t. \quad (13)$$

The load of springy elements is reflected by the dependence:

$$\begin{aligned} P_{sp} &= c(x_1 - x_2) = c \left(Vt - Vt + V \sqrt{\frac{m_2}{c}} \sin \sqrt{\frac{c}{m_2}} t + \frac{m_2 g}{c} \right) = \\ &= m_2 g + V \sqrt{m_2 c} \sin \sqrt{\frac{c}{m_2}} t \end{aligned} \quad (14)$$

The latter condition implies the presence in the system of static and dynamic components of the load, the ratio of which can be guided in the choice of parameters. For example, if you agree on their equality, we can write down:

$$m_2 g = V \sqrt{m_2 c}, \quad (15)$$

where the extremum of the dynamic component is represented on the right side of the equation.

Since the output data of the system is represented by the weight of the load and the productivity in the form of velocity V of the driving mass, then the stiffness of the pendant should act as a variable parameter, so:

$$c = \frac{m_2 g^2}{V^2}, \text{ N/m.} \quad (16)$$

$c = 153977$ N/m corresponds to the parameters $m_2 = 100$ kg and $V = 0.25$ m/s.

Changing the velocity parameter to the value of $V = 1$ m/s provides a transition to the stiffness of the springs $c = 9623.6$ N/m. It is obvious that the calculated value of rigidity corresponds to each of the ratios of parameters m_2 and V . However, the condition of static load and extreme dynamic conditions is not necessary, which creates additional possibilities in the

structural variations of the system.

Given that the interaction between the driving and driven masses of the system is estimated as a shock load, we conclude that it is advisable to complete the second stage at the time of equalization of forces \dot{x}_1 and \dot{x}_2 .

A graphical interpretation of this situation is shown in Figure 4, which, considering equation (12), corresponds to the value:

$$V \cos \sqrt{\frac{c}{m_2}} t = 0. \quad (17)$$

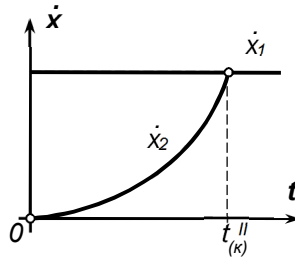


Figure 4. Graphs of the speeds of the driving and driven masses at the second stage

This means that the final time of the second stage is:

$$t''_{(f)} = \frac{\pi}{2} \sqrt{\frac{m_2}{c}}. \quad (18)$$

For values $m_2 = 100$ kg and $c = 153978$ N/m, we get $t''_{(f)} = 0.04$ s. During this time, the leading mass will perform the movement $x''_{1(f)} = V t''_{(f)} = 0.25 \cdot 0.04 = 0.01$ m, and the displacement of the driven mass is determined by the condition (11):

$$x''_{2(f)} = 0.25 \cdot 0.04 - 0.25 \sqrt{\frac{100}{153978}} \sin \sqrt{\frac{153978}{100}} \cdot 0.04 - 0.00637 = -0.00274 \text{ m} \quad (19)$$

The difference of the coordinates of the movements of the driving and driven masses is:

$$\Delta x = x''_{1(f)} - x''_{2(f)} = 0.01 + 0.00274 = 0.01274 \text{ m} \quad (20)$$

Checking the value of the springy load leads to a value

$$P_{sp} = c(x''_{1(f)} - x''_{2(f)}) = 153978(0.01 + 0.00274) = 1962 \text{ N} \quad (21)$$

which meets the previously accepted condition (15).

In the future, the calculation is related to the determination of the lengths of the links of the two-link hinge. Preliminary calculations made it possible to determine the movement of the driving and driven masses in two stages. The initial difference of coordinates is defined as $\Delta x_{(i)}$ taking into account the coordinates of the hinges D and A. $\Delta x_{(i)}$ takes into account the structural size $\Delta x_{(0)}$ and the value of deformation of the component of static load $x''_{1(f)}$ (Figure 5).

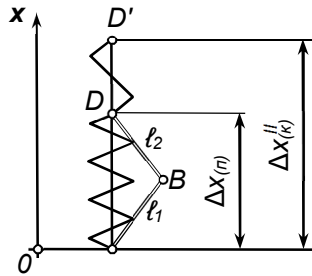


Figure 5. Scheme to determine the difference in the coordinates of the positions of the driving and driven masses

Then the condition of the hinges A and D corresponds to the completion of the second stage:

$$\Delta x''_{(f)} = \Delta x_0 + \Delta x^I_{(f)} + (x''_{1(f)} - x''_{2(f)}). \quad (22)$$

The obtained value $\Delta x''_{(f)}$ corresponds to the sum of the lengths of the links l_1 and l_2 of the double-link.

The fixed relative position of the hinges AB-D' on one vertical means the arrangement of the energy "trap". The potential energy of the deformed spring element is:

$$E = \frac{c}{2} (x''_{1(f)} - x''_{2(f)})^2. \quad (23)$$

Subsequent movements of the system along horizontal and vertical lowering movements continue at a stabilized velocity V up to the moment of unlocking of the double-link, at which the force action of the deformed spring element will exert additional influence (Figure 6).

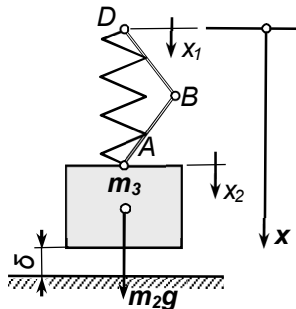


Figure 6. Scheme to the transitional process of loading goods

In this case, the driven mass will go into the transition mode with the deceleration of absolute speed according to the equations of the system:

$$\begin{aligned} x_1 &= Vt; \\ m_2 \ddot{x}_2 &= m_2 g - c(x_2 - x_1). \end{aligned} \quad (24)$$

However, the latter requires confirmation in relation to the magnitude of the fixed spring force in the deformed element. The role of the latter is present in the meaning of the initial condition $x_{2(i)}$ at the third stage. We will show this in a further analysis. Condition (24) implies:

$$\ddot{x}_2 + \frac{c}{m_2} x_2 = g + \frac{cV}{m_2} t. \quad (25)$$

Under initial conditions

$$t_{(i)} = 0; \quad x_{2(i)} = \frac{m_2 g}{c}; \quad \dot{x}_{2(i)} = V \quad (26)$$

we get:

$$x_2 = A_1 \sin \sqrt{\frac{c}{m_2}} t + B_1 \cos \sqrt{\frac{c}{m_2}} t + \frac{m_2 g}{c} + Vt \quad (27)$$

and hence integration constants $A_1 = 0$ i $B_1 = 0$.

The corresponding substitution allows to write:

$$x_2 = \frac{m_2 g}{c} + Vt; \quad \dot{x}_2 = V, \quad (28)$$

which means that there is no oscillation process at the third stage.

The transition to the new initial conditions

$$t_{(i)} = 0; \quad x_{2(i)} = \frac{2m_2 g}{c}; \quad \dot{x}_{2(i)} = 0 \quad (29)$$

leads to changes integration constants:

$$A_1 = 0 \quad \text{i} \quad B_1 = \frac{m_2 g}{c}. \quad (30)$$

Then we have:

$$x_2 = \frac{m_2 g}{c} + Vt + \frac{m_2 g}{c} \cos \sqrt{\frac{c}{m_2}} t; \quad (31)$$

$$\dot{x}_2 = V - \frac{m_2 g}{c} \sqrt{\frac{c}{m_2}} \sin \sqrt{\frac{c}{m_2}} t; \quad (32)$$

$$P_{sp} = m_2 g + m_2 g \cos \sqrt{\frac{c}{m_2}} t; \quad (33)$$

$$P_{sp.max} = 2m_2 g. \quad (34)$$

Obviously, the value $P_{sp.max}$ corresponds to the initial load, and the presence of the oscillatory process at the third stage corresponds to the graphical interpretation in Figure 7.

The first negative value of the extremum of the dynamic component of the speed of the driven mass and the zero value of the resultant comes when using the condition:

$$t_{(f)} = \pi \sqrt{\frac{m_2}{c}} \quad (35)$$

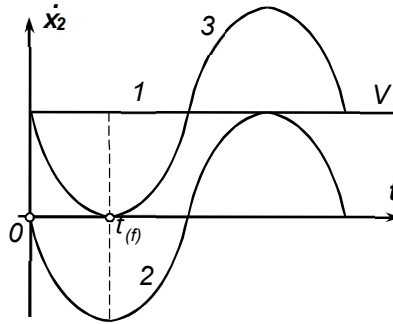


Figure 7. Schedule of change of speed of the driven mass:
 1 – static component; 2 – dynamic component; 3 – the resulting of speed

The value of the displacement of the mass m_2 at the time $t_{(f)}$ is:

$$x_{2(f)} = \frac{m_2 g}{c} + V t_{(f)} + m_2 g \cos \sqrt{\frac{c}{m_2}} t. \quad (36)$$

If the condition $x_{2(f)} = \delta$ is carried out, where δ is the gap between the mass m_2 and the receiving plane at the beginning of the transition process at stage III, we conclude that they contact at zero speed. This result is an expected positive based on the energy potential of the deformed springy element [1]. Thus, the initial conditions of the third stage, defined as the final conditions of the second, are variational factors in the third. From this point of view it is worth to note the importance of the ratio of the dynamic and static components of the load of the springy element [1]. If we take the value $x_{2(i)} = \frac{3m_2 g}{c}$, then we obtain

$P_{sp,max} = 3m_2 g$, and provided that their multiplicity is k , we obtain:

$$P_{sp,max} = k m_2 g. \quad (37)$$

The level of activation of the energy potential is estimated under the final conditions corresponding to equations (32) and (33). The value $\dot{x}_{2(f)} = 0$ leads to the conclusion that the kinetic energy of the driven mass $E_{kin(f)} = 0$ is the same as the potential energy of deformation of the springy element at $P_{sp(f)} = 0$.

Separation of the driven mass from the system of its movement at the moment of achievement $t_{(f)}$ means achievement of the mode of non-shocking loading of cargo on the receiving plane [3, 7]. The interaction between them also depends on the ratio of the masses and the receiving plane and the rigidity of the elements of its installation. It is known [3] that due to neglect of the mass of the receiving plane and at zero contact speed, the dynamic load of the springy elements is equal to static $m_2 g$, which in sum with the latter corresponds to the value of two static ones.

Conclusion

The proposed system of arrangement of the device for moving loads on the vertical and horizontal sections with their subsequent attachment to the receiving horizontal planes was intended to limit the force interactions between the driving and driven masses and energy losses associated with oscillatory processes and springy deformations. Performed theoretical studies allow to note the following:

1. The task of achieving high productivity of technological machines leads to the requirements of increasing kinematic parameters with the simultaneous intention of maintaining stable speed of the driving masses. This means the presence of modes of shock loads with dynamic amplitudes $P_{dyn} = V\sqrt{m_2c}$.
2. The ratio of the stiffnesses to the masses determines the frequency of natural oscillations, whereby such systems are conventionally divided into slow and fast decay. In oscillatory processes, internal friction results in energy losses, the limitation of which is appropriate and achievable due to the use of elements of fixation of springy elements in the deformed state with the corresponding energy potential.
3. At the stage of loading of loads, the energy potential of springy elements provides for the restriction of the speeds of contacting the loads with the supporting receiving planes with restrictions of dynamic loads.
4. The rigidity of the springy element is the regulatory parameter of influence under limited shock loads during lifting. The latter simultaneously allows to maintain speeds that satisfy a given productivity. This is an important compromise in mechanical systems for dynamic power and kinematic parameters.

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Fuzzy logic energy management system of food manufacturing processes

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Abstract

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Introduction. The research is conducted to justify the method of increasing the efficiency of electrical power use in food production processes, which is based on the algorithms of fuzzy power management system.

Materials and methods. The study is based on optimal and fuzzy control methods, such as Mamdani and Sugeno algorithms. Computer simulation was performed using MATLAB Simulink.

Results and discussion. The control criterion of power supply of food production is formulated in the form of a functional minimization problem, which depends on the expected value of active and reactive power losses, active and reactive power losses at the level of secondary substations and individual load nodes. Maintaining energy efficient voltages in the power supply system nodes is chosen as a control task. It is determined the dependence of energy efficient voltages on the nominal voltage of the power system, the active resistance of its segments, the power and the coefficient of linearized static load characteristics at the network nodes, the equivalent active resistance, the nominal power, open circuit and short circuit losses of secondary substation transformers. To solve the control problem, an algorithm is synthesized using fuzzy controllers at the levels of the primary and secondary substations. In particular, it is determined that the input signals of the secondary substation fuzzy regulator should be the deviation from the energy efficient voltages and the rate of voltage change, and the output signals should be the transformer voltage and the actuation delay. Using numerical methods it is shown that this algorithm can reduce electricity losses in food production processes up to 7% compared to classical voltage regulation methods.

Conclusions. The fuzzy system method under study ensures that energy-efficient voltage levels are maintained at the distribution network nodes when the voltage of the power source or the consumer loads are changed.

Glossary

FMP – food manufacturing processes
PSDS FMP – power supply and distribution system of FPM
DEG – district electric grid
EPG – electric power grid
SS – secondary substation
PS – primary substation
SCL – static characteristics of complex load
LV – low voltage
HV – high voltage
MFA – Mamdani Fuzzy Algorithm
SFA – Sugeno Fuzzy Algorithm
MF – membership function
SCADA – supervisory control and data acquisition

Introduction

Electricity consumption in food manufacturing is calculated in the tens of megawatts, and needs to be reduced to create more sustainable food production branch of economics and prevent waste of resources [13]. Such problem can be solved within the scope of automated control of energy consumption and supply of food manufacturing processes (FMP). Methods and models for this kind of solution are considered in wide range of scientific studies as described below.

In particular, the research [4] outlines the main tasks that should be solved by automated control systems for power supply and distribution of technological processes. At the same time, the main attention is paid to the issues of calculation of power system operation modes. The studies [22, 24, 25] consider the issues of power management by means of dispatching operations based on microprocessor technology. Methods and means of power management with the help of local systems of reactive power compensation control and voltage regulation in distribution networks are presented in [1]. The research [11] proposes a two-level voltage regulation system in the distribution electrical network, and the studies [22, 19] discuss the issue of voltage regulation in the electrical network using fuzzy logic.

According [9] it is promising to build power management systems with the use of a multifunctional meter on the lower level for operating parameters of the grid, which allows to measure voltages and currents in all three phases, active and reactive power, as well as electricity costs and has electromagnetic compatibility with 0.4-10 kV devices. The system proposed in [9] uses the digital output of the meter to connect to industrial communication networks based on Modbus and Profibus, and at the top level – SCADA systems. Analysis of this system showed that the use of SCADA systems without the use of special application software that provides control functions does not solve the problem of building effective control systems, because they perform a limited amount of functions related only to the collection of measured data, remote control switching equipment, archiving and displaying information on mnemonic circuits.

Recently, modern industrial controllers and SCADA systems have been widely used in the design of automated energy management systems [16, 5, 7]. The use of these technical tools allows to solve in real time the problems of commercial metering of power consumption, technical logging and monitoring of electrical loads of food industry

enterprises, the interaction of the operator with the information system, which is the basis for solving the problems of power management of food industry enterprise.

The analysis of studies [21, 16, 18, 8, 20] showed that the structural solutions, mathematical models and control methods presented in them provide only the function of controlling the parameters of the power system. However, these tools do not provide the implementation of the monitoring and optimal control functions which allow obtaining the main economic effect [12].

The main goal of this research is to justify designing principles of the automated energy management system of food manufacturing processes on the basis of mathematical models, methods of fuzzy logic and information technologies.

Materials and methods

Materials

Object of the study is fuzzy automated energy management system of food manufacturing processes, designed to provide energy saving without losing performance.

In this study it is used:

- Electric power supply technologies of food manufacturing;
- Control methods of electric power supply of manufacturing processes;
- Mathematical models of control methods and algorithms;
- Simulation models created in matlab simulink computer software.

Methods

Plan of studies. The studies were conducted in the following order:

- Choosing control criteria of FMP power control system;
- Determining of control tasks of power supply and distribution of FMP;
- Designing a method for determining the node voltages of
- FMP power system for providing energy-efficient operation modes;
- Developing algorithms for fuzzy voltage regulation on the 0.4 kv buses of the secondary substation transformers and on the 6-10 kv buses of the primary substation transformer;
- Performing computer simulation of the algorithm and comparing with classical voltage regulation methods.

Control of power supply system of food manufacturing should ensure the achievement of the following objectives:

- Uninterrupted power supply of consumers while complying with industry specific and general energy standards of power quality;
- Standard quality of electrical power in substation supply voltage buses and district power distribution networks;
- Minimum possible level of losses in power systems;
- Permissible level of ecological safety in the conditions of electromagnetic field influence produced by electric networks and transmission lines.

Methods of system analysis. System description of FMP power system is performed with the use of tuple definitions [10], which is the simplest, most abstract tool; it enables the inclusion of different type objects and establishing connections of different nature and content. In this method the description of a system can be written in following form:

$$\Sigma : \{ \{M\}, \{\varpi\}, \Omega \} \tag{1}$$

where $\{M\}$ is a set of elements; $\{\varpi\}$ is a set of element links; Ω are the functions of described system.

