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CONTENTS

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EXPRESS METHOD FOR ASSESSING PROTEOLYSIS IN CHEESE AND AROMATIC ADDITIVES WITH CHEESE FLAVOR

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KEY WORDS:
cheese, enzyme-modified cheese, proteolysis, OPA, Kjeldahl method

ABSTRACT

The method based on the determination of the amount of active amino groups using o-Phthaldialdehyde (OPA method) can be applied in practice to assess accurately the degree of proteolysis in cheeses. The work establishes that the OPA method gives results that strictly correlate ($R^2 > 0.80$, $p < 0.01$) with the results of assessing the degree of proteolysis by the Kjeldahl method. The results of the OPA method, expressed in the absorption intensity of the colored sample at a wavelength of 340 nm (OD340), can be converted to the content of soluble nitrogenous substances in cheese (WSN), using the calibration relationship between these indicators.

The accuracy of the calibration relationship between WSN and OD340 can be increased ($R^2 > 0.91$, $p < 0.01$) when using the OPA method in relation to a homogeneous group of cheeses produced by the same technology using the same type of milk clotting enzyme and lactic acid starter culture and having a similar shape of the molecular mass distribution of proteolysis products.

The OPA method can be used to assess the content of proteolysis products, which form cheese flavor, in EMC. The results of assessing the degree of proteolysis by the OPA method (OD340) are proportional to both the total content of soluble nitrogen and the proportion of nitrogenous substances in it with a mass of less than 0.5 kDa, which make the greatest contribution to the formation of cheese flavor.

The advantage of using the OPA method for assessing proteolysis in cheeses and EMC instead of the Kjeldahl method is a simpler measurement procedure and the possibility of studying more samples in less time.

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1. Introduction

The traditional criterion for assessing proteolysis in cheeses is the ratio of the amount of water soluble nitrogen (WSN) to the amount of total nitrogen (TN) in the cheese with the determination of nitrogen forms by the Kjeldahl method. This is a laborious and time-consuming method. Alternatively, other methods for assessing proteolysis based on other physical principles, in particular colorimetric methods, can be used. Colorimetric methods are fast, accurate and not time-consuming.

Colorimetric methods for assessing proteolysis based on the formation of colored complexes as a result of the reaction of special reagents (dyes) with reactive groups of nitrogenous substances formed during proteolysis are proposed. The degree of coloration can be used to assess the degree of proteolysis in the studied cheeses. Among the methods most widely used by researchers to study proteolysis in cheeses, there are methods based on the reaction of free amino groups:

- with 2,4,6-trinitrobenzenesulphonic acid (TNBS), which reacts with primary amines, forming colored compounds having an absorption maximum at 420 nm [1,2];
- with ninhydrin, which, as a result of a specific reaction with alpha-amino groups, leads to the formation of a bright violet color, the degree of which can be estimated on a spectrophotometer at a wavelength of 570 nm [3];
- with o-Phthaldialdehyde (OPA). Reaction between amino groups and OPA in presence of a reducing agent such as 2-mercaptoethanol forming a colored compound detectable at 340 nm in a spectrophotometer [4,5,6,7].

The method for assessing the degree of proteolysis with the OPA reagent (hereinafter referred to as the OPA method) is widely used in the analysis of the degree of protein hydrolysis in protein hydrolysates. The method is highly accurate and reproducible [8,9].

The advantages of the OPA method are:

- sufficiently short reaction time of staining (2 min) before measurements, which provides a comfortable analysis for the laboratory assistant;
- use of inexpensive VIS-range spectrophotometers ($\lambda \geqslant 340$ nm) and inexpensive optical glass cuvettes;
- no need for heating and long-term keeping of heated samples before assessing the degree of staining on a spectrophotometer, in contrast to methods using TNBS and ninhydrin [10].

Despite the obvious advantages, the OPA method has not yet found widespread use in assessing the degree of cheese maturity. This is due to the well-established tradition of assessing the degree of proteolysis in cheeses by the WSN content, as well as the absence of a strict relationship between the optical density of the colored complexes at a wavelength of 340 nm (OD340) and the WSN content in cheeses. For the introduction of the OPA method into widespread use, it is necessary to develop a method for accurately recalculating the OD340 indicator obtained using the OPA method into the WSN indicator obtained using the Kjeldahl method.

2. Materials and methods

2.1. Materials

The studies used cow's milk from one supplier-manufacturer — AgriVolga LLC (Yaroslavl Region, Uglichsky District, Burmasovo village). In the production of cheeses, a lactic acid starter culture was used, consisting of a set of cultures of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *diacetylactis*, based on bacterial

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concentrate BK-Uglich-No.4 and BK-Uglich-No.5A (FGBNU Experimental Biofabrika, Russia) with preliminary activation of the culture on sterilized milk. Milk coagulation was performed using milk clotting enzymes (MCE) of the brands: Chy-max® Powder Extra 2235 NB, Naturen® Extra 220 NB (Chr Hansen A / S, Denmark), and Fromase® 750 XLG (DSM Food Specialties, France). Proteolytic enzymes were used for the production of enzyme-modified cheese: Neutrase® 0.8 L, Flavourzyme® 1000 L (Novozymes A / S, Denmark), as well as lipolytic enzymes: of microbial origin — Palatase® 20000 L (Novozymes A / S, Denmark) and of animal origin — a lipase preparation of calves, lambs, kids (Caglificio Clerici Spa, Italy).

To assess the degree of proteolysis by the OPA method, the following reagents were used: o-Phthaldialdehyde for fluorescence ⩾ 99.0% (Sigma, Product No. 79760), dodecyl sodium sulfate 99% for ion pair chromatography (J&K, Cat. No. 953543), sodium tetraborate 99% (Aldrich, Product No. 221732), dithiothreitol 99% for molecular biology (J&K, Cat. No. 926470), L-serine Pharmaceutical Secondary Standard (Supelco, Product No. PHR1103).

2.2. Methods

2.2.1. Production of Cheese

An Edam type cheese was produced with a fat content in dry matter of 45%. Table 1 shows the technological regulations for the cheese production.

Table 1

Technological regulations for the cheese production

Stage of the technological process	Process parameters
Mass fraction of fat in milk mixture	2.8–3.0%
Milk pasteurization	72 °C; 10 sec
Calcium chloride dose	Based on 35 g of anhydrous salt per 100 kg of milk
Dose of lactic acid starter culture	0.5%
Milk acidity before coagulation	pH 6.42 ± 0.02
Milk clotting enzyme dose *	1380 ± 180 IMCU / 100 kg of milk
Coagulation temperature of milk	33 ± 1 °C
Coagulation time	41.6 ± 4.1 min
The size of the grain edges when cutting	10 ± 1 mm
Duration of processing until the cooking	27.9 ± 5.2 min
Temperature of the cooking	40.5 ± 0.5 °C
Duration of the cooking	28.2 ± 3.4 min
Duration of processing after the cooking	34.0 ± 7.2 min
Active acidity of cheese after pressing	pH 5.92 ± 0.08
Mass fraction of dry matter in cheese after pressing	53.3 ± 1.3%

Note:
* The dose of application of MCE was determined from the rennet test [11].
The table indicates (mean value ± Standard Deviation) for the duration of the stages of the technical process and pH.