Model of electrical network. It is used the combined topology of electrical network for modelling FMP power supply system on secondary substation level [15] as shown in Figure 1, where S_{NTi} – nominal power of i^{th} secondary substation transformer, S_i – power of i^{th} node load, $i=1,2,3,k$; l_1, l_2, l_3 – the lengths of 1st, 2nd and 3rd feeders of the linear segment of the network; l_k – the feeder length of radial segment of the network.

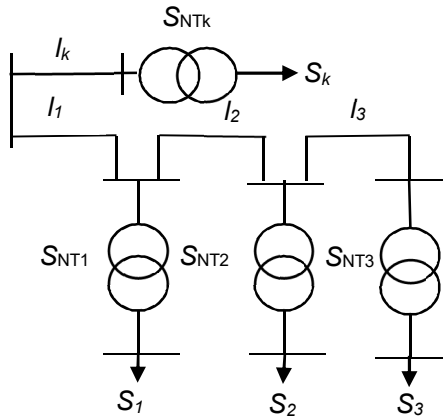


Figure 1. Combined topology network with connected SS transformers

Mathematical modelling. For mathematical modeling of control processes it is used state-space non-linear representation of control system [2].

$$\frac{dX}{dt} = F(X, Z, U, K, t) \tag{2}$$

where X is a n -dimensional state vector; F is n -dimensional nonlinear vector function; Z is m -dimensional vector of perturbations; U is l -dimensional vector of control actions; K is q -dimensional vector that determines the structural parameters of control.

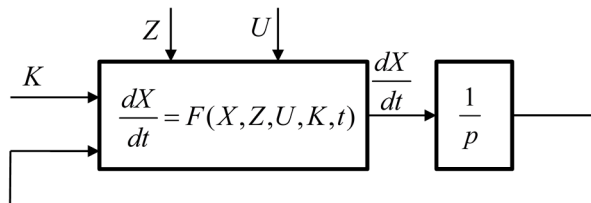


Figure 2. Structural diagram of control system

Methods of fuzzy control. For synthesis of fuzzy regulators it is used Mamdani and Sugeno fuzzy systems [14].

Methods of simulation analysis. For justifying algorithms of studied control system the simulation is used, which consist in modeling in the MATLAB Simulink environment of the operation modes of FMP power system controlled by the studied automated control system.

Results and discussion

Control tasks and choosing control criteria

Under operating conditions, the main optimal control criterion for the power supply system is just the minimum of variable component, which takes into account the costs of production, transformation and distribution of electrical power. In mathematical form, this criterion can be represented in the form of cost functional minimization [6]:

$$I(t) = \int_0^T [\bar{Y}(t), \bar{X}(t), \bar{X}] dt \rightarrow \min \quad (3)$$

where \bar{X} is the vector of unchangeable output data, $\bar{X}(t)$ is the vector of output data, $\bar{Y}(t)$ is the vector of control parameters.

If it is considered the circuit of the grid unchanged, then in the vector \bar{X} it is necessary to include data of the power system equipment, which determine the parameters of the equivalent circuit; performance characteristics of active and reactive power sources; utility power supply voltages. Vector $\bar{X}(t)$ includes data on the electrical loads of the enterprise, which change over time as the food manufacturing process continues. The control vector $\bar{Y}(t)$ contains the parameters that provide operation mode optimization by loading of active and reactive power sources, power flow at utility power supply connection node, changes of transformers turns ratios. The instantaneous value optimization problem is replaced with the control parameter optimization on a given time interval, which is equal to the averaging interval of the electric load graphs Δt . Then the multicriteria functional will look like this:

$$I(t) = \sum_{i=1}^T g(\bar{Y}_i, \bar{X}_i, \bar{X}), \quad t = \overline{1, T} \quad (4)$$

Since the loads of the individual nodes of the power supply system are probabilistic, therefore, the vector \bar{X} is a vector of random variables. To move to the deterministic formulation of the problem, the vector \bar{X} is replaced with the vector of expected values $M(\bar{X})$. Now the optimization problem is reduced to the solving n deterministic problems of minimizing the cost functional, where n is the number of load graph intervals.

The efficiency of the power supply system is estimated by the volume of electricity consumption and electricity losses in the power system.

The economic criterion of optimality is the minimum cost of electricity from the utility power system, generation of reactive power, loss of active and reactive power in electrical networks. In the deterministic formulation, the optimization problem can be formulated as follows: find the minimum of the functional of losses L

$$\min \sum_{i=1}^n L(P_i, Q_i, K_i) \quad (5)$$

Objective function J of power supply control has the form

$$J = \frac{M}{T} \int_0^T \left\{ \sum_{j=1}^{nm} \Delta P_{jLN} + K_Q \sum_{j=1}^m \Delta Q_{jLN} + \sum_{i=1}^n \Delta P_{iUS} + K_Q \sum_{i=1}^n \Delta Q_{iUS} \right\} dt \rightarrow \min_{X(t) \in \Omega} \quad (6)$$

where M is expected value of active and reactive power losses; T is the estimated time interval; ΔP_{iUS} , ΔQ_{iUS} are the active and reactive power losses at the SS level; ΔP_{jLN} , ΔQ_{jLN} are the active and reactive power losses at the i^{th} load node.

System analysis and mathematical modelling

Achieving goals described above is impossible without creation of effective mathematical model. The controlled object during the operating control is the power supply and distribution system of food manufacturing processes (PSDS FMP), which has a hierarchical structure and is operated using open tree-like schemes.

Power system of food industry enterprise is a complex nonlinear dynamic object, for which the following model can be formally described in the form (2), where X is a n -dimensional vector of parameters, that characterize the operation mode; F is n -dimensional nonlinear vector function; Z is m -dimensional vector of perturbations (time-variable active and reactive loads of consumers); U is l -dimensional vector of control actions, which is formed on the basis of a deterministic or accidental change of the technological process of the enterprise, as well as changes in the parameters of the operation mode and restrictions of the power system; K is q -dimensional vector that determines the structural parameters of PSDS FMP.

Considering the large dimension, complexity and insufficient information base of PSDS FMP, controlling procedures use its simulation models. The concept of instant circuits is applied and the dynamic model (2) is reduced to a set of static circuits. To perform the simulation procedure, the study interval T_M is divided into intervals Δt , within which the parameters X, U, K, Z are accepted unchanged.

At each simulation interval Δt , the following nonlinear system of equations is solved, which describes the steady state mode of the corresponding instant circuit:

$$F[X_k, S_k, C_k, V_k] = 0 \quad (7)$$

where X_k, S_k, C_k, V_k are parameter values of vectors X, S, C, V for k^{th} instant circuit.

The system description of PSDS FMP is performed with the use of tuple definitions (1) and can be written in this form [10]:

$$\Sigma_{PSDS} : \{ \{M\}, \{\varpi\}, \Omega \} \quad (8)$$

where $\{M\}$ is a set of PSDS FMP elements; $\{\varpi\}$ is a set of element links; Ω are the functions of PSDS FMP.

For automated PSDS FMP of modern type the element of type $\{M\}$ can be represented this way

$$\{M\} : \{M^{(t)}\}, \{M^{(l)}\}, \{M^{(ll)}\} \quad (9)$$

where $\{M^{(t)}\}$ are the technical means; $\{M^{(l)}\}$ are information technology means, including human-machine interface, software and databases; $\{M^{(ll)}\}$ are the other elements.

The links between PSDS FMP elements can be shown the following way

$$\{\varpi\} : \{\varpi^{(MX)}\}, \{\varpi^{(E)}\}, \{\varpi^{(ME)}\}, \{\varpi^{(I)}\} \quad (10)$$

where $\{\varpi^{(MX)}\}$ are the mechanical links; $\{\varpi^{(E)}\}$ are electrical links; $\{\varpi^{(ME)}\}$ are electromechanical links, created by electromagnetic field; $\{\varpi^{(I)}\}$ are informational links.

It was noted above that the PSDS FMP actively interacts with the electric power grid (EPG), which should be taken into account when creating a mathematical model. Then it can be written in the form

$$\{M\} : \bigcup_{r=l}^R \{M^{(r)}\}; \{\varpi\} : \bigcup_{r=l}^R \{\varpi^{(r)}\}; \quad (11)$$

where $R = 3$; $\{M^{(1)}\}$ corresponds to the EPG elements that must be taken into account in a mathematical model of the entire system; $\{M^{(2)}\}$ – the set of elements of PSDS FMP; $\{M^{(3)}\}$ are elements of district electric grids (DEG), the consideration of which is necessary for an adequate description of the processes in the combined system of EPG, PSDS FMP and DEG.

A symbolic description of the PSDS FMP processes can be represented as follows:

$$S_n [X(t_0)] = X(t), X \in \mathfrak{R}^n, t \in T \quad (12)$$

The process S_{n_0} is a rule of transition from the state with the parameter value (time) t_0 to the state with the value $t > t_0$ through all its intermediate continuous or discrete values. The process S_{n_0} is associated with a mapping of sets $T \times X \rightarrow X_t$.

To study the processes S_{n_0} it is used a combined method: determination of static load characteristics in the interactive mode and calculation of the parameters of the operation mode.

A formal description of the PSDS FMP model can be presented as a tuple

$$\sum_{PSDS}^{(M)} : \{X, A, B, t, \mathfrak{N}\}, X \in \mathfrak{R}^n, A \in \mathfrak{R}^m, B \in \mathfrak{R}^w, t \in T \quad (13)$$

where X is a time-variable parameter vector; B is a input parameter vector; A is a time-invariant parameter vector, which includes structural parameters of substations, distribution system and transmission lines; \mathfrak{N} is a rule of determining parameters of X . The vector B includes load powers $V = V(t)$ and structural parameters of PSDS FMP model that varies depending of power consumer configuration changes.

For modeling of operation modes of the open PSDS FMP electrical network, the voltages at network nodes are determined by the equations [17]:

$$U_i = U_N - \frac{1}{U_N} \sum_{i=1}^n (P_i R_i + Q_i X_i) \quad (14)$$

Without taking into account the power losses in the enterprise distribution network, the voltage losses are calculated in separate sections of the line by fixing loadings of SS (S_1, S_2, \dots, S_n) or the powers (P_1, P_2, \dots, P_n) that flow over the sections. The total voltage loss in the line from the power source to any node i is determined by the sum of the voltage loss in each section and calculated by the formula:

$$\Delta U_{0i} = \frac{1}{U_N} \sum_{i=1}^n (p_i r_i + q_i x_i) = \sqrt{3} \left(\sum_{i=1}^n i_{ai} r_i + \sum_{i=1}^n i_{pi} x_i \right)$$

To model the modes of PSDS FMP closed electrical networks it is used the node potential method [17]. The system is described using the linear system of equations:

$$\underline{\mathbf{Y}} \cdot \underline{\mathbf{U}} = \underline{\mathbf{I}} \quad (15)$$

where $\underline{\mathbf{U}}$ is a vector of node potentials; $\underline{\mathbf{I}}$ is a vector of node currents; $\underline{\mathbf{Y}}$ is a matrix of node admittances, which consists of $\underline{\mathbf{Y}}_{ii}$ – the sum of admittances that connected to node i and $\underline{\mathbf{Y}}_{ij} = \underline{\mathbf{Y}}_{ji}, i \neq j$ – negative values of admittances between nodes i and j .

Therefore, $\underline{\mathbf{Y}}$ is a symmetric matrix and the sum of all columns and rows is zero.

Unknown node voltages can be obtained using an inverted matrix:

$$\underline{\mathbf{U}} = \underline{\mathbf{Y}}^{-1} \cdot \underline{\mathbf{I}} \quad (16)$$

In the simplest case, the voltage at the power supply node is specified, and the power of the consumers must be set as a voltage dependent according to (15). Using the nominal voltage, the power consumer admittance matrix, which is part of the node admittance matrix, is determined once:

$$\underline{\mathbf{Y}}_k = \frac{S_k^*}{U_n^2} \quad (17)$$

This process is called linear power flow. However, if the nodal powers of the consumers, calculated by nodal voltages and the consumers' admittance matrices, do not match the set values, then the calculated voltages are taken to be equal to the nominal voltage. The resulting variations are acceptable if the deviation of the calculated voltage from the nominal voltage is low or the consumer admittance is considered constant.

At the given constant power of the nodes, the relation between the full power and the voltage is calculated by (18).

$$S_k = U_k^2 \cdot \underline{\mathbf{Y}}_k^* \quad (18)$$

It is shown on the basis of (18) that the problem of load current is reduced to a nonlinear quadratic system of equations for node voltage. The above system of equations is solved by some iterative method such as Newton-Raphson method [11].

Justification of FMP power supply control algorithms

In order to provide energy efficient modes of power transmission in PSDS FMP, energy efficient voltage levels are maintained at the nodes of the system [2] by regulating reactive power sources, as well as the voltage of secondary substation (SS) and primary substation (PS) transformers.

The values of effective voltage levels at the PS level are calculated on the basis of real-time static characteristics of the complex load (SCL) [11], and voltages at the PS transformers level – on the basis of the calculation of 6-10 kV distribution electrical network modes.

The SS voltage regulator reference values are calculated on the basis of active P_{LV} and reactive power Q_{LV} values, which are measured on SS low-voltage side, parameters of SS 10 kV power supply system (radial, linear or combined connection schemes), cable line lengths L_k , transformer technical data, static load characteristics on the SS low-voltage (LV) side $\Delta P_{SS^*}(U_{SS^*}) = A_1 \cdot \Delta U_{SS^*}$, identified for the main operation modes in real-time.

The reference values are calculated in the following order. The following factors are determined: SS transformer load $\alpha = \sqrt{P_{LV}^2 + Q_{LV}^2} / S_{LV}$; reactive power $tg\varphi = Q_{LV} / P_{LV}$;

active power $\cos \varphi = P_{LV} / \sqrt{P_{LV}^2 + Q_{LV}^2}$; active load $\alpha_A = \alpha \cdot \cos \varphi$, and the degree of reactive power compensation $\beta_Q = Q_{BKN} / Q_{PN}$. Using equilibrium coefficient of the secondary network for active power A_{li} , obtained by SCL of the i^{th} SS transformer, it is calculated the value of the equilibrium voltage on the bus 0.4 kV of i^{th} SS.

We take the equilibrium voltage on the LV SS buses as the reference value SS voltage regulator.

The equivalent circuit of combined topology network (Figure 1) is shown in Figure 3, where $R_{EC1}, R_{EC2}, R_{EC3}$ are equivalent circuit resistances of 1st, 2nd and 3rd branches of linear segment; R_{ECk} is equivalent circuit resistance of radial segment branch; R_{TSi} – equivalent resistance of i^{th} transformer; S_i – power of i^{th} node load, $i=1,2,3,k$; l_1, l_2, l_3 – the lengths of 1st, 2nd and 3rd feeders of the linear segment of the network; l_k – the feeder length of radial segment of the network; $r_{01}, r_{02}, r_{03}, r_{0k}$ – per unit length resistance of feeders; U_N – nominal voltage of network, and

$$R_{EC1} = \frac{\left(1 + \frac{2(S_2 + S_3)}{S_1}\right) r_{01} l_1}{3U_N^2}; \quad R_{EC2} = \frac{\left(1 + \frac{2S_3}{S_2}\right) (r_{01} l_1 + r_{02} l_2)}{3U_N^2};$$

$$R_{EC3} = \frac{(r_{01} l_1 + r_{02} l_2 + r_{03} l_3)}{3U_N^2}; \quad R_{ECk} = \frac{r_{0k} l_k}{3U_N^2}; \quad R_{TSi} = \frac{\Delta P_{SCL}}{S_{NTi}^2}.$$

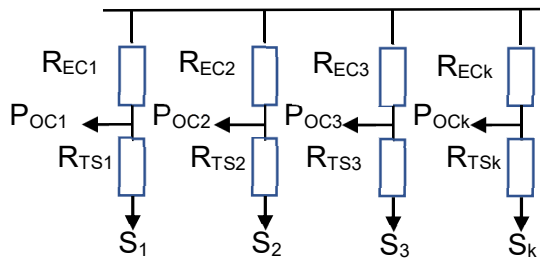


Figure 3. Equivalent radial circuit of combined topology network

Using the technical specifications of electrical equipment and the current values of active and reactive power of the generalized electric receiver, which are measured on the buses of LV SS, for all segments of the SS connection (Figure 1), it's obtained the following expressions for the equilibrium voltage:

$$\Delta U_{SSi^*0} = \frac{\Delta P_{OCi} + S_i^2 \left[R_{ECi} + \frac{\Delta P_{SCL}}{S_{NTi}^2} \right]}{A_{li} \cdot P_{Ni}} = \frac{\Delta P_{OCi} + S_i^2 [R_{ECi} + R_{TSi}]}{A_{li} \cdot P_{Ni}}, i=1,2,3,k. \quad (19)$$

where A_{li} – coefficient of linearized static characteristic of i^{th} node load; P_{Ni} – nominal active power of i^{th} node load, ΔP_{OCi} – open circuit power losses; ΔP_{SCL} – short circuit power losses.