Fresh cheese was salted by immersion in an 18% salt solution for 1 day at a temperature of 4 ± 2 °C. After salting, the cheeses were packed under vacuum (negative pressure 1 bar; evacuation time 20 s) in bags made of AMIVAC CH-B polymer film (ATLANTIS-PAK, Russia). The cheeses ripening of half of the batch of cheeses was carried out at a temperature of 6–8 °C, the other half of the batch of cheeses — at a temperature of 10–12 °C. Ripening duration was 45 days. After ripening, the cheeses were stored at a temperature of 3 ± 1 °C. Samples for analysis were taken after 2 and 10 months of storage.

2.2.2. Production of Enzyme-Modified Cheese

Aromatic additives with a cheese flavor ("enzyme-modified cheese" — EMC) were produced according to the two-step approach method described in [12] with a number of modifications. A mixture was prepared from fresh cheese curd having a pH of 6.35 ± 0.05, Na caseinate, a complex phosphate additive "Phonakon" (Reatex, Russia) consisting of a mixture of phosphate salts-emulsifiers (E451i, E450i, E450ii, E339i, E339ii) and water. Mass fraction of dry matter in the mixture is 42%, fat in dry matter of the mixture is 40%. The mixture was homogenized using an immersion blender for 3 min in a water bath at a temperature of (80–85) °C. The resulting mixture was cooled to a temperature of 45 ± 1 °C, after which proteolytic enzyme preparations were added to the mixture: Neutrase® 0.8 L (0.6% w/w) and Flavourzyme® 1000 L (0.2% w/w).

After stirring, the mixture was incubated at 48 ± 1 °C for 4 hours. After 4 hours, the mixture was stirred again and lipase preparations were added to it: Palatase® 20000 L (0,4% w/w) or animal lipases (0,5% w/w). After stirring, the fermentation of the mixture was continued at a temperature of 48 ± 1 °C for 36 hours. After the completion of the fermentation, the mixture was heat-treated in a water bath at a temperature of (80–85) °C for 30 minutes to inactivate enzymes and microorganisms. The finished EMC was transferred into glass containers with caps and stored at a temperature of 3 ± 1 °C.

2.2.3. Methods for Studying the Cheese Properties

Determination of active acidity — 10 g of grated cheese was ground in a mortar with 10 cm^3 of deionized water; active acidity was determined on a pH-150MI pH meter (Measuring equipment, Russia).

The determination of the mass fraction of moisture was carried out by drying at a temperature of 102 ± 2 °C according to the Russian state standard GOST 3626–73.

Determination of the mass fraction of total and soluble nitrogen was carried out by the Kjeldahl method according to the Russian state standard GOST R54662–2011.

The degree of proteolysis in cheeses was expressed as a percentage of the absolute content of water soluble nitrogen (WSN) relative to the absolute content of total nitrogen (TN).

Sample preparation

The extraction of water soluble nitrogen from the cheeses was carried out according to the method [13] in the modification: 20 g of grated cheese was mixed with 40 cm^3 of deionized water, homogenized on a FSH-2A high-speed homogenizer (Jiangsu Jinyi Instrument Technology Company Limited, China) for 1 min. The resulting mixture (slurry) was transferred to a volumetric flask and the volume was made up to 100 ml with deionized water. The mixture was kept at 40 °C for 1 h with continuous shaking for 200 min^{-1} on an SK-O180-E orbital shaker (DLAB Scientific Co., Ltd, China). The samples were centrifuged at 3000 g for 30 min. After centrifugation, the samples were cooled to 4 °C and the upper fat layer was removed. The supernatant was separated and filtered on cellulose acetate filters with a pore size of 0.45 μm (Vladipor, Russia). The resulting filtrate was used to determine the content of water soluble nitrogen according to Kjeldahl and to analyze the molecular mass distribution. To study the OPA method, the obtained extract was mixed with deionized water in a ratio of 1:9 (vol/vol).

The extraction of water soluble nitrogen from EMC was carried out after preliminary defatting. Defatting was carried out with ice-cold hexane according to the modification method [14]. For this, 20 g of EMC was mixed with 60 ml of hexane in a 500 ml Erlenmeyer flask with a ground stopper and kept in a refrigerator at minus 18 °C for 1 h with periodic shaking. A portion of

hexane with the extracted fat was decanted, after which a fresh portion of hexane was added, and the defatting procedure was repeated. The fat was extracted 3 times. The defatted sample was dried under a stream of air at room temperature for 3 h, after which another cycle of defatting and drying was repeated. To obtain an aqueous extract, a weighed portion of 2.5 g of dried EMC was mixed with 50 ml of deionized water, kept for 30 min with continuous shaking, and then homogenized on a high-speed homogenizer. The mixture was transferred to a volumetric flask, made up to 100 ml with deionized water and filtered through filter paper. The resulting filtrate was used to determine the content of water soluble nitrogen according to Kjeldahl. To analyze the molecular mass distribution, the obtained extract was mixed with deionized water in a ratio of 4:6 (vol/vol) and filtered on cellulose acetate filters with a pore size of 0.45 μm. To study the OPA method, the obtained extract was mixed with deionized water in a ratio of 1:9 (vol/vol).

Molecular mass distribution

Determination of the molecular mass distribution of soluble nitrogenous substances in the aqueous extract from cheeses and EMC was carried out by high-resolution gel filtration method using a Superose 12 10/300 GL column (GE Healthcare, Sweden). The eluent is an aqueous solution of 0.05 M Na_2HPO_4 + 0.15 M NaCl, the flow rate of the eluent is 0.5 ml/min; detector wavelength — 280 nm. The column was calibrated according to the retention time of protein substances with a known molecular mass: IgG (180 kDa), aldolase (158 kDa), BSA (69 kDa), ovoalbumin (43 kDa), β-Lg (36.0 kDa), α-La (14.4 kDa), cytochrome C (12.3 kDa), tryptophan (0.204 kDa). The calibration graph was approximated by the logarithmic regression model [15]. The molecular mass distribution was expressed as the fraction of the peak area of the nitrogen fraction from the total area of the chromatogram. Results are expressed as the percentage of peptide material in various molecular mass ranges [16].

Degree of hydrolysis by the OPA method (OD340)

The degree of hydrolysis (DH) was determinated according to Nielsen et al [8] with modifications. The OPA reagent was prepared as follows: 4.020 g anhydrous sodium tetraborate and 200 mg dodecyl sodium sulfate (SDS) were dissolved in 150 mL deionized water. 160 mg o-Phthaldialdehyde 97% (OPA) was dissolved in 4 mL ethanol. The OPA solution was then transferred quantitatively to the above-mentioned solution. 176 mg dithiothreitol 99% (DTT) was added to the solution by rinsing with deionized water. The solution was made up to 200 mL with deionized water.

Optical density was determined in a mixture of 3 ml of OPA reagent and 0.4 ml of the test sample. A mixture of 3 ml of OPA reagent and 0.4 ml of deionized water was used as a blank. The absorption was determined on a spectrophotometer of LEKI model SS1207UV (MEDIORA OY, Finland) in quartz cuvettes with an optical path length of 10 mm at a wavelength of λ = 340 nm. The absorbance values for the interaction of amino groups with OPA were taken after 2 min standing. The result was expressed as the concentration of meqv serine of NH_2 group in a liter of the sample solution based on the calibration straight line built in the range of serine concentrations 0–2.0 meqv/L.

Mathematical processing was performed using Microsoft Excel and StatSoft Statistica software packages.

3. Results and discussion

Table 2 shows the indicators of the composition of cheeses and EMC studied during the experiment.

Table 2

Indicators of the composition of cheeses and EMC

Indicator	Cheeses (n = 16)	EMC (n = 7)
Mass fraction of dry matter, %	59.28 ± 1.76	43.19 ± 0.55
Mass fraction of fat in dry matter, %	46.03 ± 0.44	38.99 ± 0.18
Mass fraction of total nitrogen, %	4.51 ± 0.21	2.88 ± 0.15
Mass fraction of soluble nitrogen, %	1.24 ± 0.30	2.21 ± 0.20
Active acidity, pH	5.25 ± 0.06	5.70 ± 0.21
Average content of nitrogenous fractions with molecular mass:		
< 0,5 kDa	14%	34%
0,5–1 kDa	14%	20%
1–2 kDa	14%	23%
2–5 kDa	15%	17%
5–10 kDa	8%	5%
10–20 kDa	10%	1%
> 20 kDa	24%	0%

Note: the table shows Mean ± Standard Deviation.

OPA reacts with free amino groups of peptides and free amino acids. The results of the OPA method (OD340) depend on both the amount of protein material (WSN) in the test sample and the degree of hydrolysis of the protein material that proportional concentration to free of amino groups. The results of the Kjeldahl method (nitrogen content in the sample) depend only on the amount of protein material in the sample and do not depend on its degree of hydrolysis. Due to the different physical nature of the action of these two methods, it is necessary to substantiate the applicability of the OPA method to control the degree of proteolysis in cheeses and EMC, proving the possibility of recalculating the values of the OPA method into the values obtained by the Kjeldahl method.

Table 3 shows the degree of correlation between the results of the OPA method and the indicators of proteolysis of cheeses and EMC.

Table 3

Correlation between the variables of OPA method and the indicators of proteolysis in samples of cheeses and EMC

Variable	Cheese (n=16)		EMC (n=7)	
	WSN	OD340	WSN	OD340
WSN	1.00	0.91	1.00	0.89
Amount of peptide material with a diapason of molecular weight				
< 0.5 kDa	0.12	0.15	0.14	0.51
0.5–1 kDa	0.41	0.52	-0.50	-0.57
1–2 kDa	0.39	0.51	-0.39	-0.71
2–5 kDa	0.74	0.68	0.58	0.56
5–10 kDa	0.30	0.35	0.40	0.18
10–20 kDa	-0.53	-0.66	0.13	-0.03
> 20 kDa*	-0.44	-0.49	—	—

Note:
WSN — water soluble nitrogen, OD340 — optical density at wavelength 340 nm.
R — correlation coefficient; p — significance level.
* Peptides with mass > 20 kDa are absent in EMC.

As follows from the data in Table 3, the results of the OPA method (OD340) for cheeses and EMC depend on the total con-

tent of nitrogenous substances (WSN) and the molecular mass distribution of hydrolysis products.

Assessment of proteolysis in cheeses

In cheeses, OD340 has a strong correlation with WSN (R = 0.91). The strong correlation between OD340 and WSN is due to the specificity of proteolysis in cheeses. During the proteolysis process in cheeses, proteins and large peptides are broken down and converted into smaller peptides and free amino acids. The number of fractions with a mass of more than 10 kDa decreases, and the number of fractions with a mass of less than 10 kDa increases. In the studied cheeses, peptides with a mass of less than 10 kDa leave most (on average 65%, see Table 2) of compounds of water soluble nitrogen (WSN), therefore, WSN correlates with the content of fractions weighing less than 10 kDa.

At the same time, with an increase in the degree of proteolysis in cheeses, the increase in the content of individual nitrogenous fractions occurs in a balanced way and no fraction prevails over the other. Figure 1 (1) shows chromatograms of the molecular mass distribution of proteolysis products in cheeses with different degrees of proteolysis. Figure 1 (2) shows the dependence of OD340 on the WSN content in cheese.

For the reasons described above, much of the variation of the response variable in OD340 can be attributed to variation in the single influencing factor (WSN). Therefore, the regression equation describing the dependence of OD340 on WSN has a high coefficient of determination $R^2 = 0.83$. The results obtained coincide with the results of the studies by Rohm et al [7], which revealed a strong relationship ($R^2 = 0.965$) between the content of free NH_2 groups in Swiss cheese, determined by the OPA method, and WSN, determined by the Kjeldahl method.

Some of the variation in the OD340 response variable is related to the influence of other influencing factors that are not taken into account in the regression equation shown in Figure 1.2. The results of the correlation analysis (Table 3) show that OD340 significantly correlates with the content of individual peptide fractions with different molecular masses in cheeses. This indicates that differences in molecular mass distribution between cheeses will influence on the form of regression dependence of the OD340 on WSN.

The reasons for the differences in molecular mass distribution can be associated with the different specificity of proteolysis in cheeses. Most of the proteolysis products in cheeses such as Gouda and Cheddar are formed as a result of the action of MCE. Especially on Cheddar and Gouda, the enzymes in rennet (chymosin or rennet substitute) are mainly responsible for initial proteolysis and the production of most of the water-soluble N [17].

In the production of cheeses, MCEs of different origins were used: recombinant calf chymosin (Chy-max® Powder Extra 2235 NB), calf rennet (Naturen® Extra 220 NB) and microbial rennet based on the *Rhyzomucor miehei* protease (Fromase® 750 XLG). MCEs of different origins have different proteolytic activity and different specificity of proteolysis. Microbial rennet has the highest proteolytic activity among other MCE types; they break down a large number of peptide bonds in casein, which leads to the accumulation of an increased amount of peptides with low molecular mass. Recombinant chymosin has low proteolytic activity and cleaves a limited number of peptide bonds in casein. Among the products of proteolysis in cheeses with recombinant chymosin, peptides with a large molecular mass prevail. Calf rennet (a mixture of chymosin and pepsin) occupies an intermediate position in proteolytic activity between microbial rennet and recombinant chymosin [18]. Figure 2 (1) shows the molecular mass distribution of proteolysis products in cheeses produced with different types of MCEs and having the same degree of proteolysis (WSN/TN = 31%).

Earlier, it was suggested that for cheeses that have different shapes of molecular mass distribution, there will be a different form of regression dependence of OD340 on WSN. Since the shape of the molecular mass distribution of proteolysis products depends on the type of MCE used for the cheese production, in order to test the proposed assumption, the total data sample for all cheeses (n=16) was divided into 3 subsamples for cheeses produced with different types of MCE: Naturen (n=8), Chy-max (n=4) and Fromase (n=4). For each subgroup, a regression relationship between OD340 and WSN was constructed. The results are presented in Figures 2 (2) — 2 (4).