When adjusting SS voltage, the equilibrium voltage is the reference value of the SS voltage regulator and is calculated for the main operation modes of the power supply system using static load characteristics on the SS buses according to the voltage, which are determined in the interactive mode.

In order to maintain the energy efficient modes of FMP, the PSDS FPM provides for the use of fuzzy voltage regulators of the SS transformers and PS transformer.

Justification of energy efficient 6-10 kV distribution network voltages

The voltage regulation in the 6–10 kV distribution network is carried out by adjusting the voltage of the PS transformer by means of on-load tap changer [11]. The main purpose of voltage regulation is to reduce power losses in the 6-10 kV electrical network and to provide conditions for voltage regulation of the SS transformers when changing the voltage on the high voltage (HV) side of the SS, depending on the load and the voltage on the HV side of the PS transformer (maximum and minimum load of the grid). This implies maintaining voltage levels on the 6–10 kV buses the electrically closest and most distant SS, which provide conditions for the effective operation of PSDS FPM at all SSs under load change. Energy efficient voltage levels in the 6–10 kV network are calculated on the basis of a real-time mathematical model for the characteristic modes of the power system based on the measured currents and the voltage of the PS transformer [3]. Determination of the farthest and the closest connections is made in real time using measured currents, voltages and power of individual connections based on fuzzy logic using the following parameters: load power S , line length L , voltage regulating effect $P(U)$, load distribution along the line $R(L)$, availability of reactive power compensation devices C . A block diagram of the fuzzy controller for selecting the most distant and the closest lines to the power center of is shown in Figure 4.

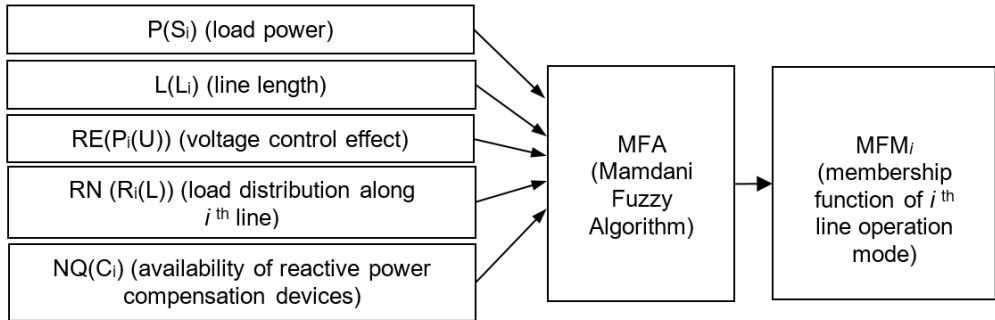
Synthesis of fuzzy regulation of the voltage of the SS transformer

When adjusting the voltage in the secondary electrical network should be provided:

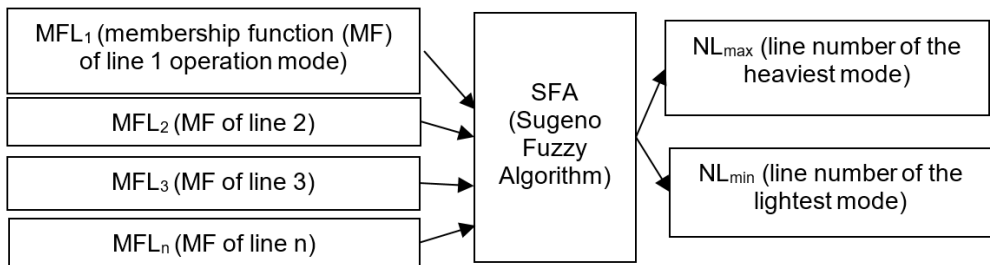
- Compliance of voltage indicators with the requirements of the official standard;
- Compliance of the voltage level with the value permissible for the equipment of the electrical networks, taking into account permissible operational increases of the industrial frequency voltage on the electrical equipment (according to the data of manufacturers and circular saws);
- Minimum consumption and loss of electricity in the secondary electrical networks.

Voltage modes are selected depending on the type of secondary electrical network (radial, linear, combined), the nature of the consumers connected to the secondary network, their distance from the secondary substation.

An algorithm for fuzzy voltage regulation of the SS transformer with an electronic switch is built using the Mamdani algorithm [2]. The structure of the fuzzy regulator of the SS transformer is presented in Figure 5. For the implementation of control functions, a database was created for a fuzzy SS voltage regulator.



a – algorithm of fuzzy determining of i^{th} line operation mode



b – algorithm of fuzzy determination of the closest and the farthest line connection

Figure 4. Block diagram of fuzzy connection selection with the heaviest and lightest operation modes of the closest and the most distant connections

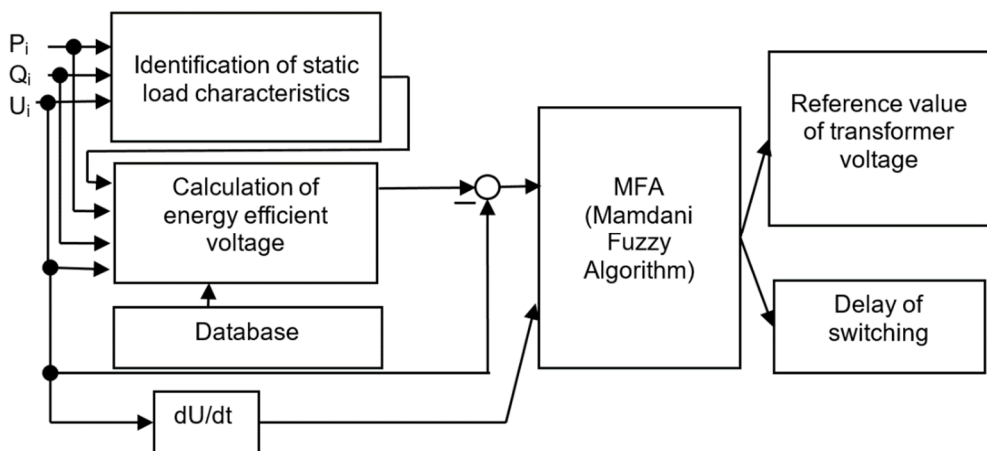


Figure 5. Block diagram of a fuzzy voltage regulator of the SS transformer

Synthesis of fuzzy voltage regulation at the PS transformer level

The structure of the fuzzy regulator of the on load tap changing PS transformer is presented in Figure 6.

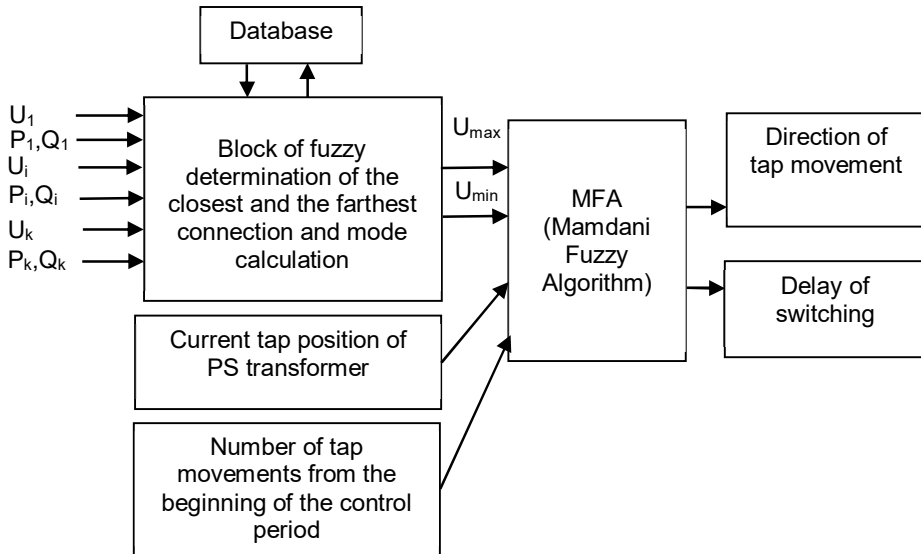


Figure 6. Block diagram of fuzzy voltage regulator of PS transformer

The values of the fuzzy output variable "direction" were defined as follows: at a voltage below nominal ("very low", "low"), the tap changer direction should correspond to the values "down", and at high voltage ("high", "very high") the tap changer direction is up. In cases where the voltage is approximately equal to the rated ("normal"), the tap changer does not fire ("stop").

Simulation results of PS fuzzy controller operation

To study proposed algorithms and mathematical model in Simulink environment on the PS level, it was created a computer model of a 110/10 kV substation, to which a non-linear dynamic load is connected. The block diagram of the simulated system is presented in Figure 7, where Source 110 kV is a block describing the 110 kV power system; Transformer – block describing model of three-phase power transformer 110/10kV; Line – blocks that define the parameters of the line; V-I measurement – blocks of measurement of current values of current and voltage; Dynamic load – blocks that simulate three-phase dynamic loading; Power demand – units that have the task of changing the power consumption for the dynamic load units; Fuzzy-logic controller – fuzzy controller block; On-load regulator – blocks of voltage regulation of the power transformer on load.

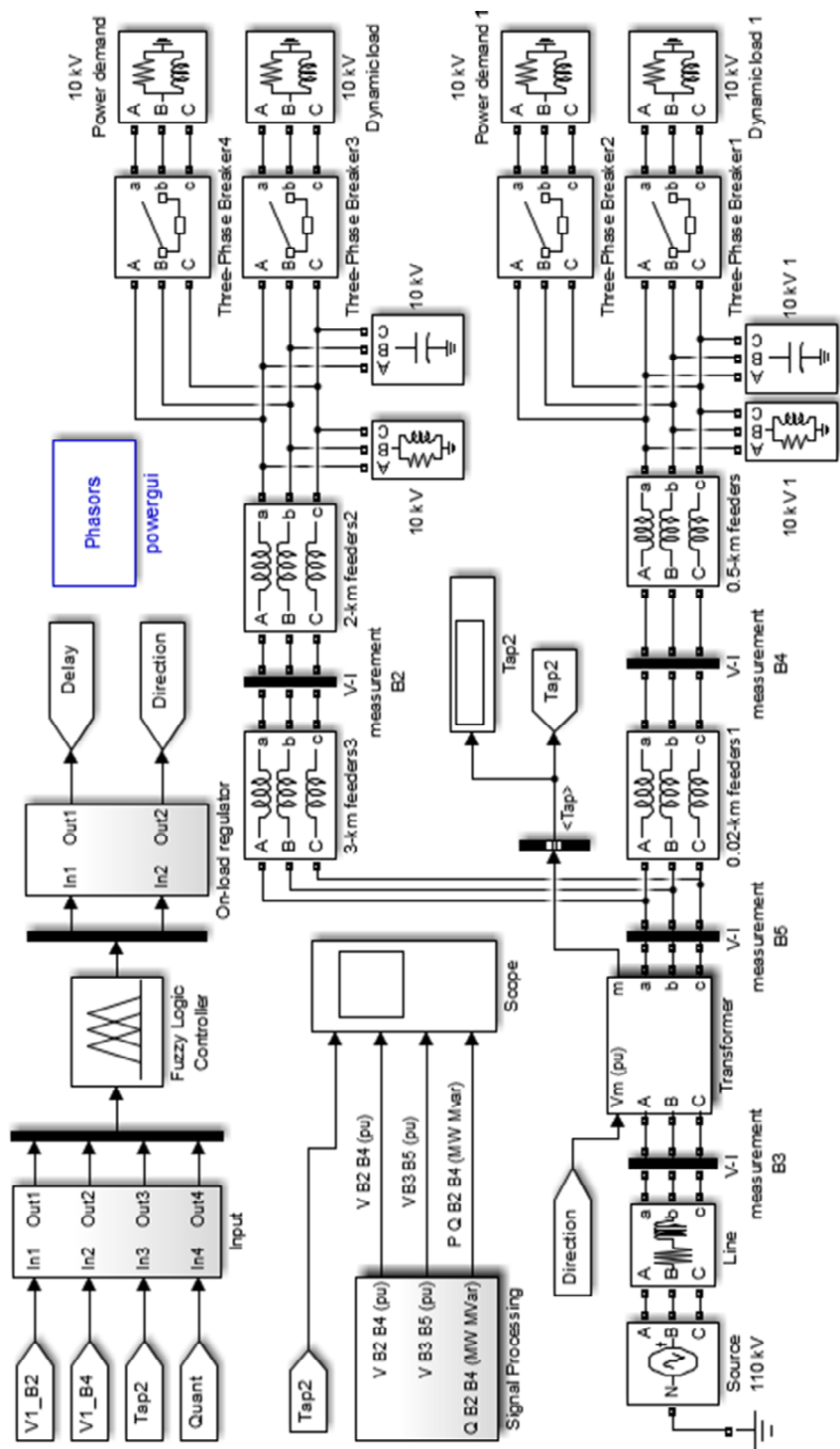


Figure 7. Model of 110/10 kV substation with fuzzy controller and tap changer

Using the model it is obtained graphs of voltage changes depending on the load in time. Figure 8 shows graphs of voltage changes at 110 kV (V_{B3}) and 10 kV substations (V_{B5}), voltage and load on the remote (V_{B2}) and near-consumer (V_{B4}) bus loads. The analysis of the obtained voltage graphs showed that the developed algorithm of voltage regulation based on fuzzy systems ensures the maintenance of the given voltage levels at the nodal points of the distribution network when changing the voltage of the power source and when changing the load of consumers according to different schedules.

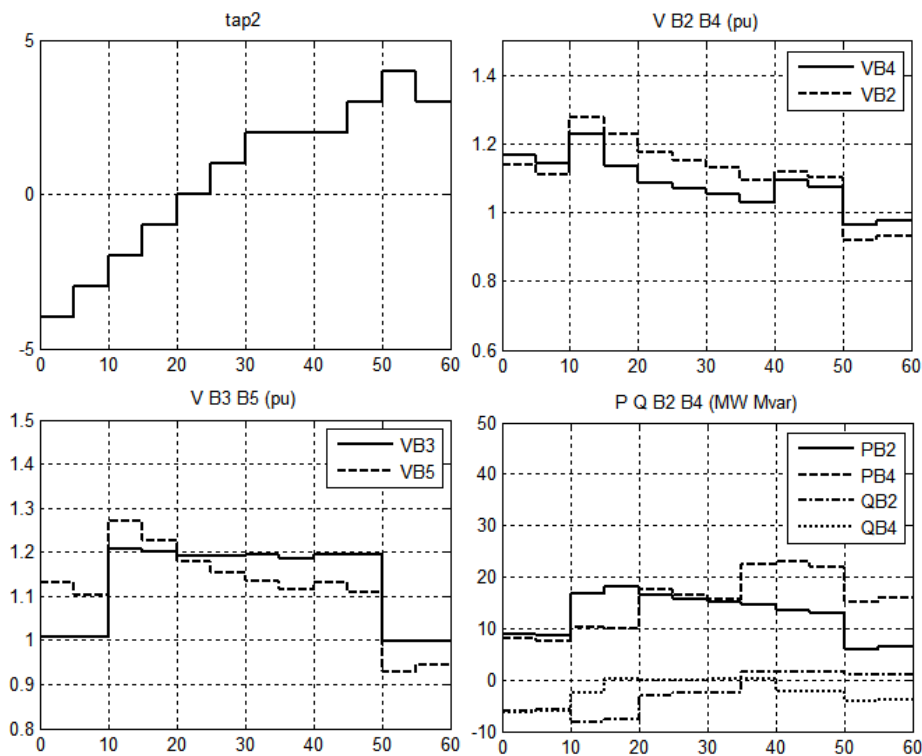


Figure 8. Graphs of voltage and load changes in the distribution network.

The implementation of the proposed algorithm for voltage regulation based on fuzzy sets allows to maintain rational levels of voltage in the distribution network, which are obtained on the basis of analysis and optimization of the modes of the electrical distribution network, which will ensure a rational level of electricity losses.

Simulation results of SS fuzzy controller operation

As a research object in the Simulink environment at the SS level, a computer model of a 10/0.4 kV substation was created, to which a nonlinear dynamic load is connected to the tires. The block diagram of the simulated system is presented in Figure 9, where Source 10 kV is a unit describing the 10 kV power system; Transformer – block describing the model of three-phase power transformer 10/0.4kV; Line – blocks that define the parameters of the airline; V-I measurement – block of measurement of current values of current and voltage.

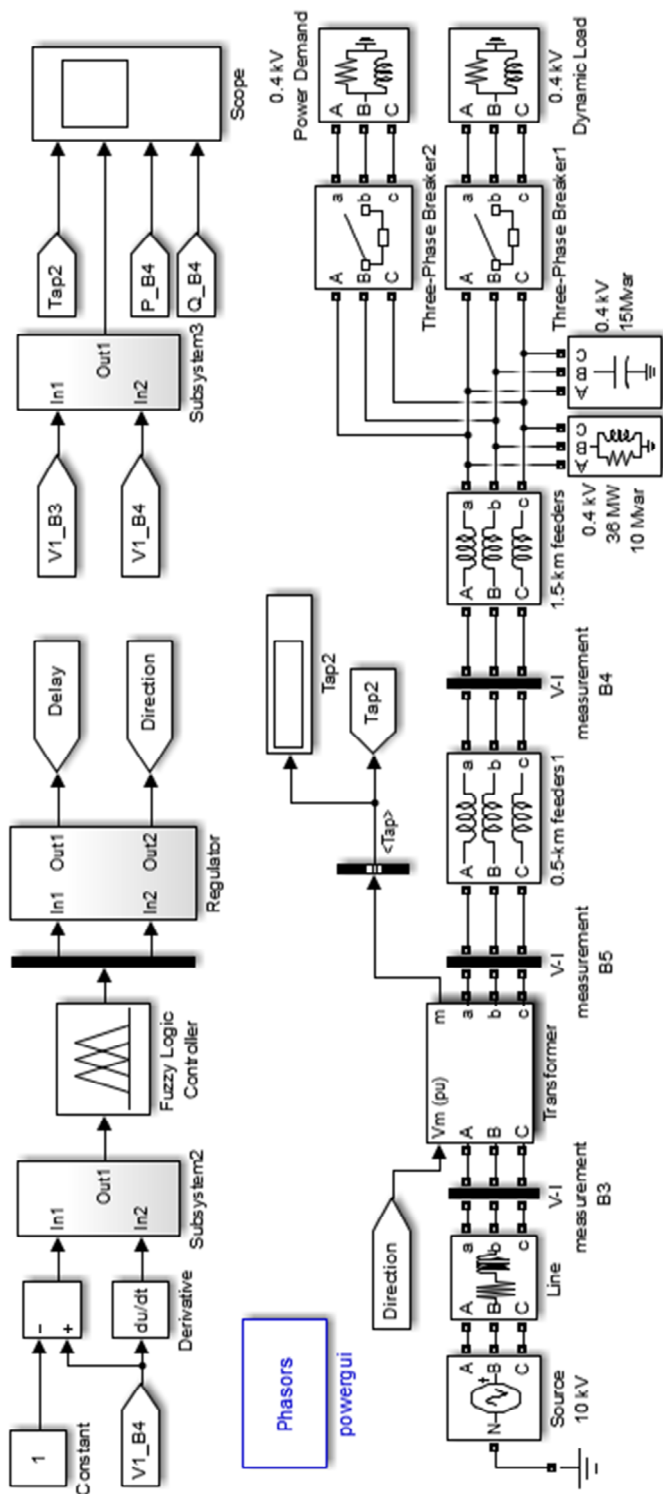


Figure 9. Simulation model of voltage fuzzy control system of the SS transformer in MATLAB/Simulink

The results of computer simulation of the SS fuzzy controller operation under different modes of the secondary network: loading and dropping load; switching capacitors on and off; change of supply voltage are presented in Figure 10.

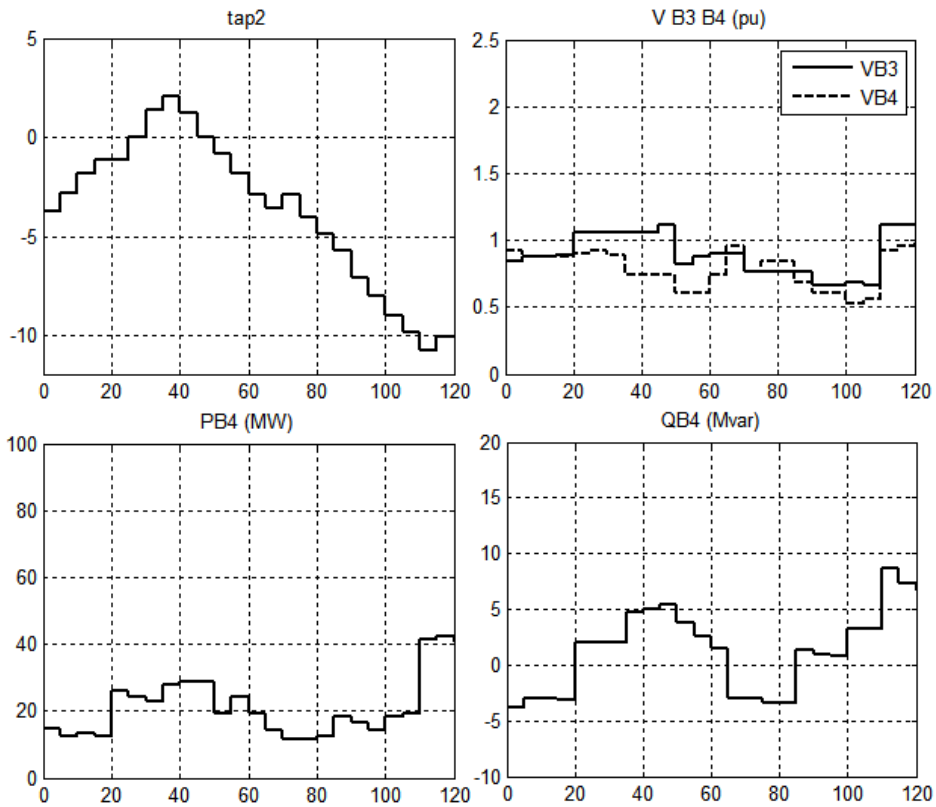


Figure 10. Graphs of voltage and load changes in the secondary network

Figure 11 shows the daily graphs of power loads of food manufacturing process W (pu) and voltages V (pu). Graph of voltages which demonstrates the results of two different ways of controlling the voltage regime: classical [11] and fuzzy, as well as process without control.

The results of the computer simulation made it possible to estimate the losses of electrical energy in the electrotechnical complex in relative units when comparing the results of the operation of the classical regulator of the load regulation device and the fuzzy controller. As it can be seen in Figure 12, the use of fuzzy control of transformer with an electronic switch allows to reduce electricity losses by 7% compared to the classical voltage regulation algorithm.

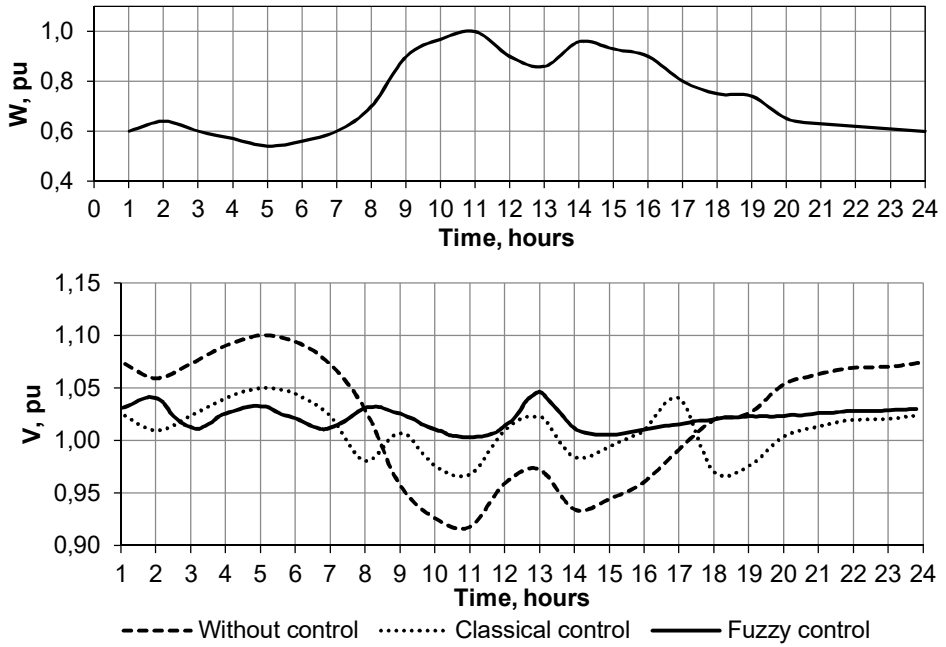


Figure 11. Changing the voltage deviation on the SS buses during the day

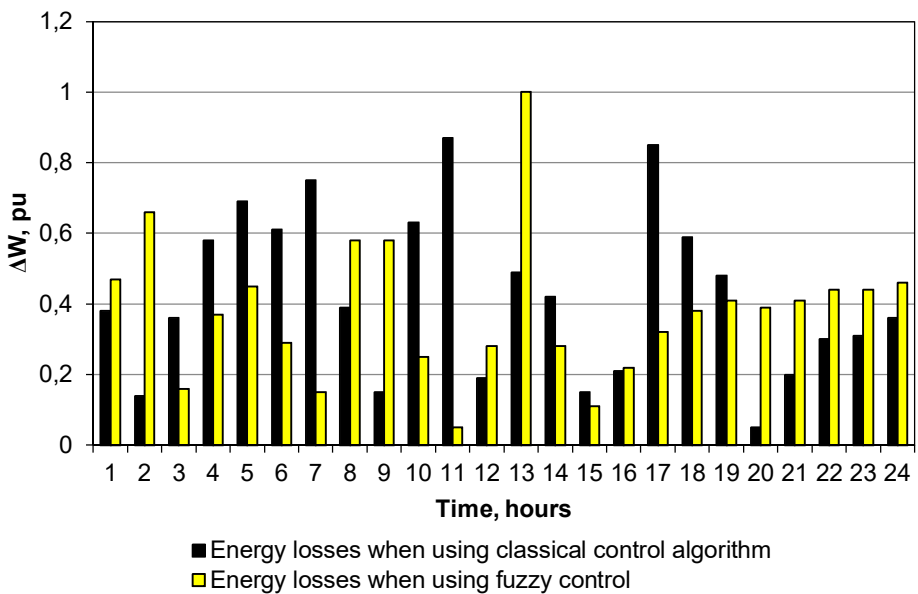


Figure 12. Changing the relative losses of electricity during the day with different methods of voltage regulation on SS buses

Conclusion

Based on methods of system analysis such as tuple definition and decomposition methods it was identified links between heterogeneous elements of the energy supply system of food manufacturing processes and built the mathematical model. This model made it possible to estimate energy efficient modes of the power supply system. Fuzzy controllers at SS and PS level were developed to maintain these energy efficient modes during food manufacturing processes.

Using the results of the computer simulation of algorithms of SS and PS fuzzy controllers in MATLAB Simulink environment the losses of electrical energy were estimated comparing to the results of the classical regulator operation in main operation modes of food manufacturing. The results showed that the use of our method allows reducing electricity losses up to 7% compared to the classical voltage regulation algorithm.

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Price transmission along the Lithuanian pigmeat supply chain

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Abstract

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Introduction. The paper analyses structural changes of pig farming in Lithuania and explores price behaviour along the Lithuanian pigmeat supply chain.

Materials and methods. The conducted study uses annual indicators collected by Statistics Lithuania and weekly prices published by SE ‘Agricultural Information and Rural Business Centre’ (AIRBC). Methods of comparative analysis and graphical representation allow investigating the most important changes of the Lithuanian pig farming. Price behaviour is studied employing econometric tests showing the characteristics of the analysed pigmeat price series and different aspects of price relations in the short- and long-term perspective.

Results and discussion. The share of small-sized farms is decreasing in the structure of pig farms, while farmer and family farms have lost their key role in pig farming, in particular between 2004 and 2018. In 2004, the share of pigs that were grown on farmer and family farms accounted for 56.7% of the population, while in 2018 a drop to a critically low 24.9% level was demonstrated. During the period from 2007 to 2016, the decrease in the share of farms with 10 animals and more was from 3.1 to 2.5%, while the share of farms with 3–9 pigs increased from 34.7 to 47.9%. This development direction of pig farming was caused by multiple factors, including the change of the business environment after 2004, the transformation of agricultural support model and aftermaths of price hikes, the impact of the governmental intervention due to the integration into the Eurozone, as well as animal health issues.

Price transmission analysis demonstrates that the pork market had faced several critical shocks that had an impact on price behaviour and stakeholders’ welfare. However, the Johansen co-integration tests show that the most significant structural break was in 2013. The Granger causality test confirms that the price setting direction runs from retail to farm, while, in the long run, the hypothesis of the asymmetric behaviour is not supported. According to the results of Vector Error Correction Model, pigmeat prices return to the described equilibrium with a speed 3.6% for the analysed period.

Conclusions. The study confirms the dramatic change of the Lithuanian pig farming sector. A test for price symmetry does not show market efficiency problems, but in the short-run one-way causality is present.

Introduction

According to OECD-FAO agricultural outlook [22], in Europe, pork meat consumption per capita was the highest among all meat varieties since 1990. However, on a global scale this trend had changed in 2007 and poultry became the most popular globally consumed meat variety, while pork meat was the second most important meat in the world.

Thus, the topic of pigmeat price transmission attracted a huge attention from academic society around the world. The most recent research covers studies of domestic supply chains in Australia [15], Czech Republic [7, 25], China [8, 10, 28], Denmark [19], Finland [20], France [19], Germany [19, 29], Hungary [3], Ireland [19], Italy [6], the Netherlands [19], Poland [18], Slovenia [5], Switzerland [1], the United Kingdom [19, 23], the USA [13, 21], and etc. Studies investigate different aspects of pork market efficiency and therefore apply various sets of econometric techniques.

For example, [1, 3, 4, 6, 8, 10, 13, 15, 19, 20, 21, 25] focus on issues of asymmetric price behaviour along the pork supply chain employing various research methods allowing to investigate both short- and long-term relationships among the supply chain. Some studies have a particular interest in impact of different factors [8] and the regime-dependent prices behaviour [13]. [2, 3, 4, 5, 6, 10, 18, 23, 28] describe the relations between prices on different level of the supply chain. According to research results, academics find both symmetric and asymmetric behaviour of prices along the domestic supply chain, research findings do not allow making a generalized conclusion on the leading price setting stakeholder or describe price relations and equilibrium adjustment speeds as similar. The findings of the aforementioned studies imply that price behaviour and relevant market efficiency challenges depend on the country. It should be noted that even the common market of the EU is rather a set of sufficiently diverse supply chains reflexing market peculiarities of the countries.

This fact makes the study of the Lithuanian pigmeat supply chain an interesting topic, because the previous research on price transmission in this country is scarce due to data availability. Based on the previous research, this paper selects a framework of econometric tests that show multiple aspects of price behaviour along the supply chain. It is important to note that since Lithuania had joined the European Union (EU), the domestic pig farming sector got through the serious structural transformations. The overall population of pigs reduced, while the dominant share of animals on farmer and family farms was replaced by the leading role of agricultural companies and enterprises. The aforementioned changes could have a significant impact on price behaviour along the pigmeat supply chain and influence the welfare of stakeholders along the pigmeat supply chain.

The paper is aiming to analyse the structural changes of pig farming in Lithuania and explore the price behaviour along the Lithuanian pigmeat supply chain. The study identifies the main factors that have had an impact on the pork sector evolution and focuses on prices as an important component that could have an impact on pig farming development trends in Lithuania.

Materials and methods

Materials

The study relies on main annual indicators of pig farming collected by Statistics Lithuania and weekly upstream and downstream prices of pigmeat published by AIRBC. The upstream level is measured by the average purchase price of pigs (confirmation class E)

collected from the Lithuanian enterprises on weekly basis. The downstream price level is measured by the average retail price of ham without bones calculated from retail prices of the main network supermarkets in Lithuania. The price transmission study is carried out for the period 2010–2017.

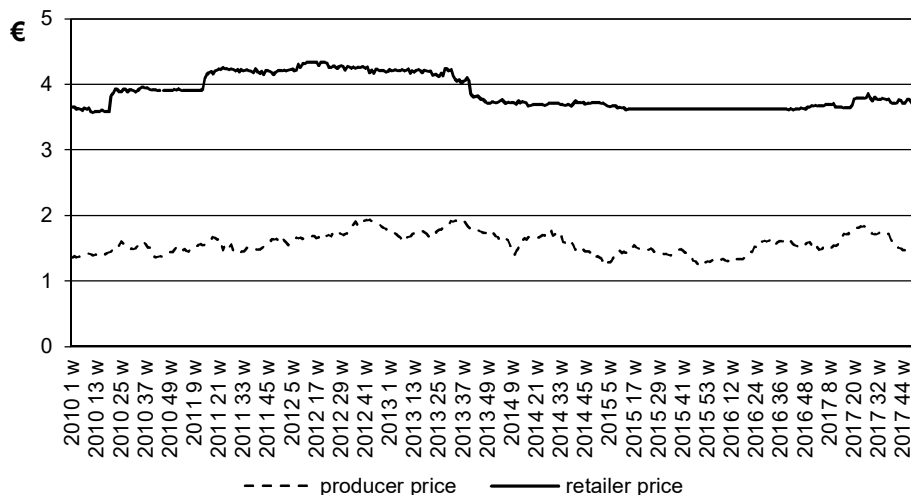


Figure 1. Pigmeat prices on producer and retailer levels

Source: AIRBC, own calculations.

According to Figure 1, the gap between upstream and downstream prices is changing during the analysed period. Downstream prices are less volatile than the prices on upstream level. Starting from 2014, an interesting behaviour of the price on downstream level is observed. Retail price stabilizes for the long period and does not respond to price fluctuations on producer level, while starting from 2017 it becomes more dynamic. This situation could be a result of couple inter-related factors. For example, the influence of the legislation controlling price hikes before and after the entrance to the Eurozone, as well as the reaction of retailers to the threat of African swine fever on domestic market and the change of the situation after the Russian ban.