Assessment of proteolysis in EMC

When assessing the degree of proteolysis in EMC using the OPA method (Table 3), the OD340 value significantly correlates

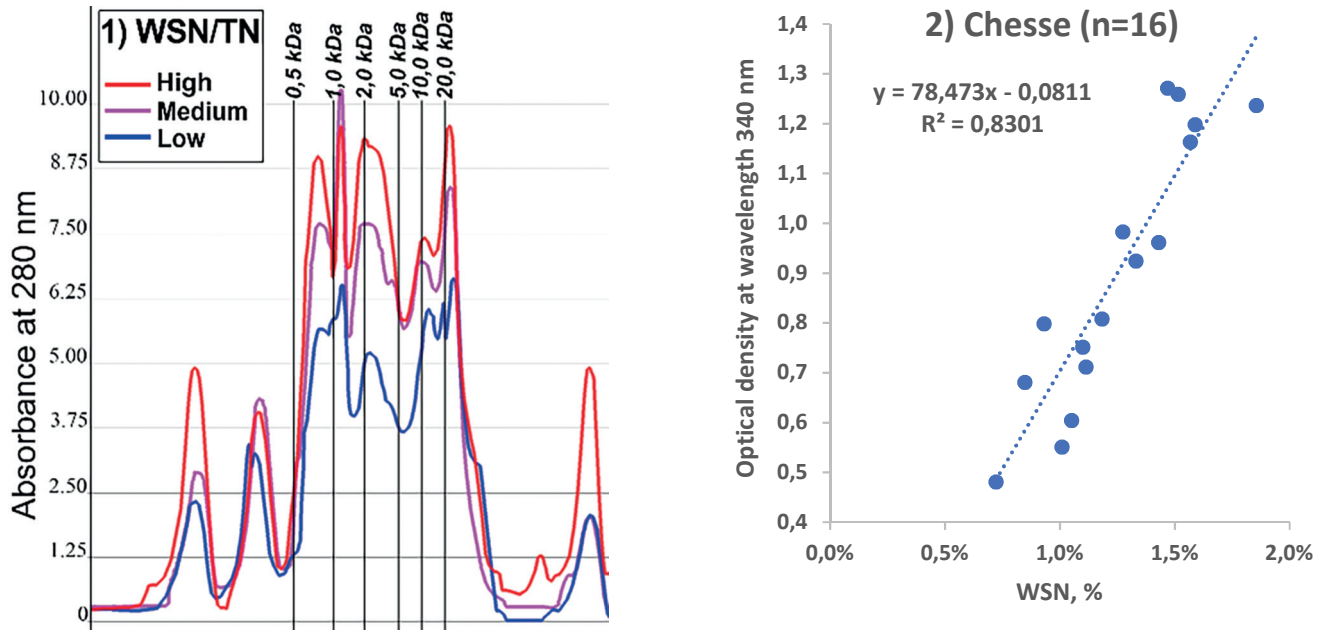

Figure 1. 1) Molecular mass distribution of proteolysis products in cheese samples with minimum, average and maximum proteolysis levels in the sample: Low (WSN/TN = 17%); Medium (WSN/TN = 28%); High (WSN/TN = 39%) and 2) dependence of the degree of staining with OPA reagent (OD340) on the content of water soluble nitrogen (WSN) in cheeses.

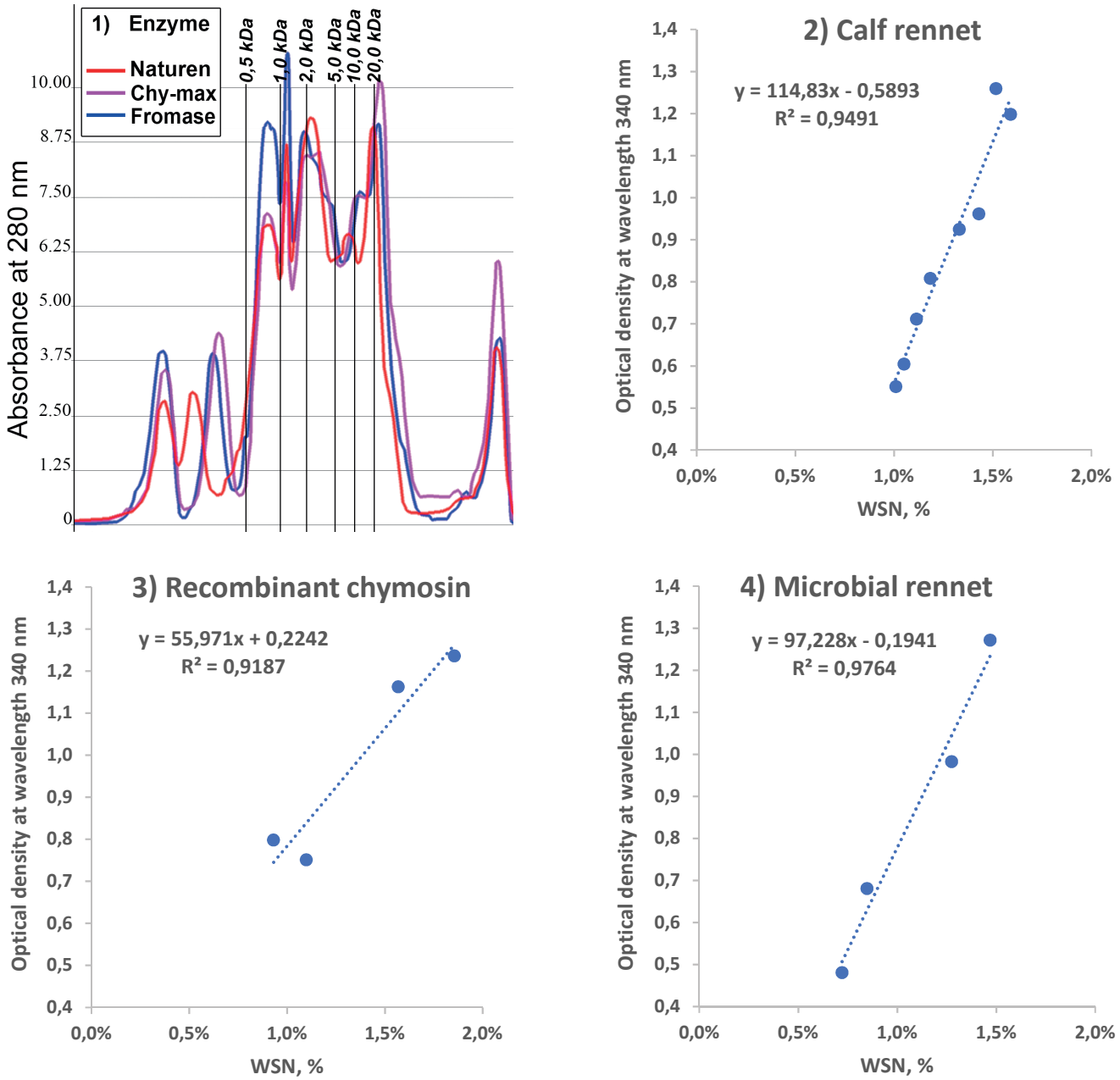

Figure 2. 1) Molecular mass distribution in cheese samples produced using different types of MCE and having the same degree of proteolysis (WSN/TN = 31%); and 2), 3, 4) — the dependence of the degree of staining with the OPA reagent (OD340) on the content of water soluble nitrogen (WSN) for cheeses with different types of MCE

with WSN (R = 0.89) and with the content of nitrogenous fractions with a mass of less than 5 kDa.

Proteolysis in EMC has a specificity that differs from proteolysis in cheeses: with an increase in the degree of proteolysis, the content of nitrogenous fractions with a mass of less than 0.5 kDa increases to the greatest extent in EMC (Figure 3 (1)). The EMC production technology is optimized to obtain the maximum amount of proteolysis products with a molecular mass of less than 0.5 kDa. In particular, the Flavourzyme® enzyme preparation is intended for this, which contains exopeptidases that break down protein to form free amino acids.

The hydrolysis products of casein, small peptides and free amino acids, have a pronounced taste and make a direct contribution to the formation of cheese flavor [19, 20]. High levels of low molecular weight peptides and free amino acids (FAA) important in cheese flavor [12]. As a result of numerous studies, it was found that among the products of proteolysis, peptides with a mass of less than 0.5 kDa make the greatest contribution to the formation of the flavor of Cheddar cheese [17]. Commercial EMC samples are characterized by a WSN/TN ratio of up to 80% and higher, with a proportion of proteolysis products with a molecular mass < 0.5 kDa up to 90% [21]. Therefore, the absolute content of peptide material with a molecular mass of < 0.5 kDa can be selected as a criterion for assessing proteolysis in EMC. Amount of peptide material with a MW < 0.5 kDa is calculated using the following equations:

$$A_{0,5\,kDa} = S_{0,5\,kDa} \times WSN$$

where:

WSN — water soluble nitrogen content in the sample (g/100 g);

$S_{0.5kDa}$ — part area under chromatographic curve in region molecular mass < 0.5 kDa (%).

Figure 3 (2) shows the regression dependence of the degree of staining with OPA reagent (OD340) on the amount of peptide material with a MW < 0.5 kDa in EMC.

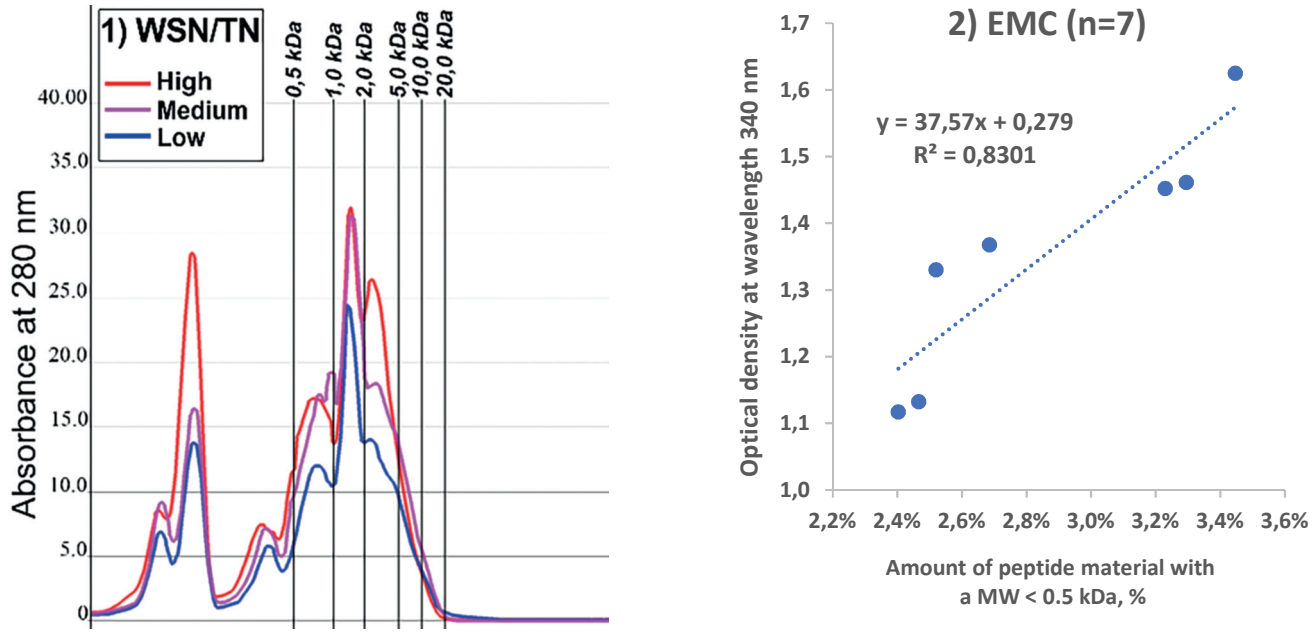

Figure 3. 1) Molecular mass distribution in EMC samples with the minimum, median and maximum levels of proteolysis in the sample: Low (WSN/TN = 64.5%); Median (WSN/TN = 79.2%); High (WSN/TN = 80.1%);
2) Dependence of the degree of staining with the OPA reagent (OD340) on the amount of peptide material with a MW < 0.5 kDa in EMC

4. Conclusions

The OPA method, based on the determination of the amount of active amino groups, can be applied in practice to assess accurately the degree of proteolysis in cheeses. The OPA method gives results that strictly correlate ($R^2 > 0.80$, $p < 0.01$) with the results of assessing the degree of proteolysis by the Kjeldahl method. The results of the OPA method, expressed in the absorption intensity of the colored sample at a wavelength of 340 nm (OD340), can be converted to the content of soluble nitrogenous substances in cheese (WSN), using the calibration relationship between these indicators.

The accuracy of the calibration relationship between WSN and OD340 can be increased ($R^2 > 0.91$, $p < 0.01$) when using the OPA method in relation to a homogeneous group of cheeses produced by the same technology using the same type of MCE and lactic acid culture and having a similar shape of molecular mass distribution of proteolysis products.

The OPA method can be used to assess the content of proteolysis products, which form cheese flavor, in EMC. The results of assessing the degree of proteolysis by the OPA method (OD340) are proportional to both the total content of soluble nitrogen and the proportion of nitrogenous substances in it with a mass of less than 0.5 kDa, which make the greatest contribution to the formation of chees flavor.

The advantage of using the OPA method for assessing proteolysis in cheeses and EMC instead of the Kjeldahl method is a simpler measurement procedure and the possibility of studying more samples in less time.

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Review paper

DNA AUTHENTICATION TECHNOLOGIES FOR PRODUCT QUALITY MONITORING IN THE WINE INDUSTRY

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ABSTRACT
Identification of wine product authenticity is a topical question in the Russian Federation. A solution to this problem can be DNA authentication of wines, which is a technological process of product authenticity control using genetic identification of the main plant ingredient — wine grape varieties. This type of wine verification is carried out by analyzing residual amounts of Vitis vinifera L. nucleic acids extracted from cell debris of final products by molecular genetic methods. The aim of this work is the analysis of the existing methods for extraction of nucleic acids from grapes, wine raw materials and commercial wines, as well as description of the molecular genetic approaches to technical genetic identification of grape varieties and authentication of wines made from them. The obtained data suggest suitability of DNA authentication of wine products as a supplement to earlier approved analytical methods (documentary, visual, sensory, physico-chemical).

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1. Introduction

One of the priorities in Russia over the last decade has been provision of the population with high-quality and safe food products. The alcohol industry is of great importance for economy of the Russian Federation [1]. With that, the wine industry accounts for a significant volume of manufactured products.