The investigated Lithuanian pigmeat supply chain demonstrates a higher price volatility on producer level, while retail prices are more stable. In fact, the analysed case is similar to the functioning of pigmeat supply chains in Czech Republic [25] and Poland [18], while the opposite price development trend is evidenced in Slovenia where retail prices demonstrate higher volatility than prices on farm [5]. However, some studies provide examples of quite similar volatility on both levels of the country, for example, price development in China [10], Italy [6], and Finland [20]. Hence, the behaviour of prices on different supply chain levels of the same commodity depends on the country.

Methods

At the first stage the study applies methods of comparative analysis and graphical representation to investigate changes on the Lithuanian pig farms. The findings are drawn on the basis of the analysis of main indicators published by Statistics Lithuania.

At the second stage the price transmission along the pigmeat supply chain is explored. Firstly, the nature of data is investigated in order to characterize price series as stationary or non-stationary. For this purpose, Augmented Dickey Fuller (ADF) [9] test is run. This test allows to judge about reliability and validity of the data [27]. Additionally, we deployed the Bai-Perron test [2] in order to take into account potential structural breaks. It assists in avoiding the rejection of the null hypothesis (H_0) [24] when the results could be meaningful.

At the third step the Johansen co-integration test [16, 17] is carried out to answer the question if there is a co-integrating vector or vectors between downstream and upstream pigmeat prices. This step reveals if prices on different levels of the pigmeat supply chain repeat the movements related to price hikes and reductions in the long run. The absence of the co-integrating vector alerts about possible problems in price transmission resulting in market inefficiency issues.

At the fourth step the Granger causality test [14] is carried out. The results of this test allow to identify the direction of price running causality in the short-term perspective. The efficient market could be characterised by the two-way causality, while in case of price setting leadership on downstream or upstream level the welfare of farms or consumers could be violated.

At the fifth step the relations between upstream and downstream prices are described by vector error correction model (VECM). Characteristics and the application issues of this model are described in [30].

Finally, the threshold autoregressive (TAR) model [11, 12] is deployed. The initial step tests the H_0 that there is no cointegration between prices in the long run relationship. Next, the H_0 of symmetric adjustment mechanism between the farm and retail prices in the long-run equilibrium is investigated.

Results and discussion

Structural changes of pig farming in Lithuania

Over the last decades, the structure of the pig population on Lithuanian farms overcame a significant transformation. According to Statistics Lithuania, when Lithuania entered the EU in 2004, the share of pigs that were grown on farmer and family farms accounted for 56.67% of the population, while in 2018 a drop to a critically low 24.93% level was demonstrated. For the investigated period 2010–2018, the gradual increase of the share of animals at agricultural companies and enterprises and the corresponding changes in pig farming structure are demonstrated in Figure 2.

Another important characteristic is the structure of pig farming by herd size. The share of pigs on farms that had 1–2 pigs decreased very sharply from 10.22% in 2007 to 4.23% in 2016 [33]. At the same time, the share of pigs on farms with the herd size from 3 to 9 pigs dropped from 14.01% to 10.09%. The share of pigs on farms with 10 pigs and more increased from 75.77% to 85.68%. Furthermore, the corresponding decrease in the share of farms with 10 animals and more was from 3.06% to 2.51%, while the share of farms with 3–9 pigs increased from 34.74% to 47.93% during the period from 2007 to 2016 [33].

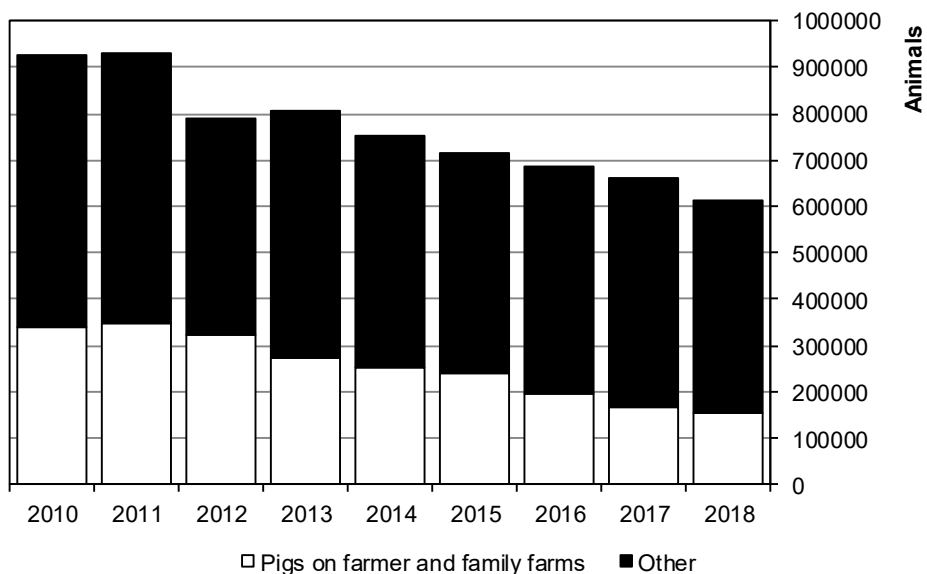


Figure 2. Structure of pig population by farm type in Lithuania for the period 2010–2018
Source: Statistics Lithuania [32].

Table 1

Main indicators of supply balance sheets for pigmeat, thousand tonnes

	2010	2011	2012	2013	2014	2015	2016	2017	2018	Change (2010 = 100%)
Produced	73.3	74.9	79.4	86.9	84.9	84.3	74.0	71.5	72.0	98.2
Import	78.5	83.2	85.4	90.6	84.1	91.8	83.8	89.0	92.1	117.3
Export	15.3	23.2	27.6	35.7	22.3	27.6	17.0	19.7	23.3	152.3
Total domestic uses	136.9	136.7	135.4	141.9	147.0	147.6	141.3	141.0	140.7	102.8

Source: Statistics Lithuania [34], own calculations.

During the period 2010–2018, domestic production was growing until 2013, later, the Russian ban and African swine fever had an impact on the produced amounts of pigmeat and foreign trade. In 2018, both import and export of meat products (estimated in meat equivalent) increased, as compared to the year 2010. Total domestic uses also showed a sign of the moderate increase and statistics reacted to embargo and animal health problems (Table 1).

Hence, the current situation of the Lithuanian pig farming was determined by multiple factors inside and beyond pork sector. A crucial aspect was the change of the farming environment after the integration into the EU in 2004. Representatives of pig farming quickly

realized that they could not compete with the leading pigmeat producing countries that were equipped with modern material facilities allowing to offer their product at a better price. The first years of the competition within the common market started from the clear understanding of two serious problems. First, there was a need to invest in modernisation of farms that produced pigmeat. Second, a change of traditional breeds of pigs to the new breeds, preferred by European consumers, was compulsory. However, the national agricultural support model did not spend a decent attention to this situation.

The introduction of the Common Agricultural Policy determined the establishment of the novel support model and gave another signal for the development of the national agriculture creating more favourable conditions for crop production. Global price hikes for agricultural commodities in 2007–2008 and 2010–2011 contributed to the growth of pig farming costs bolstering pig feeds. Direct payments and the growth of crop prices made crop production a more attractive farming niche.

In 2015, Lithuania became a member of the Eurozone and farmers faced a new dilemma. On the one hand, the prices for the related services were rising, on the other hand, the competition on the EU market did not allow to follow the general price development direction. Hence, for smaller farmers, the choice between the generously supported crop production and unprofitable pig farming became more obvious. The exit of small farms from pig farming often resulted in land rent and its further use for crop production on larger farms.

Another important aspect that contributed to the structural changes in pig farming was animal health. The outbreaks of classical swine fever in 2009 and 2011 had a significant impact on export restrictions of live pigs and the transformation of the foreign trade structure. At that time, the Russian Federation was the main trading partner for the Lithuanian pork sector; however, the outbreak of the classical swine fever in 2011 had stopped the export of live pigs until the second half of 2013. Hence, this situation encouraged farmers to look for new markets and switch from export of live animals to pigmeat products.

The subsequent opening of the Russian market was short, because in January 2014 the first outbreak of African swine fever was confirmed in Lithuania. This outbreak led to the confusion and the disturbance of foreign trade. The strength of the common market became the weakness as the European Commission could not quickly respond to a new challenge and propose the zoning, while a free cross-border movement within the EU became a threat. Later, the Russian market was closed due to import ban on EU agricultural commodities.

Nevertheless, the impressive geographical spread of African swine fever virus in wild nature and on Lithuanian pig farms was reported until 2018, while only in 2019 the figures started to fall down. As a comparison, according to statistics of the State Food and Veterinary Service [31], 1,446 spots in a wild nature and 3,098 infected wild boars were found in 2018, while by November 26th, 2019 only 430 infected places and 644 wild boars were documented.

The aforementioned disease led to the polarization of pig farming society in Lithuania, because a huge number of small farms was represented as a serious threat for commercial farms. The detection of virus on a small farm resulted in an export ban for larger commercial farms belonging to the same zone. It was argued that the spread of this virus in pig farming was rapid in countries that had a significant share of small farms [26]. The vulnerability of small farms was widely recognized due to the careless implementation of biosafety measures on those farms. However, the proposals to slaughter pigs and prohibit pig farming on small farms did not achieve enough support in Lithuanian agriculture.

Finally, a political decision to keep a diverse farming structure was made. On the one hand, the larger farms were proposed to get funding for farm modernization and the improvement of biosafety measures. Small and medium farms with less than 100 pigs, located in districts with African swine fever spots, were offered two types of the

compensatory support from autumn 2018. The first type of the support assisted in improvement of biosafety measures on farms, while the second measure compensated a switch from pig farming to other livestock farming activities.

Pigmeat vertical price transmission in Lithuania

ADF test shows that pigmeat raw prices on downstream and upstream levels are not stationary, because the absolute value of ADF test statistic is lower than critical values (Table 2). However, pigmeat prices at both levels of the supply chain become stationary in first difference and it could be concluded that pigmeat prices are integrated of order one.

Table 2

Results of unit root test for pigmeat prices

H_0 : has a unit root		lproducer		D(lproducer)		lretailer		D(lretailer)	
	Level	<i>t</i> -stat	Prob.	<i>t</i> -stat	Prob.	<i>t</i> -stat	Prob.	<i>t</i> -stat	Prob.
ADF test statistic		-2.166	0.219	-16.68	0.000	-1.239	0.659	-20.199	0.000
Test critical values:	1%	-3.446		-3.446		-3.446		-3.446	
	5%	-2.868		-2.868		-2.868		-2.868	
	10%	-2.570		-2.570		-2.570		-2.570	
Lag Length: 1 (SIC, maxlag=17)				Lag Length: 0 (SIC, maxlag=17)					

Source: own calculations.

According to the previous studies, the similar price behaviour is documented in studies on price series in Switzerland [1], Czech Republic [25], China [10], the United Kingdom [23]. Nevertheless, some studies evidence that data stationarity issues depend on supply chain level and pigmeat product, for example, case studies of Polish [18], Slovenian [5], and Czech [7] supply chains. It is not often the case that price series confirm an assumption of data stationarity, for instance the USA case study [21].

The Johansen co-integration test does not indicate the co-integrating equation and states that in the long run pigmeat prices do not move together. The results show that market faces efficiency problems, however, in some cases the explanation of such problems could be structural breaks that allow to find co-movements between breakpoints.

It should be noted that some researchers identify structural breaks and split price series analysis into sub-periods [5, 10] or integrate structural breaks into research [4, 23]. The majority of the cases justifies the presence of such breaks in price series by crises [10, 19], animal diseases [1, 5, 23], governmental interventions [5], and other factors.

Thus, Bai-Perron test is run to investigate pigmeat prices for the presence of structural breaks. Bai-Perron test applies the break specification method ‘ $L+1$ vs L sequentially determined breaks’ and identifies five breaks for the investigated period: 1) 4/01/2011, 2) 7/27/2012, 3) 10/04/2013, 4) 2/20/2015, 5) 10/21/2016. During the period 2010–2017, the Lithuanian pigmeat supply chain faced shocks of various origins, i.e. the change of the business environment, entrance to the Eurozone, price hikes, swine fever outbreaks, and the change of foreign trade partners as well as main trading commodities. The aforementioned factors influenced the price development and the efficiency of the Lithuanian pork market and contributed to the rise of the identified structural breaks.

Further, the Johansen co-integration tests show that the most significant structural break was in 2013 and the inclusion of this dummy into the estimation process allows to receive meaningful results.

The Johansen test allows rejecting hypothesis that pigmeat prices on both levels are not co-integrated in the long-term perspective (Table 3). However, the hypothesis of one or two co-integrated equations cannot be rejected. Thus, the conclusion could be drawn that prices along the pigmeat supply chain are co-integrated.

Summarising the previous findings, it could be concluded that studies apply specific tests for the co-integration or the co-integration becomes an initial step of tests for symmetric price behaviour. The absence of the co-integration in Swiss pork sector is found by [1] applying the Engle-Granger test, however, tests for asymmetry finds the evidence of the co-integration. One co-integrated equation is found in the United Kingdom [23], or couple co-integrating vectors for the investigated period in China [10], while in case of Slovenia the split of time series into sub-periods allowed to find only one co-integrating equation instead of two [5]. Thereby, the situation on the Lithuanian market is not unique, and previous research shows quite different situation for the countries.

Table 3

Results of the Johansen co-integration test with linear deterministic trend and break in 2013 for pigmeat prices

<i>H₀</i> :	Eigenvalue	Statistic	Critical Value (0.05)	Prob.
Trace test				
No CEs*	0.059	35.950	29.797	0.009
At most 1 CE	0.022	11.445	15.495	0.186
At most 2 CEs	0.006	2.545	3.841	0.111
Maximum Eigenvalue test				
No CEs*	0.059	24.506	21.132	0.016
At most 1 CE	0.022	8.900	14.265	0.295
At most 2 CEs	0.006	2.545	3.841	0.111

* rejects the null hypothesis at the 0.05 level. Lags interval (in first differences): 1 to 4.
Source: own calculations.

The next step explores if prices on different levels help to explain price behaviour on the opposite supply chain level in the short run. Table 4 introduces results of the Granger causality test for 2 lags. According to estimated values, the Lithuanian pigmeat market demonstrates features of one-way causality that runs from retailer to producer.

Table 4

Results of the Granger causality test for pigmeat prices

<i>H₀</i> :	F-Statistic	Prob.
'Lproducer' does not Granger Cause 'lretailer'	0.179	0.67
'Lretailer' does not Granger Cause 'lproducer'	3.673	0.05

Source: own calculations.

It should be noted that the previous research also found evidences of one-way causality, however, the direction often runs from farm to retail level [4, 13, 21] and corresponds to the price determination theory arguing that the causality should run from upstream to downstream sectors.

The VECM is assisting in describing the Lithuanian pigmeat market. The estimated VECM includes a structural break in 2013 as an addition parameter (Table 5). The estimated error correction term (ECT) shows that after shocks pigmeat prices return to the described equilibrium with a speed 3.6% for the analysed period.

Table 5

Estimation of VECM for pigmeat prices with break in 2013

Co-integrating equation for Lithuanian case	
lretailer(-1)	1.000
lproducer(-1)	-0.222 (0.099) [-2.242]
D2013(-1)	0.125 (0.021) [6.041]
C	-1.331
Error Correction:	D(lretailer)
ECT	-0.0357 (0.0084) [-4.279]

Source: Own calculations.

The main results of TAR model with constant and structural break for 2 lags are provided in Table 6. The selected threshold value is zero. The comparison of F -joint (6.45) with the critical value 5.81 allows rejecting the H_0 of ‘no co-integration’ and accepting the alternative that the series are co-integrated. Moreover, the H_0 of the symmetric price behaviour is not rejected, because F -equal (0.53) is lower than the critical value (2.88). This means that increases and decreases of the prices are transmitted from the retailer to the producer – in the long run – with the same intensity.

Table 6

Results of TAR with break in 2013 for pigmeat prices

Variable	Coefficient	Std. Error
Above Threshold	-0.040	0.0180
Below Threshold	-0.060	0.021
Differenced Residuals ($t-1$)	0.040	0.050
Differenced Residuals ($t-2$)	0.039	0.050
F -equal:	0.537	(2.882)*
T -max value:	-2.199	(-2.094)*
F -joint (Phi):	6.445	(5.811)*

Source: own calculations.

It is true to note that different tests for presence of asymmetry is one of the most often investigated issues. Academic studies show that different countries demonstrate both symmetric [3, 15] and asymmetric [1, 8, 13, 18, 25] price behaviour or combine both types in a longer period [10] or in a long- and short-term perspectives [4], as well as on different levels of supply chain [21].

Conclusion

Over the period from 2010 to 2018, significant structural changes in the Lithuanian pig farming took place. The overall population of pigs had decreased, while the dominant role of agricultural companies and enterprises in pig production was growing. Small farms were disappearing from Lithuanian pig farming, because farmers exited pig farming or switched to other farming types, while the role of medium-sized pig farms in the country was not important in terms of production.