In Russia, wine quality is determined by several normed physico-chemical indices [2]. As the experience shows, these indices cannot guarantee the objective conclusion about wine authenticity. Due to the widespread presence of falsified products on the market, the problem of new method development became a topical issue in product quality and safety assessment. Consequently, the key task is extension of the assessment criteria area with more modern methodological base, in particular, the DNA authentication technologies.

DNA authentication of wines is a technological process of product authenticity control by genetic identification of the main plant ingredient — wine grape varieties. This type of wine verification is carried out by analyzing residual amounts of *Vitis vinifera* L. nucleic acids extracted from cell debris of final products by molecular genetic methods. [3].

2. Main part

Analysis of the literature on residual DNA extraction from wine cell debris indicates the following key methods: Pereira [4], Savazzini & Martinelli [5], and Nakamura [6], as well as their modifications [7]. The first two methods mentioned above have the similar extraction stage: precipitation of wine plant debris. This stage is performed using precipitators such as sodium chloride, 2-propanol and sodium acetate with the following centrifugation [4,5]. The method for residual DNA extraction described by L. Pereira et al. [4] is most effective due to high yield of extracted residual nucleic acids (Figure 1).

Methods for DNA authentication of wine raw materials and commercial wines are based on using several genetic markers of nuclear, mitochondrial and chloroplast DNA (Table 1) [5,6].

One of the methods for DNA authentication of wine raw materials is the use of highly polymorphic DNA microsatellite loci. Initially this method was intended for genetic identification of grape varieties [8,9,10,11,12]. Table 2 presents the basic set for identification and certification of grape varieties and hybrids [4,5,6, 13,14,15,16,17,18,19,20].

The *SSR* fragments were amplified by multiplex PCR, which enabled combining several analyzed loci. It is conventional to use this amplification algorithm when working with DNA obtained from grape plant parts (fruit, leaf, stem, root); however, it is not efficient when analyzing the extracted residual nucleic acid from wine [5,6,13,14,15].

Another type of *SSR* markers targeted to chloroplast DNA (*spSSR*) [21,22,23,24,25] has several advantages compared to the analysis of nuclear DNA (*nSSR*) due to the higher copy number of a target per cell, higher resistance to the exonuclease action and lower susceptibility to degradation because of its content in organelles with the double membrane [5, 7].

Analysis of microsatellite loci of chloroplast DNA remains to be an alternative approach to varietal genetic identification of *Vitis vinifera* L. as this type of *SSR* markers has the low discriminatory ability.

Table 1

Markers used for wine DNA authentication

Methods for DNA authentication of wine raw materials and wine products		
SSR- markers of nuclear, mitochondrial and chloroplast DNA of *Vitis vinifera* L	*STS*- markers of nuclear, mitochondrial and chloroplast DNA of *Vitis vinifera* L	*STS*- markers of nuclear, mitochondrial and chloroplast DNA of *Vitis vinifera* L

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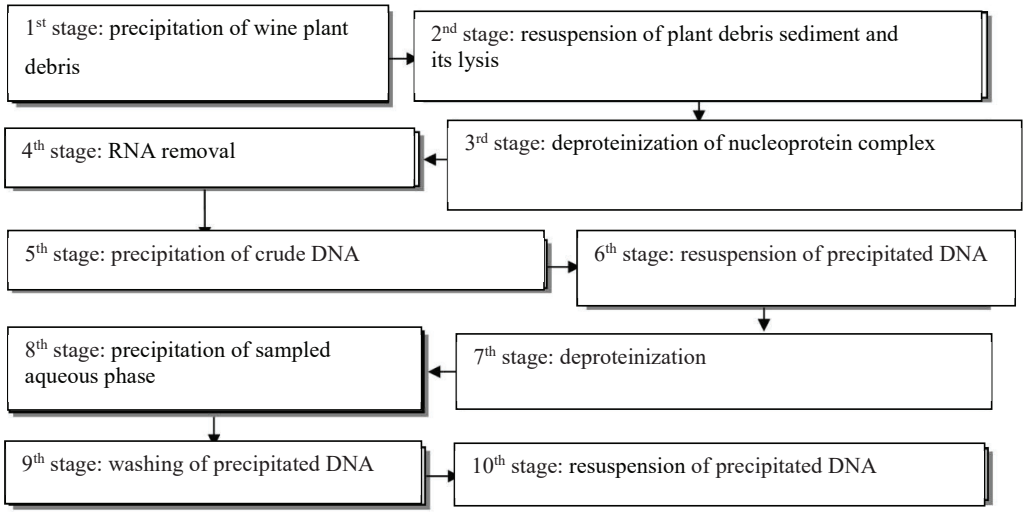

Figure 1. Stages of sample preparation and extraction of Vitis vinifera L. DNA from wine by the Pereira method

Table 2

***SSR* markers of nuclear DNA used for large-scale identification and certification of grape varieties and hybrids partly suitable for DNA authentication of wines**

No	SSR locus	Sequence of oligonucleotide primers	Range of allele lengths, bp
1	VVS2	5′-CAGCCCGTAAATGTATCCATC-3′	123–165
		5′-AAATTCAAAATTCTAATTCAACTGG-3′	
2	VVMD5	5′-CTAGAGCTACGCCAATCCAA-3′	220–268
		5′-TATACCAAAAATCATATTCCTAAA-3′	
3	VVMD7	5′-AGAGTTGCGGAGAACAGGAT-3′	231–267
		5′-CGAACCTTCACACGCTTGAT-3′	
4	VVMD25	5′-TTCCGTTAAAGCAAAAGAAAAAGG-3′	243–275
		5′-TTGGATTTGAAATTTATTGAGGGG-3′	
5	VVMD27	5′-GTACCAGATCTGAATACATCCGTAAGT-3′	173–223
		5′-ACGGGTATAGAGCAAACGGTGT-3′	
6	VVMD28	5′-AACAATTCAATGAAAAGAGAGAGAGAGA-3′	216–285
		5′-TCATCAATTTCGTATCTCTATTTGCTG-3′	
7	VVMD32	5′-TATGATTTTTTAGGGGGGTGAGG-3′	234–272
		5′-GGAAAGATGGGATGACTCGC-3′	
8	VrZAG62	5′-GGTGAAATGGGCACCGAACACACGC-3′	173–219
		5′-CCATGTCTCTCCTCAGCTTCTCAGC-3′	
9	VrZAG79	5′-AGATTGTGGAGGAGGGAACAAACCG-3′	236–270

The use of microsatellite DNA as a source of *STS* is also mentioned in the literature. Sequence Tagged Site (STS) is a short unique sequence, which amplified profiles serve as molecular genetic markers [4, 13]. For example, S. Nakamura et al. (2007) [6] developed the experimental *STS* primer sets for certain *SSR* loci of mitochondrial and chloroplast DNA [10, 21] and tested them in PCR for identification of *Vitis vinifera* L. varieties, as well as DNA authentication of wines produced from them.

As for *SNP* markers [26], they are also suitable for DNA authentication of wines [12]. *SNP* markers have the following advantages:

- differentiation of individual *Vitis vinifera* L. genotypes in single-varietal wines and assemblage wines with the possibility of quantitative assessment of plant ingredients
- efficiency in the analysis of the fragmented DNA of low quality.

Table 3 presents the primer and probe sets for real-time PCR with fluorescent hybridization detection, which are used in genetic identification of the *Sangiovese* variety and DNA authentication of wine produced from it by the single-nucleotide polymorphism (*SNP*) analysis in three analytical positions (98, 222 and 244) [7].

Another variant for application of *SNP* markers is to use the knowledge about single nucleotide polymorphism in several genes of *Vitis vinifera* L. incorporated into the method for high-resolution melting (*HRM*) curve analysis based on the real-time PCR platforms [12, 26, 27].

HRM analysis is an effective genotyping technology [28,29] with combined PCR stages and highly specific and sensitive detection with a possibility to differentiate several genotypes within one analysis, which is also suitable for wine DNA authentication [12, 26].

3. Conclusion

Analysis of methods for extraction of residual nucleic acids from final alcoholic products indicates the topicality and prospects of using DNA authentication as a molecular genetic

method for controlling safety of alcoholic beverages and detecting adulteration. The use of DNA technologies facilitates the most reliable determination of product authenticity in the wine industry. Molecular marker systems are suitable for identification of wine grape (*Vitis vinifera* L.) varieties and can ensure traceability throughout the life cycle of a final product.

Table 3

Real-time PCR primers and probes for three *SNP* positions applied in genetic identification of the *Sangiovese* variety and DNA authentication of wine produced from it

SNP	PPCR Round	Oligonucleotide primers and TaqMan probes	PCR product
98	1st PCR round with external primers	5′-TTCAAAGCGAAGAACCAG-3′ 5′-ACCCTTCAACAAACCAAC-3′	790 bp
	2nd PCR round with nested primers and TaqMan probes	5′-GTTAGTGTAAGGTGATGCC-3′ 5′-TTTCCTAATCCTTGTTGG-3′ 5′-FAM-TAGGATTTATGAAGGGAAG-3′-BHQ1 5′-VIC-TAGGATTTATGAAGGCAAG-3′-BHQ2	136 bp
222	1st PCR round with external primers	5′-AGACTGACTTTTGAAACACC-3′ 5′-TTCCTGGATTGGGTATG-3′	889 bp
	2nd PCR round with nested primers and TaqMan probes	5′-AAGACACCCACCAAGTTC-3′ 5′-CCAGGCAAGTAACACAAG-3′ 5′-FAM-AGCAATGTGGGCTGA-3′-BHQ1 5′-VIC-AGCAATGTGGGCCGA-3′-BHQ2	128 bp
244	1st PCR round with external primers	5′-AAACGCAGGAGAATGTC-3′ 5′-TTCAACCTGATGCCTAAC-3′	721 bp
	2nd PCR round with nested primers and TaqMan probes	5′-AATCCCCATCCCGAAGTG-3′ 5′-CCCAGTTCCATTCCTACACC-3′ 5′-FAM–CCTTTCTGGGTTGAACA-3′-BHQ1 5′-VIC–CCTTTCTGGGTTGCACA-3′-BHQ2	136 bp

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FEATURES OF MICRO- AND ULTRASTRUCTURE OF LOW-FAT BUTTER AND ITS LOW-FAT ANALOGUES

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KEY WORDS:
low-fat butter, micro- and ultrastructure, structure features

ABSTRACT

The aim of the research was to study the features of the structure of low-fat butter and butter pastes, which, in terms of composition and properties, more fully meet the requirements of a healthy diet than high-fat types of butter. The objects of research were: butter with fat content of 72.5%; butter with fat content of 55% made with the addition of skimmed milk powder; butter of the same fat content with the addition of stabilizers based on guar and xanthan gums and emulsifiers based on mono- and diglycerides of fatty acids; butter pastes with fat content of 45% with similar additives used to increase the stability of the process of butter formation and improve the texture. The microstructure was studied using an MBI-6 microscope, and the ultramicrostructure was studied using a Phillips electron microscope. In the first case, the sample was prepared by crushing the sample, in the second one — by the method of ultrafast freeze-fracture and etching. Researches have shown that the use of the introduced ingredients improves the homogeneity of the structure of the studied products. Due to the ability of milk proteins and stabilizers to retain moisture, it is more evenly distributed and well retained in the fat matrix of the product, formed from crystalline and liquid fat in the form of a continuous phase, which is confirmed by a sufficient penetration depth of the fat-soluble dye. Plasma droplets in butter with fat content of 72.5% and 55% are more isolated than in butter pastes, as indicated by the greater penetration depth of the water-soluble dye. The average diameter of isolated moisture droplets in low-fat products was 3.3–5.4 μm, and the average diameter of the fat globules that form the basis of the crystalline framework was 5.4–7.4 μm, depending on the composition of the product. For butter with fat content of 72.5%, the values of these indicators were 2.8 and 4.0 μm. The results of the study indicate the presence of differences in the sizes of structural elements, but at the same time confirm the uniformity of the structure of low-fat products, allowing them to be attributed to dispersions «water-in-oil».

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1. Introduction

In the classical sense, butter is meant to be a product obtained from cow's milk with a mass fraction of fat of at least 80% (international standard CXS279–1971 «Codex standard for butter», as amended in 2003, 2006, 2010 and 2018). In fact, it is a fat concentrate with evenly dispersed milk plasma. The composition, ratio and distribution of phases in this product predetermines its structure and texture, physicochemical and rheological properties, flavour benefits and consumption areas [1,2,3]. In accordance with the Russian standard GOST R52253–2004 "Butter and butter paste from cow's milk. General specifications" a product of this composition is classified as a classic butter. Such butter retains its properties well during transportation under controlled conditions (not higher than 5 °C) and can be stored for a long time at low subzero temperatures, therefore it is classified as a strategic product used to provide the population in extreme conditions. However, under normal conditions, the consumer prefers butter with a lower fat content or its low-fat analogues, which, according to the international classification, are called «milk spreads», and in our country they are referred to as «low-fat butter» (with fat content from 50% to 79%), "butter pastes" (with fat content of 40 to 49%) and "cream pastes" (with fat content of 39% or less). They put less stress on the human body in terms of calorie content, saturated fatty acids and cholesterol [4,5]. Among these products, the most widespread is low-fat butter with a fraction of milk fat of 72.5%, which has a plastic texture, a pronounced flavour bouquet and good storage capacity at low temperatures above zero, therefore it is often taken as a reference standard when developing new types of low-fat products based on milk fat.