Other important factors, contributing to structural changes and demotivating to run medium-sized farms, were the growth of farming costs, animal diseases, and disturbances of foreign trade. The current negative trends could be changed introducing specific support measures targeting at fostering a specific pig farming structure.

The empirical research on price transmission along the Lithuanian pigmeat supply chain demonstrates that the pigmeat market experienced several critical shocks over the investigated period. Those shocks had different nature (governmental intervention, animal health issues, global price hikes) and made an impact on price behaviour and co-movements. Tests suggest that in the short-run the leading price setter is retailer. This result challenge for the investigation of farmers welfare issues.

In the long-term perspective, prices are transmitted from the retailer to the producer with the same intensity and do not demonstrate asymmetric behaviour. Asymmetry was not found within the investigated market for the period studied and this fact suggests that policies in place were working effectively during the analysed period. Hence, it should be noted that introduction of additional details (for example, more stakeholders along the supply chain or investigation of specific periods) could enrich the knowledge about the functioning of the Lithuanian market.

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Анотації

Харчові технології

Технологія і чинники впливу на якість йогурту грецького. Огляд

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Вступ. В аналітичному дослідженні розглянуто основні способи виробництва йогурту грецького, зокрема умови формування фізико-хімічних характеристик кислотних молочно-білкових згустків і показників якості готового продукту.

Матеріали і методи. Огляд ґрунтується на вагомих наукових публікаціях за обраним напрямом дослідження.

Результати і обговорення. На основі аналізу різних способів виробництва (традиційного, сепараторного, із застосуванням мембранних технологій, прямої рекомбінації) встановлено, що йогурт, виготовлений з їх застосуванням, суттєво відрізняється за показниками якості. Два найважливіші параметри – текстурна характеристика та вміст зв'язаної води, визначають якість йогурту та його органолептичне сприйняття споживачами. Численні технологічні параметри, як-от високотемпературне оброблення, підвищений вміст сироваткових білків стосовно казеїну, висока температура сквашування, склад і надмірна кількість заквашувального препарату, обумовлюють вади консистенції питного йогурту – зернистість (відчуття часточок згустку) або поверхнева шорсткість (нерівність білкової матриці згустка). Саме спосіб прямої рекомбінації має переваги серед інших, оскільки він більш екологічний і підвищує харчову цінність готового продукту. Водночас реологічні властивості рекомбінованого йогурту дещо гірші за характеристики класичного йогурту або йогурту, виготовленого УФ-методами, внаслідок утворення слабшого білкового згустка. Тому для виробництва йогурту з підвищеним вмістом сухих речовин типу грецького рекомендовано використовувати різні сухі молочні компоненти, зокрема з підвищеним вмістом сироваткових білків.

Висновки. Перспектива подальших досліджень має бути спрямована на вдосконалення технології йогурту грецького шляхом прямої рекомбінації.

Ключові слова: *йогурт, грецький, казеїн, гель, реологія.*

**Біологічно активні сполуки з натуральних харчових джерел
для ферментованих молочних продуктів**

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Вступ. Це дослідження спрямоване на виявлення і кількісне визначення цінних сполук з натуральних продуктів, таких як мед, волоський горіх і обліпіха з метою подальшого виробництва нових ферментованих продуктів з коров'ячого молока.

Матеріали і методи. Як матеріал дослідження використовувався бджолиний мед поліфлора району Дрегешань (Румунія), а для виявлення і кількісного визначення летких компонентів використовувалася система ГХ-МС. Якісний і кількісний аналізи поліфенолів проводився з використанням системи ВЕРХ Agilent 1200, що складається з фотодіодного детектора і електророзпилювального іонізаційного мас-детектора.

Результати і обговорення. Хроматографічний аналіз бджолиного меду поліфлора дав змогу визначити вміст фенольних кислот (79,284 мг/100 г зразка), ізопреноїдів (127,449 мг/100 г зразка) і флавоноїдів (168,475 мг/100 г зразка). У меді виявлено широкий спектр ароматичних сполук: терпенових з'єднань, вищих спиртів, альдегідів і кетонів, складних ефірів. Вміст альдегідів склав 7,889 мг/100 г, кетонів – 2,333 мг/100 г, вищих спиртів – 3,212 мг/100 г, ефірів – 8,993 мг/100 г. За отриманими хроматограмами, вміст поліфенолів у ядрі волоського горіха склав 786,553 мг ЕГК/100 г, а в ягодах обліпіхи – 343,229 мг ЕГК/100 г. Також найвища концентрація значущих токоферолів виявлена у формі α -токоферолу в кількості 33,245 мг/100 г і β -токоферолу 12,723 мг/100 г масла. Більш низькі значення 4,553 мг/100 г і 1,286 мг/100 г визначені у γ -токоферолі та δ -токоферолі відповідно.

Висновки. Виявлений склад трьох видів сировини (мед поліфлора, ядро волоського горіха, ягоди обліпіхи) і його кількісна оцінка сприятиме отриманню гармонійного ароматичного профілю з певними смаковими властивостями у ферментованих молочних продуктах.

Ключові слова: молоко, мед, волоський горіх, обліпіха.

Дослідження комплексів фери-йонів з біолігандами пробіотичного походження

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Вступ. Досліджено особливості отримання стабільних, легкозасвоюваних і безпечних комплексів фери-йонів з біолігандами пробіотичного походження та їхні характеристики.

Матеріали і методи. Як біоліганди використовували продукти метаболізму та переробки біомаси *Lactobacillus delbrueckii subsp. Bulgaricus B-3964*, зокрема молочну кислоту, амінокислоти, низькомолекулярні пептиди та мурапептиди. Деструкцію пептидогліканів біомаси проводили шляхом почергової обробки ультразвуком і папаїном. Хід комплексоутворення контролювали за допомогою методу турбідиметрії.

Результати і обговорення. У результаті деструкції пептидогліканів отримали суміш амінокислот, низькомолекулярних пептидів і муропептидів, концентрація яких складала, відповідно, 10,24 мг/см³, 6,45 мг/см³ та 2,25 мг/см³. Для утворення комплексів Fe³⁺ використовували три полідентантні системи: продукти деструкції пептидогліканів; молочну кислоту; суміш продуктів деструкції та молочної кислоти. Встановлено, що досліджувані системи біолігандів зв'язують йони Fe³⁺ у кількості 32, 40 та 46 моль/дм³·10⁻² відповідно. У формуванні комплексу заліза (III) та біолігандів пробіотичного походження беруть участь електростатичні та координаційні взаємодії. Вивчено поведінку комплексів при різних значеннях рН середовища та температур. Найбільш стабільним є комплекс, утворений за участі системи біолігандів, що містить продукти деструкції пептидогліканів біомаси та молочну кислоту. Така система біолігандів забезпечує формування хелатних комплексів фери-йонів, стабільних у діапазоні рН 1–10 од. Доведено, що отриманий комплекс є перспективним інгредієнтом дієтичних добавок та оздоровчих продуктів харчування, технологія яких передбачає високотемпературну обробку, оскільки комплекс стабільний в інтервалі температур 20–122°C. При досягненні температури 122–125°C втрата маси становить для комплексу 3%, а для механічної суміші – 14%. У діапазоні температур 122–178 °C спостерігається ендотермічна реакція при термічній обробці комплексу, причому термічних ефектів при обробці МС не спостерігається. Втрата маси комплексу у цьому діапазоні температур становить 22%, механічної суміші – 16%. Ендотермічний пік на кривій диференційного термічного аналізу комплексу може свідчити про наявність у його структурі хелатних зв'язків, при руйнуванні яких відбуваються зміни ентальпії процесу.

Висновки. Результати досліджень свідчать про ефективність застосування полідентантних змішанолігандних систем пробіотичного походження для комплексоутворення з фери-йонами.

Ключові слова: *фери-йон, хелат, біоліганд, пептидоглікан, муропептид.*

Застосування рослинних натуральних добавок для поліпшення біоактивних властивостей органічних крафтових сирів

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Вступ. Мета дослідження полягала в тому, щоб збільшити вміст біоактивних речовин і покращити антиоксидантні властивості органічних крафтових сирів шляхом застосування вибраних органічних рослинних добавок у виробництво сирів.

Матеріали і методи. У вироблених органічних сирах, що містять відібрані органічні рослинні добавки, отриманий антиоксидант визначали методом FRAP. Ряд спектрофотометричних вимірювань проводили для визначення вмісту поліфенольних речовин та інших відібраних біоактивних компонентів, включаючи каротиноїди, лікопен, хлорофіл, антоціани, флавоноїди та беталаїни.

Результати і обговорення. Отримані результати показали, що антиоксидантна активність досліджуваних продуктів становила від 1,48±0,11 μmol Trolox/g (варіант контрольного сиру після дозрівання) до 4,1±0,3 μmol Trolox/g (сир після дозрівання, що містить томати). У свою чергу, вміст загальних фенольних сполук у сирах відразу після дозрівання становив від 141,51±2,38 mg GAE /100 g (у контрольному варіанті) до

289,9±9,9 mg GAE /100 g (у сири з додаванням сушених томатів). Кінцеві продукти з додаванням броколі та томатів після зберігання в холодильнику мали найвищі антиоксидантні властивості порівняно з іншими тестованими продуктами. Крім того, після процесу дозрівання загальний вміст каротиноїдів в органічних сирах, виготовлених з органічними добавками моркви й томатів, відповідає результатам аналізу антиоксидантної активності. Більш того, сири, що містять сушені томатні добавки, характеризувалися найвищою кислотністю, пов'язаною з вмістом молочної кислоти після дозрівання ($2,33 \pm 0,02$ g/100 g сиру) та після зберігання в холодильнику ($2,51 \pm 0,03$ g/100 g сиру); водночас ці продукти демонстрували найвищі значення загального фенольного вмісту. Охолоджене зберігання сирів знижувало значення твердості та адгезії лише для сирів, що виробляються з додаванням цибулі, тоді як в інших варіантах цей показник збільшувався після закінчення зберігання в холодильнику. Усі рослинні добавки сприяли збільшенню червоного кольору в сирах.

Висновки. Досліджувані добавки підвищили рівень біоактивних компонентів і антиоксидантні властивості сирів після дозрівання, а також позитивно вплинули на їх колір.

Ключові слова: *сир, органічні, молочна кислота, антиоксидант, біоактивний.*

Вплив параметрів переробки на функціонально-технологічні властивості ягідних коагулянтів

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Вступ. Метою досліджень є визначення впливу параметрів гідротермічної обробки і протирання ягідної сировини на вміст розчинних пектинів в ягідних пюре. Звернено увагу на відповідність отриманих пюре вимогам до плодово-ягідних коагулянтів, які можна використовувати для отримання молочно-білкових копреципітатів зі сколотин.

Матеріали і методи. Досліджувалася ягідна сировина із журавлини та калини. Обробку ягід гострою парою здійснювали за допомогою електричної пароконвекційної печі інжекторного типу за температури 105–107 °C протягом 5–30 с. Для протирання ягід використано дробарно-фінішерну установку з діаметрами отворів сит 0,4–1,2 мм.

Результати і обговорення. Вміст пектинових речовин в обох ягідних пюре збільшується прямо пропорційно збільшенню тривалості гідротермічної обробки з 5 с до 60 с. Пюре калини характеризується повільнішим збільшенням кількості пектинових речовин порівняно з журавлинним пюре, що пояснюється різницею в структурі молекул протопектину.

Втрати вітаміну С для обох ягідних пюре відносно низькі в перші 15 с обробки, тоді як з підвищенням температури вони швидко зростають через прискорення розривів гідроксильних зв'язків в структурі аскорбінової кислоти.

Рациональною визнано обробку ягідної сировини гострою парою при температурі 105–107 °C протягом 15–30 секунд для ягід журавлини та 5–15 секунд для ягід калини,

що відповідає збільшенню кількості пектинових речовин на 19–22% та 11–19% у журавлинному та калиновому пюре відповідно.

Найбільший вихід пюре журавлини досягався при використанні сита з діаметром отворів 0,8–1,2 мм, тоді як для отримання пюре калини раціональним є використання сита з діаметром отворів 0,6–0,8 мм. Збільшення виходу пюре журавлини, якщо порівняти з пюре калини, можна пояснити більш вираженою сприйнятливістю грубого харчового волокна калини до механічних навантажень порівняно з більш тонкою журавлинною клітковиною.

Хімічний склад отриманих ягідних коагулянтів порівняно з вихідною сировиною характеризується збільшенням вмісту пектинових речовин на 20% та 21% і зменшенням вмісту вітаміну С на 30% та 29% для пюре журавлини та калини відповідно. Отримані коагулянти характеризуються вмістом органічних кислот у кількості 2,0% та 1,7% для пюре журавлини та пюре калини відповідно.

Висновки. Встановлені раціональні режими обробки ягід журавлини та калини гострим паром та їх подрібнення до стану пюре. Значне збільшення вмісту розчинних пектинів і наявність органічних кислот дають змогу використовувати отримані ягідні пюре як коагулянти в технології молочно-білкових копреципітатів зі сколотин.

Ключові слова: *молоко, білок, концентрат, ягода, пюре, пектин.*

Якісні характеристики та антиоксидантна активність йогурту з козячого молока і фруктів

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Вступ. Метою дослідження є оцінка фізико-хімічних, мікробіологічних, сенсорних характеристик та антиоксидантного потенціалу йогурту з козячого молока і фруктів.

Матеріали і методи. Йогурт був виготовлений з козячого молока з додаванням бланшованих фруктів (10%) з аронії (чорноплідної горобини L, сорт Nero), персиків (*Prunus persica*, сорт Молдова), малини (*Rubus idaeus*, сорт Cusma de Guguța), полуниці (*Fragaria xanassa*, сорт Selva), яблука (*Malus domestica*, сорт Голден). Показники якості та антиоксидантного потенціалу визначали за стандартними методиками.

Результати і обговорення. Доданий тип плодів дуже вплинув на значення титрованої кислотності та рН. Персиковий йогурт мав рН 4,68±0,019. Більш висока кислотність була отримана для малинового йогурту, 103±0,076 Т. Кількість сухих речовин складала 20,40±0,45% для полуничного йогурту. Вміст сухих речовин обернено пропорційний значенню активності води, максимальні значення склали 0,904±0,038 для яблучного, персикового, малинового йогурту.

Мінімальні значення в'язкості отримані для йогурту з аронії – 5450±4,85 Па·с, а максимальні – для полуничного йогурту 8960±4,45 Па·с. Значення загальної кількості мікроорганізмів в йогурті є задовільними, найвищий результат був для йогурту з персиків – 1,8 log КУО/мл. Максимальна кількість молочної кислоти в яблучному йогурті становить 7,16±0,40 log₁₀ КУО/мл. Дріжджів і цвілі виявлено не було. Аронієвий йогурт має найвищий загальний вміст поліфенолів (187,15 мг ЕГК 100 г⁻¹), антоціанів (56,45/100 г) та антиоксидантної активності (3,9%). Максимальний вміст каротиноїдів (0,452 мг/100 г) отримано для персикового йогурту, аскорбінової кислоти

(25,77 мг/100 г) – для полуничного йогурту. Органолептичні властивості зразків йогурту показують, що полуничний йогурт володіє кращими характеристиками, набравши 19,25 балів з 20.

Висновки. Додавання аронії, полуниці, малини та персика позитивно впливає на біологічну цінність і показники якості йогурту з козячого молока і фруктів.

Ключові слова: *йогурт, козяче молоко, аронія, полуниця, персик, яблуко.*

Вплив аерації на колір, фізико-хімічні та текстурні характеристики кондитерських пін

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Вступ. Метою дослідження є визначення впливу аераційного процесу і виду сировини на якісні характеристики пін, що використовуються в кондитерських виробках.

Матеріали і методи. Застосовано дві різні методики та використано сировину різного походження (вершки – S2, S4 і рослинні вершки – S1, S3), яка була основою для отримання зразків піни та яку проаналізовано за хімічним складом, пористістю, показниками кольору і властивістю текстури. Пористість зразків вимірювали за допомогою програмного забезпечення ImageJ (NIH Image), а первинні та вторинні параметри текстури визначені з використанням текстурного аналізатора Mark 10-ESM 301.

Результати і обговорення. Найвища жирність кондитерських пін спостерігалася для зразків на основі вершків (20,35%), тоді як зразки на основі рослинних вершків мали нижче значення жирності (15%). Вміст вологи у зразках кондитерських виробів коливався від 43,59 до 47,68%, різниця вмісту вологи за класифікацією зразків (S1-S4) була значною ($p < 0,01$). Найвищі концентрації розчинних речовин – 26,25 та 26,40 представлені зразками на основі рослинних вершків; зразки піни S1 і S3 належать до однієї статистичної групи. Водна активність зразків піни становила від 0,804 до 0,824, найвищі значення виявляли зразки на основі вершків S4. Пористість зразків коливалася між 15,27 та 7,04%. Діаметр повітряних бульбашок коливався від 4,36 мм до кількох мікрометрів. Пористість зразків негативно впливає як на первинні ($r = -0,946^*$), так і на вторинні ($r = -0,967^*$) властивості текстури. Яскравість зразків на основі рослинних вершків показала найвищі значення (92,77 та 93,64); отже ці зразки біліші та яскравіші, ніж піни на основі вершків (89,74 та 86,89); при цьому показник забарвлення b^* , що характеризує жовто-синю вісь, показав високі значення для зразків на основі вершків (28,71 та 34,09). Результати аналізу текстурного профілю показали, що найбільшу твердість демонструє зразок S3 – 3,48 Н, інші зразки піни мають близькі значення в межах від 1,52 до 1,76 Н. Зразки з низькою пористістю мали високі показники твердості, високий рівень ламкості (0,24 Н), що характеризує більш компактний і крихкий продукт з високою в'язкістю (0,64), липкістю (1,53), жувальністю (1,31), міцність внутрішніх зв'язків (39,82).

Висновки. Крім впливу на твердість та інші параметри текстури, процес аерації змінює зовнішній вигляд продукту, колір і смак.

Ключові слова: *піна, кондитерський виріб, текстура, колір.*

Характер зв'язків води у гідратованих молочно-білкових системах

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Вступ. Досліджено характер зв'язку та характеристики процесу релаксації кластерів і водних доменів у молочно-білкових системах з метою прогнозування їх функціональних та технологічних властивостей.

Матеріали і методи. Особливості релаксації кластерів і доменів води у колоїдних розчинах молочних білків вивчали методом термостимульованої деполаризації (ТСД). Електрофоретичний аналіз фракційного складу білків молока здійснювали за модифікованим методом Laemmli.

Результати і обговорення. Вивчено особливості розподілу води в об'ємних матрицях білків у молоці та в гідратованих молочно-білкових концентратах методом ТСД. На основі порівняльного аналізу ТСД-спектрів релаксації дипольних структур води в низькотемпературній і у високотемпературній областях у зразках свіжого знежиреного молока, відновленого сухого знежиреного молока та розчину казеїнату натрію доведено суттєву різницю характеру кластероутворення води в цих системах. Встановлено, що молочно-білкові концентрати за зниженої енергії активації деполаризації води у гідратованому стані формують просторові сітки з меншими розмірами комірок, ніж білки молока натурального. Виявлений ефект пояснюється тим, що під впливом теплового оброблення та сушіння відбуваються суттєві конформаційні зміни білкових сполук, спричинені денатурацією більшості сироваткових білків, зокрема імуноглобулінів, сироваткового альбуміну, β -лактоглобуліну і α -лактальбуміну. Зроблено висновок про доцільність застосування у складі харчових продуктів молочно-білкових концентратів, білкові матриці яких мають просторові обмеження й утримують кластери води, менші за такі для молока натурального. Результати наукового дослідження мають практичне значення, оскільки дають змогу цілеспрямовано формувати і стабілізувати просторову структуру білоквмісних харчових систем.

Висновки. Встановлено особливості характеру зв'язку води у гідратованих молочно-білкових системах різного ступеня теплового оброблення.

Ключові слова: *молоко, білок, концентрат, вода, зв'язок.*

Вплив ультратонкого помелу і високого тиску на функціональні властивості побічних продуктів сої

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Вступ. Вміст розчинних харчових волокон (SDF), а також смак та якість побічних продуктів переробки сої можна покращити за допомогою комбінації технології ультратонкого помелу та високого тиску.

Матеріали і методи. Побічні продукти переробки сої; установка надтонкого помелу KCW-701S; установка високого тиску FPG5620YHL; значення високого тиску: 0, 50, 100, 150, 200 і 300 МПа; співвідношення матеріал-рідина: 1:3, 1:5, 1:7, 1:9 та 1:11; тривалість обробки: 5, 10, 15, 20 та 25 хв.

Результати і обговорення. Ультратонкий помел має значний вплив на SDF побічних продуктів переробки сої. Із зменшенням частоти подрібнення вміст SDF поступово збільшувався. Коли частота становила 30 Гц, їхній вміст досягав найвищого значення – 27,11%, і зростав на 8,1% порівняно з контролем. Коли частота була менша 30 Гц, вміст SDF різко зменшувався до 24,12%. Тому частота ультратонкого помелу була найкращою на 30 Гц, а вміст SDF у побічних продуктах сої – найвищий.

При тиску 150 МПа, співвідношенні матеріал-рідина 1:7 і тривалості обробки 10 хв вміст SDF досягав максимуму 28,76% у побічних продуктах сої, що більше на 12,76% порівняно з контрольною групою.

Розчинність у воді, набухання, вологоутримуюча та оліеутримуюча здатність у побічних продуктах сої були нижчими порівняно з контрольною групою. При тиску 150 МПа розчинність у воді була 11,24%. Але цей показник становив 10,39% при тривалості обробки 15 хв. При 150 МПа та 10 хв набухання становило 8,2 мл/г, але при 20 хв показник набухання збільшився до 8,8 мл/г. Подібні тенденції мають показники вологоутримуючої та оліеутримуючої здатності. При 100 МПа показники досягали максимального значення, при 150 МПа значення цих показників були найменшими, але коли час обробки перевищував 10 хв показники вологоутримуючої та оліеутримуючої здатність незначно збільшилися.

Висновки. Ультратонкий помел при 30 Гц забезпечував вихід SDF на рівні 27,11%. Обробка SDF високим тиском 150 МПа при співвідношенні матеріал-рідина 1:7 та тривалості обробки 10 хв забезпечила підвищення виходу до 28,76%.

Ключові слова: помел, тиск, соя, відходи, волокна, функціональність.

Вплив помелу зернових культур на вміст фітинової кислоти та засвоюваність мінеральних речовин і білків

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Вступ. Метою дослідження є визначення впливу процесу подрібнення, який застосовувався для зернових культур при виробництві борошна різними способами, вмісту фітинової кислоти, мікроелементів та засвоюваності *in-vitro*.

Матеріали і методи. Під час подрібнення зернових (пшениця, ячмінь, жито, овес, рис) визначалися вміст білка, золи, фітинової кислоти, мінеральних речовин та показники засвоюваності білка і мінералів. Вміст мінералів визначали за допомогою методу ICP-OES, вміст білка – методом Кельдаля, засвоюваність мінеральних речовин та вміст фітинової кислоти – методами *in-vitro*.

Результати і обговорення. Процес лущення збільшив коефіцієнт засвоюваності білка до 65% і значно знизив вміст фітинової кислоти ($p < 0,05$) в зернових крупинках, крім жита. Засвоюваність мінералів підвищилася для всіх проб зерна, але вона була оцінена статистично незначною ($p > 0,05$). Рисове борошно було зразком, який мав найнижчий вміст фітинової кислоти (921,87 мг/100 г) і найвищий показник засвоюваності мінеральних речовин (58,35%). Хоча вміст загальної золи та деяких

мінералів (Na, Ca, K, Mg, Zn, Fe, Ba та P) зменшився у борошні, особливо у пшениці та рисі, норма деяких мінералів (Na, Mg, Zn, Fe та Al) збільшилися через їх розподіл і локалізацію в зернових шарах, особливо в житі та вівсі.

Рівень мінеральних речовин змінювався залежно від вмісту висівок у зернах, а процес подрібнення був більш ефективним щодо вмісту фітинової кислоти та засвоюваності білка, ніж засвоюваності мінералів. Вміст фітинової кислоти був локалізований на зовнішньому шарі зерна і зменшувався у ядрі.

Висновки. Хоча вміст фітинової кислоти, яка впливає на засвоюваність зернових, зменшувався в процесі подрібнення, для покращення харчових якостей зернових можна запропонувати поєднання таких різних видів обробки, як замочування і ферментація.

Ключові слова: зернові, луцення, мінерал, фітинова кислота, подрібнення, біодоступність.

Вплив комплексного поліпшувача направленої дії на споживчі властивості булочних виробів

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Вступ. З метою покращення споживчих властивостей булочних виробів, вироблених за прискорених технологій, проведено дослідження використання комплексного хлібопекарського поліпшувача направленої дії.

Матеріали і методи. Досліджувалися споживчі властивості булочного виробу – батону «Свіжість» (з борошна пшеничного вищого сорту, дріжджів пресованих хлібопекарських, солі кухонної харчової, маргарину столового, цукру білого). Технологічний процес проводився з тривалістю відлежування тіста – 20 хв та використанням поліпшувача «Свіжість +», який дозували в кількості 2,0% до маси борошна. Якість готових виробів оцінювали за фізико-хімічними та органолептичними показниками.

Результати та обговорення. Використання поліпшувача у кількості 2,0% до маси борошна сприяє збільшенню питомого об'єму виробів на 11%, покращення формостійкості, пористості та зменшення втричі тривалості бродіння до 20 хв. Це зумовлено використанням в складі поліпшувача ферменту амілолітичної дії, який сприяє збільшенню газоутворювальної та цукроутворювальної здатності. Вироби з доданням поліпшувача краще зберігають свіжість, що підтверджено збільшенням загальною деформації м'якушки на 26,0%, меншим підскоринковим шаром та меншою кількістю прошарків повітря у м'якушці виробів протягом 72 год без пакування. Спостерігається більше накопичення декстринів та бісульфітзв'язуючих речовини у виробках у разі використання поліпшувач, що вказує на гальмування процесів черствіння виробів та покращання споживчих властивостей. Це пояснюється тим, що вироби вносяться додатково вологоутримувальні добавки, а саме мальтодекстрин та клейстеризований крохмаль, які під час зберігання виробів утримують осмотично та адсорбційно зв'язану вологу. Також мальтодекстрин є водорозчинним гідроколоїдом, який підвищує ступінь утримання вологи, і утворює тривимірну сітку, яка гальмує взаємодію клейковини та крохмалю, в результаті чого сповільнюється ретроградація крохмалю.

Висновки. Використання поліпшувача уповільнює ретроградацію крохмалю внаслідок утворення його складовими з борошном суцільної маси коагульованих під час випікання білків, в середині яких вкраплені набухлі, частово клейстеризовані зерна крохмалю і збільшенням кількості декстринів.

Ключові слова: поліпшувач, булочні вироби, черствіння, аромат, декстрини, свіжість.

Вплив екструдованого кукурудзяного борошна на стабілізацію бісквітного тіста для виробництва безглютенового бісквіту

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Вступ. Визначено вплив екструдованого кукурудзяного борошна на стабілізацію бісквітного тіста, показники вологоутримувальної здатності борошна, стійкості піни та густини бісквітного тіста з метою створення бісквітного напівфабрикату для безглютенового харчування.

Матеріали і методи. Дослідження вологоутримувальної здатності борошняної сировини здійснювали методом центрифугування. Стійкість піни визначали як співвідношення висоти стовпа піни після витримання протягом 24 год за температури 18–20 °С до загальної висоти стовпа піни зразка. Мікроскопічні дослідження структури продукту проводили за допомогою цифрового біокулярного мікроскопа серії «MicroMed».

Результати і обговорення. Оптимальне значення густини бісквітного тіста 0,444–0,446 кг/м³. Підвищення концентрації борошна кукурудзяного екструдованого вище 20 мас.% призводить до значної густини тіста, що є небажаним у виробництві бісквітних напівфабрикатів, оскільки робить їх густішими і менше пористими. Проте наявність густини бісквітного тіста, за відсутності пшеничного борошна вищого сорту, вказує на можливість створення бісквітного напівфабрикату безглютенового виключно з використанням борошна кукурудзяного. У зразку бісквітного тіста з використанням борошна кукурудзяного екструдованого 100 мас.% бульбашки піни практично однакових розмірів, крім цього, помітно, що між ними утворилися канали, які сприяють вирівнюванню тиску повітря всередині пінної системи бісквітного тіста, що сприяє стабілізації пінної системи. Результати оптимізації бісквітного напівфабрикату з використанням борошна кукурудзяного екструдованого мають такі інтервали оптимізаційних параметрів: 100 мас.% – заміна пшеничного борошна на борошно кукурудзяне екструдоване із кількісним співвідношенням рецептурних компонентів яйця: цукор: борошно – 2,1:1:1,02.

Висновки. Борошно кукурудзяне екструдоване сприяє стабілізації пінної системи бісквітного тіста, що дає змогу створити бісквітний напівфабрикат безглютеновий для дієтичного харчування.

Ключові слова: бісквіт, безглютеновий, борошно, кукурудза, екструдовання, стабілізація.

Біотехнологія, мікробіологія

Вплив триптофану на ауксин-синтезувальну здатність продуцента поверхнево-активних речовин *Acinetobacter calcoaceticus* IMB B-7241

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Вступ. Метою дослідження є визначення оптимальної концентрації триптофану та часу його внесення в середовище культивування продуцента поверхнево-активних речовин (ПАР) *Acinetobacter calcoaceticus* IMB B-7241 для досягнення максимального синтезу ауксинів.

Матеріали і методи. Культивування проводили у рідкому поживному мінеральному середовищі з використанням як субстрату етанолу та відходів виробництва біодизелю (технічного гліцерину). Триптофан додавали в середовище у вигляді 1% розчину в кількості 100, 200 або 300 мг/л на початку процесу або в кінці експоненційної фази росту (48 год культивування). Фітогормони виділяли шляхом триразової екстракції органічними розчинниками із супернатанту культуральної рідини після екстракції ПАР. Якісне та кількісне визначення гіберелінів проводили за допомогою високоефективної рідинної хроматографії.

Результати і обговорення. Встановлено, що незалежно від моменту внесення триптофану в середовище культивування штаму IMB B-7241 із технічним гліцирином спостерігали значне підвищення синтезу ауксинів порівняно з показниками на середовищі без цього попередника. Найвищої концентрації ауксинів досягнуто при додаванні 300 мг/л триптофану в середовище з обома субстратами. *A. calcoaceticus* IMB B-7241 синтезував 1404,73, 1295,04 та 4850,98 мкг/л ауксинів на гліцириновому середовищі за внесення 100, 200 та 300 мг/л триптофану в кінці експоненційної фази росту (без попередника концентрація ауксинів становила всього 175,4 мкг/л). Підвищений синтез ауксинів штамом IMB B-7241 корелював з активністю триптофантрансамінази: при культивуванні на технічному гліцирині без попередника вона становила 163 нмоль·хв⁻¹·мг⁻¹ білка, в той час як за наявності 300 мг/л триптофану, доданого в кінці експоненційної фази росту, підвищувалася у 3,2 раза (до 526 нмоль·хв⁻¹·мг⁻¹ білка). Найвища концентрація ауксинів при культивуванні на етанолі (2261,66 мкг/л) досягалася за внесення 300 мг/л триптофану на початку культивування.

Висновок. Результати дослідження підтверджують можливість підвищення на один-два порядки концентрації синтезованих ауксинів у разі додавання в середовище культивування *A. calcoaceticus* IMB B-7241 невисоких концентрацій попередника їх біосинтезу.

Ключові слова: попередник біосинтезу, індол-3-оцтова кислота, *Acinetobacter calcoaceticus* IMB B-7241.

Процеси і обладнання

Форми стану рівноваги мембран вакуумних кришок на основі енергетичного критерію

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Вступ. Проведені дослідження форм стану рівноваги мембран вакуумних кришок для скляної тари з метою опису зміни енергії, яка обумовлює стабільну роботу мембран у заданому режимі обробки продукції.

Матеріали і методи. Об'єктом досліджень є скляна упаковка. Предметом – гнучкі мембрани кришок. Визначення прогину і товщини мембрани здійснювалось інструментальними методами. Дослідження форм стану рівноваги й енергетичних рівнів рівноваги мембран здійснювалось шляхом математичного моделювання енергетичним та обчислювальними методами.

Результати і обговорення. Після втрати стійкості мембрана переходить в інше положення стійкої рівноваги. Результати вимірювань показали, що у стані втрати стійкості додатковий прогин центра мембрани $f=0,07$ мм, що значно менше за початковий прогин центра мембран $f_{нч}=0,25$ мм. Тобто в результаті втрати стійкості не відбувається дзеркальної деформації робочого конуса мембрани. Такі параметри прогину гарантують працездатність мембран у широкому діапазоні різниці між протитиском системи (в автоклаві) та внутрішнім тиском у тарі протягом усього процесу термообробки продукції.

Повна енергія круглої пластини представлена у вигляді суми енергії безмоментного напруженого стану, енергії вигину та роботи зовнішнього тиску. Виведено розрахункове рівняння повної енергії мембрани, отримані її енергетичні рівні у різних положеннях стійкої рівноваги.

Підставивши експериментально отримане значення f у стані втрати стійкості в розрахункове рівняння повної енергії мембрани, отримано розрахункову для розглянутого випадку, величину тиску втрати стійкості мембран кришок $P_I=0,0326 \cdot 10^6$ Па. Розрахункова величина P_I є близькою до значення тиску втрати стійкості мембран кришок $P_I=0,03$ МПа, яке наводиться виробником кришок. На відхилення розрахункової величини тиску P_I від реальної може впливати твердість жерсті, з якої виготовлена кришка з мембраною.

Отримані енергетичні рівні різних положень рівноваги мембрани при двох різних навантаженнях, які відповідають критичним тискам втрати стійкості P_1^* та розвантаження P_2^* . Графіки енергетичних рівнів обох тисків, побудовані за допомогою розрахункового рівняння, мають мінімуми потенціальної енергії. Такий вигляд кривих енергетичного критерію для критичних тисків повністю відповідає теоремі Лагранжа-Діріхле, оскільки тиску $P_{1\min}^*$ відповідає мінімальна щодо інших суміжних станів енергія.

Висновки. Отримано розрахункове рівняння повної енергії мембрани та енергетичні рівні станів її рівноваги, що відповідають критичним тискам.

Ключові слова: *стійкість, енергія, пластина, прогин, тиск, рівновага.*

Вплив геометричних і динамічних параметрів водополімерного струменя на показники процесу гідрорізання харчових продуктів

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Вступ. Проведено дослідження процесу різання харчових продуктів водополімерним струменем з метою підвищення ефективності методу гідрорізання шляхом модифікації робочої рідини.

Матеріали і методи. Досліджено процес розрізання філе курки бройлера, риби чека, яловичого і свинячого м'яса. Досліди проводилися за температур $-7\text{ }^{\circ}\text{C}$ і $-25\text{ }^{\circ}\text{C}$, зміни тиску від 50 МПа до 150 МПа, діаметра сопла $0,35\cdot 10^{-3}$, $0,6\cdot 10^{-3}$ м та швидкості переміщення водополімерного струменя відносно зразка харчового продукту 0,015, 0,025, 0,050 і 0,100 м/с. Концентрація ПЕО з молекулярними масами $6\cdot 10^6$, $4\cdot 10^6$ і $3\cdot 10^6$ варіювалася від 0 до 0,05%.

Результати і обговорення. Глибина розрізу в замороженому харчовому продукті досить різко зростає зі збільшенням концентрації та молекулярної маси ПЕО і досягає максимуму за деяких оптимальних концентрацій ($C_{\text{опт}}$). Для ПЕО з молекулярною масою $3\cdot 10^6$ оптимальна концентрація дорівнює 0,0150,020%, а для молекулярних мас $4\cdot 10^6$ і $6\cdot 10^6$ – 0,007-0,010% і 0,0015-0,0020% відповідно. Збільшення діаметра отвору сопла за незмінного тиску водного розчину ПЕО призводить до збільшення глибини розрізу в харчовому продукті. Відмічений характер впливу діаметра вихідного отвору сопла гідрорізальної струменеформуєчої голівки на глибину розрізу пов'язується з тим, що використання сопел з відносно великим діаметром вихідного отвору за незмінних швидкості переміщення водополімерного струменя відносно зразка харчового продукту і тиску водного розчину ПЕО перед соплом повинно призводити до збільшення кількості енергії, яка припадає на одиницю поверхні харчового продукту, що розрізається. Щоб досягти однакової глибини різання заморожених харчових продуктів (в діапазоні від $-7\text{ }^{\circ}\text{C}$ до $-25\text{ }^{\circ}\text{C}$) достатньо тиску водополімерного струменя лише 45-65% від водяного струменя, і навпаки, за однакового початкового тиску спостерігається збільшення глибини і швидкості різання водополімерним струменем в 1,5–2,5 рази, що свідчить про особливий механізм його взаємодії з харчовим продуктом. Отримано залежність безрозмірної глибини розрізу водополімерним струменем у заморожених харчових продуктах від безрозмірної відстані до зрізу сопла. Використання високошвидкісного струменя водного розчину ПЕО для розрізання заморожених харчових продуктів суттєво підвищує ефективність процесу різання та якість поверхні розрізу.

Висновки. Для суттєвого підвищення ефективності гідрорізання харчових продуктів найдоцільніше використовувати як робочу рідину струмись водного розчину ПЕО.

Ключові слова: розрізання, струмись, поліетиленоксид, сопло, тиск.

Обмеження динамічних силових параметрів в перехідних процесах

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Вступ. Стаття присвячена аналізу перспектив комплексного використання накопиченої потенціальної енергії на прикладі механізму для піднімання, горизонтального переміщення і вкладання вантажів, наприклад, у транспортну тару з обмеженням динамічних навантажень.

Матеріали і методи. Дослідження теоретичне, виконане на основі законів і принципів механіки зі створенням та аналізом відповідних математичних формалізацій, які стосуються енергетичних і механічних трансформацій з кінцевим результатом поєднання підвищених кінематичних параметрів та обмежених силових дій.

Результати і обговорення. Синтез технологічних систем має за мету подолання протиріч між кінематичними параметрами високопродуктивної машини, енерговитратами і динамічними навантаженнями.

На основі аналізу особливостей ударних навантажень у двомасових системах визначена можливість використання жорсткості пружної підвіски як варіативного фактора для досягнення заданих співвідношень між статичними і динамічними навантаженнями за перебігу їх у гравітаційному полі. Використано ідею різкого збільшення і фіксації жорсткості системи підвіски за максимальних деформацій та навантажень. Механічна трансформація пружної системи в коливальному процесі від низькочастотної до високочастотної здійснюється паралельним підключенням до пружних елементів високої жорсткості у формі дволанкового шарніра.

Фіксоване положення деформованого пружного елемента дає змогу виконувати роль «утримувача» потенціальної енергії поля пружних сил, використання якої здійснюється на етапі вкладання вантажів для обмеження швидкостей їх контактування з опорними приймальними площинами. Виключення з системи низькочастотних коливань означає обмеження енергетичних втрат на внутрішнє тертя в пружних елементах.

Розроблені математичні формалізації підтвердили правомірність ідеї використання системи змінної жорсткості, в якій, окрім позитивного результату обмеження екстремальних динамічних навантажень на етапі перехідного процесу, досягаються позитивні енергетичні та динамічні ефекти на заключних етапах.

Висновки. Ідея використання системи зі змінними жорсткостями пружних елементів дає змогу обмежувати протиріччя між значеннями кінематичних і динамічних параметрів.

Ключові слова: *динаміка, жорсткість, деформація, маса, навантаження.*

Нечітка система управління електропостачанням процесів виробництва харчових продуктів

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Вступ. Проведено дослідження з метою обґрунтування методу підвищення ефективності використання електричної енергії в процесах виробництва харчових продуктів, який базується на алгоритмах нечіткої системи керування електропостачанням.

Матеріали і методи. Дослідження ґрунтується на таких методах оптимального та нечіткого управління, як алгоритми Мамдані та Сугено. Комп'ютерне моделювання проводилося за допомогою MATLAB Simulink.

Результати і обговорення. Сформульовано критерій керування електропостачанням виробництва харчових продуктів у вигляді задачі мінімізації функціонала, що залежить від математичного очікування втрат активної та реактивної потужностей, втрат активної та реактивної потужності на рівні цехових підстанцій та окремих вузлах навантаження. Як задачу керування обрано підтримання енергоефективних напруг у вузлах системи електропостачання. Визначено залежність енергоефективних напруг від номінальної напруги мережі, активного опору її сегментів, потужності та коефіцієнта лінеаризованих статичних характеристик навантаження у вузлах мережі, еквівалентного активного опору, номінальної потужності, втрат холостого ходу та короткого замикання цехових трансформаторів. Для вирішення задачі керування синтезовано алгоритм з використанням нечітких регуляторів на рівнях головної та цехових підстанцій. Зокрема визначено, що вхідними сигналами нечіткого регулятора цехової підстанції мають бути відхилення напруги від енергоефективної і швидкість зміни напруги, а вихідними – напруга трансформатора і затримка спрацювання. За допомогою чисельних методів продемонстровано, що такий алгоритм дає змогу зменшити втрати електроенергії в процесах харчового виробництва до 7% порівняно з класичними методами регулювання напруги.

Висновки. Досліджуваний метод на основі нечітких систем забезпечує підтримання енергоефективних рівнів напруги у вузлових точках розподільної мережі при зміні напруги джерела живлення або навантаження споживачів.

Ключові слова: енергоефективність, електропостачання, контроль.

Економіка

Зміни цін у ланцюжку поставок свинини на прикладі Литви

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Вступ. Аналізуються структурні зміни сектору свинарства Литви та зміни цін у ланцюжку поставок свинини.

Матеріали і методи. У дослідженні використовуються річні показники Департаменту статистики Литви і щотижневі ціни, опубліковані ДП «Центр сільськогосподарської інформації і сільського бізнесу». Ключові зміни сектору свинарства Литви досліджуються на основі методів порівняльного аналізу та графічного зображення. Зміни цін на свинину досліджуються із застосуванням економетричних тестів, які надають можливість описати властивості ряду даних і визначити різні аспекти взаємодії цін у короткостроковій і довгостроковій перспективах.

Результати і обговорення. У період з 2004 р. по 2018 р. у структурі сільського господарства зменшилася частка малих фермерських і сімейних господарств, що займаються свинарством, які в минулому відігравали важливу роль у секторі свинарства Литви. У 2004 р. частка свиней, вирощуваних на фермерських і сімейних господарствах, становила 56,7% всієї популяції свиней, а в 2018 р. вона зменшилася до критично низького рівня і склала 24,9%. З 2007 р. по 2016 р. кількість господарств, які вирощують 10 свиней і більш, зменшилася з 3,1 до 2,5%, а частина господарств з 3-9 тваринами збільшилася з 34,7 до 47,9%. Цей напрямок розвитку свинарства обумовлено численними факторами, включаючи зміни бізнес-середовища після 2004 р. та еволюцію системи засобів підтримки сільського господарства Литви, наслідки різких стрибків цін, державної інтервенційної політики, пов'язаної з входом в євросону, і проблеми захворювань тварин.

Результати аналізу зміни цін демонструють, що на ринку свинини відбулося кілька шоків, які вплинули на формування цін і добробут учасників ланцюжка поставок. Однак результати коінтеграційного тесту Йохансена показали, що найбільш значущий структурний розрив зафіксований у 2013 році. Тест причинності Грейнджера підтвердив, що формування цін відбувається в напрямку від роздрібною торгівлі до фермерів, незважаючи на те що в довгостроковій перспективі гіпотеза асиметричної поведінки цін не підтверджується. Згідно з результатами моделі корекції помилок, ціни на свинину повертаються до рівноваги із швидкістю 3,6% за період.

Висновки. Дослідження підтверджує значущі зміни у секторі свинарства Литви. Тест цін на симетрію не виявив проблем ефективності функціонування ринку, проте в короткостроковій перспективі є одностороння причинність ціноутворення.

Ключові слова: *ринок, свинина, зміна ціни, ланцюжок поставок*

Instructions for authors



Dear colleagues!

The Editorial Board of scientific periodical
“**Ukrainian Food Journal**”
invites you for publication of your research results.

Requirements to all texts:

Language – English.

Recommended size of the article – 15–20 pages.

Font – Times New Roman, font size – 14, line intervals – 1, margins on both sides – 2 cm.

The structure of the article:

1. The title of the article
2. Authors (full name and surname)
3. Institution, where the work has been performed.
4. Abstract (2/3 of a page). The structure of the abstract should correspond to the structure of the article (Introduction, Materials and methods, Results and discussion, Conclusion).
5. Keywords.
6. The main body of the article should contain the following parts:
 - Introduction
 - Materials and methods
 - Results and discussion
 - Conclusion
 - References

If you need you can add another parts and/or divide them into subparts.

7. The information about the author (Name, surname, scientific degree, place of work, email and contact phone number).

All figures should be made in graphic editor, the font size 14.

The background of the graphs and charts should be only in white color. The color of the figure elements (lines, grid, text) – in black color.

Figures and EXCEL format files with graphs additionally should be submitted in separate files.

Photos are not recommended to be used as graphical materials.

Website of Ukrainian Food Journal: <http://ufj.ho.ua>

Email for all submissions and other inquiries: ufj_nuft@meta.ua

Шановні колеги!

Редакційна колегія наукового періодичного видання «**Ukrainian Food Journal**» запрошує Вас до публікації результатів наукових досліджень.

Вимоги до оформлення статей

Мова статей – англійська.

Мінімальний обсяг статті – **10 сторінок** формату А4 (без врахування анотацій і списку літератури).

Для всіх елементів статті шрифт – **Times New Roman**, кегль – **14**, інтервал – **1**.

Всі поля сторінки – по **2 см**.

Структура статті:

1. УДК.
2. **Назва статті.**
3. Автори статті (ім'я та прізвище повністю, приклад: Денис Озеряно).
4. *Установа, в якій виконана робота.*
5. Анотація. **Обов'язкова** структура анотації:
 - Вступ (2–3 рядки).
 - Матеріали та методи (до 5 рядків)
 - Результати та обговорення (пів сторінки).
 - Висновки (2–3 рядки).
6. Ключові слова (3–5 слів, але не словосполучень).

Пункти 2–6 виконати англійською і українською мовами.

7. Основний текст статті. Має включати такі обов'язкові розділи:
 - Вступ
 - Матеріали та методи
 - Результати та обговорення
 - Висновки
 - Література.

За необхідності можна додавати інші розділи та розбивати їх на підрозділи.

8. Авторська довідка (Прізвище, ім'я та по батькові, вчений ступінь та звання, місце роботи, електронна адреса або телефон).
9. Контактні дані автора, до якого за необхідності буде звертатись редакція журналу.

Рисунки виконуються якісно. Скановані рисунки не приймаються. Розмір тексту на рисунках повинен бути **співрозмірним (!)** тексту статті. **Фотографії можна використовувати лише за їх значної наукової цінності.**

Фон графіків, діаграм – лише білий. Колір елементів рисунку (лінії, сітка, текст) – чорний (не сірий).

Рисунки та графіки EXCEL з графіками додатково подаються в окремих файлах.

Скорочені назви фізичних величин в тексті та на графіках позначаються латинськими літерами відповідно до системи СІ.

В списку літератури повинні переважати англомовні статті та монографії, які опубліковані після 2010 року.

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.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

1. 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.

2. Посилання на книгу:

Автори (рік), Назва книги (курсивом), Видавництво, Місто.

Ініціали пишуться після прізвища.

Всі елементи посилання розділяються комами.

Приклад:

2. Wen-Ching Yang (2003), *Handbook of fluidization and fluid-particle systems*, Marcel Dekker, New York.

Посилання на електронний ресурс:

Виконується аналогічно посиланню на книгу або статтю. Після оформлення даних про публікацію пишуться слова **Available at:** та вказується електронна адреса.

Приклади:

1. (2013), *Svitovi naukovometrychni bazy*, available at:
http://www1.nas.gov.ua/publications/q_a/Pages/scopus.aspx
2. Cheung T. (2011), *World's 50 most delicious drinks [Text]*, 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, а з російської – стандарт МВД (в цих стандартах використовуються символи лише англійського алфавіту, без хвостиків, апострофів та ін).

Зручні сайти для транслітерації:

З української мови – <http://translit.kh.ua/#lat/passport>

З російської мови – <http://ru.translit.net/?account=mvd>

Додаткова інформація та приклад оформлення статті – на сайті

<http://ufj.ho.ua>

Стаття надсилається за електронною адресою: ufj_nuft@meta.ua

Ukrainian Food Journal публікує оригінальні наукові статті, короткі повідомлення, оглядові статті, новини та огляди літератури.

Тематика публікацій в Ukrainian Food Journal:

Харчова інженерія	Процеси та обладнання
Харчова хімія	Нанотехнології
Мікробіологія	Економіка та управління
Фізичні властивості харчових продуктів	Автоматизація процесів
Якість та безпека харчових продуктів	Упаковка для харчових продуктів

Періодичність виходу журналу 4 номери на рік.

Результати досліджень, представлені в журналі, повинні бути новими, мати чіткий зв'язок з харчовою наукою і представляти інтерес для міжнародного наукового співтовариства.

Ukrainian Food Journal індексується наукометричними базами:

Index Copernicus (2012)
 EBSCO (2013)
 Google Scholar (2013)
 UlrichsWeb (2013)
 Global Impact Factor (2014)
 Online Library of University of Southern Denmark (2014)
 CABI full text (2014)
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 InfoBase Index (2015)
 Chemical Abstracts Service Source Index (CASSI) (2016)
 Emerging Sources Citation Index (2018)

Рецензія рукопису статті. Матеріали, представлені для публікування в «Ukrainian Food Journal», проходять «Подвійне сліпе рецензування» двома вченими, призначеними редакційною колегією: один є членом редколегії і один незалежний учений.

Авторське право. Автори статей гарантують, що робота не є порушенням будь-яких авторських прав, та відшкодовують видавцю порушення даної гарантії. Опубліковані матеріали є правовою власністю видавця «Ukrainian Food Journal», якщо не узгоджено інше.

Політика академічної етики. Редакція «Ukrainian Food Journal» користується правилами академічної етики, викладених в роботі Miguel Roig (2003, 2006) "Avoiding plagiarism, self-plagiarism, and other questionable writing practices. A guide to ethical writing". Редакція пропонує авторам статей і рецензентам прямо слідувати цьому керівництву, щоб уникнути помилок у науковій літературі.

Інструкції для авторів та інша корисна інформація розміщені на сайті
<http://ufj.ho.ua>

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Головний редактор:

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