However, the true standard of comparison is butter of classic composition, produced by the traditional method — the method of cream churning. The basis of such butter, according to [2,3,6,7,8,9], is fat in various states of aggregation (in liquid and in the form of triglyceride crystals). Crystalline formations of milk fat, in contact with each other, form a spatial structure — a crystalline framework. The type of connection of structural elements in this crystal framework determines the type of structure: coagulation and crystallization. According to Rebinder P. A. et al. [9] good texture of butter of classical composition corresponds to a mixed type of structure — crystallization and coagulation one with a predominance of the latter, which characterizes the elasticity, plasticity and thixotropic recoverability of the product. The mixed type with a predominance of the coagulation structure is formed, as a rule, when it is produced by cream churning. In the case of the production of classic butter by the method of converting high-fat cream, the crystallization type of structure prevails, which determines the excessive hardness and fragility of the butter. The same method is used to produce low-fat butter and butter pastes, although in international practice, for the production of low-fat products, the homogenization (dispersion) process can be used, which is a simpler technological solution that does not require a particularly complex hardware design. With such technological methods, there are practically no restrictions on the minimum acceptable content of the fat phase in the product. However, obtained products with the use

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of homogenization, especially with a mass fraction of fat up to 40%, are characterized by the presence of an empty, unexpressed flavour, a feeling of wateriness. To improve their flavour and aroma, milk-protein additives, stabilizers and emulsifiers [10] are added to the source raw material before heat treatment and homogenization [10], which, along with ensuring the fullness of the flavour, increase the homogeneity of the system and prevent moisture separation when the structure is destroyed. This processing provides the product with a pasty texture with good spreadability. When homogenization is used in low-fat products, an "oil-in-water" product structure is predominantly formed. The aqueous phase in direct type products is continuous, contains proteins, carbohydrates, vitamins, mineral components and therefore is a good breeding ground for the development of spoilage microorganisms. The most common cause of deterioration in this type of food is mold caused by the development of Penicillum and Cladosporium during storage. To prevent mold growth, product manufacturers have to use preservatives [10], the attitude of the consumer towards which is unambiguously negative. Considering this, many researchers propose the formation of a "water-in-oil" structure, which provides a high degree of plasma dispersion as an alternative way to prevent microbial spoilage of low-fat products. Glaeser H. in [11] indicated that to obtain an emulsion of this type, the fat content in the product must be at least 34%. However, the study of products even with a high fat content (38–40%) shows that complete closure of moisture droplets in the continuous fat phase does not occur. In products of this composition, the aqueous phase is additionally structured due to its binding to proteins. As a result, in analogues of butter with a reduced fat content, the aqueous phase is simultaneously both in emulsified form and in the form of a continuous aqueous phase, i. e. such products are mixed dispersions, which are less susceptible to microbiological deterioration in comparison with direct "oil-in-water" dispersions [10].

When fatty products are produced by the method of converting a high-fat mixture, a crystallization structure is formed, the severity of which depends on the composition of the product and the operating parameters of the butter churn. The plastic texture of a product of low fat content is formed with an increase in the intensity of mechanical treatment, achieved by a decrease in the productivity of the butter by 15–30% and the introduction of milk-protein additives, stabilizers and emulsifiers into the normalized mixture [12]. The butter plasticity, in addition to the fat phase, is influenced by the distribution of its dispersed phase — milk plasma, most of which in high-fat types of butter is in the form of tiny droplets isolated from each other, and a small part of them is connected by the finest ducts and channels that permeate the entire mass product. Part of the plasma exists in a bound state and is firmly retained on the surface of fatty aggregates formed from solid and liquid fat, and on the shells of fat globules that are not destroyed in the process of butter production [6]. With an increase in the proportion of plasma in butter from 16–25% to 30–35%, its dispersion decreases by 1.3–1.6 times [13]. This leads to a decrease in the strength characteristics of the product and an increase in its fragility [14].

With an increase in the proportion of plasma, its importance in the formation of the product structure increases. An important element of this structure in products of low fat content (40–55%), according to Gulyaev-Zaitsev S.S. and Belousov A. P. [15], is "the presence of a large number of continuous, thin liquid layers of milk plasma, permeating the entire monolith of the product and including a significant fraction of its aqueous phase. The stabilization of the thin-layer structure of the aqueous phase in the liquid fat is achieved by interfacial adsorption layers with mechanical strength, formed by milk plasma proteins and other surfactants on both surfaces of each water layer. Such a distribution of the unencapsulated aqueous phase achieves a rather strong fixation of it in the product structure, as a result of hydration of the surfaces of both interfacial layers. The combination of a large number of interfacial adsorption layers with mechanical strength and associated with both phases can be considered as the second skeletal structure of such products." This is the main distinguishing feature of low-fat butter compared to conventional butter. The better the plasma is distributed and the adsorption layers are stabilized, the more stable the structure of the low-fat butter is.

Studies carried out at VNIIMS on the effect of the composition of low-fat types of butter and butter pastes in the fat content range of 45–55% on the process of butter formation and the formation of the main structural, mechanical and rheological characteristics are described in [16]. These studies have shown a positive effect of the introduced skim milk, stabilizers and emulsifiers on the process of butter formation and the formation of a good texture of low-fat products. Of interest is to study the effect of these additives on the micro- and ultrastructure of foods that reflect the internal structure of low-fat foods. This article is devoted to this issue.

2. Materials and methods

The objects of research were experimental samples of butter with a mass fraction of fat of 72.5% (control), experimental samples of butter with a mass fraction of fat of 55%, as well as butter pastes with a mass fraction of fat of 45%, made by converting the corresponding normalized mixtures in a cylindrical butter churn. To ensure the stability of the structure formation process and good moisture retention in low-fat products, the Palsgaard 0291 emulsifier based on mono- and diglycerides with an iodine number of 60 g I_2/100 g and the complex stabilizer Palsgaard 5232, based on guar and xanthan gums, in the following quantities: for a product with fat content of 55% — 0.5% and 0.2%; for a product with fat content of 45% — 0.5% and 0.25%, were added to the normalized mixtures. The manufacturer of these additives is the Danish company of the same name.

Low-fat butter and butter pastes with the addition of skimmed milk powder were also used as objects of research. For butter, its amount was calculated in such a way as to obtain the mass fraction of milk-solids-non-fat (MSNF) of 7.5% in the product, and for butter pastes — 15.0%. Processing parameters of mixtures: pasteurization temperature of normalized mixtures, composed on the basis of high-fat cream, buttermilk and added additives, was 85 °C with holding for 15 minutes; temperature of the normalized mixture at the inlet to the laboratory cylindrical butter churn — 75 °C; product outlet temperature — 15–17 °C; coolant temperature (ice water) — 2 °C; intensity of mechanical action on the processed product — 35.8–40.3 W/kg.

The continuity of the fat and water phases of the studied products was assessed by the method of diffusion of fat- and water-soluble dyes described in [15]. To do this, a glass tube with a diameter of 10 mm and a height of 5 cm was carefully pressed into the product monolith to a depth of 3 cm; the tube with the product was removed, and placed vertically in a stand rod. A fat-soluble dye Sudan III and a water-soluble dye of methylene blue in an amount of 3 cm^3 were applied to the surface of the product. The sample was left at room temperature for 20 days and the depth of penetration of the dyes was measured.

Microstructure studies were performed using an MBI-6 (Russia) and an analogue-digital camera «Olympus». The specimens were prepared by crushing: a small amount of the product was placed on the slide with a metal needle; a cover slip was carefully placed on top, which was pressed against the slide with a 100 g weight; left for 1–2 minutes at room temperature. Microscopic examination was performed in transmitted light in a bright field with direct illumination of a transparent light filter. During the work, a 40^x objective with a $K7^x$ eyepiece was used. The prepared

specimens were examined under a microscope and the most typical fields were photographed. Electron microscopic studies were carried out using a transmission electron microscope EM-410 «Phillips» (Netherlands). Specimens for research were prepared by the method of ultrafast freeze-fracture and etching with carbon replication and contrasting with platinum according to the methods described in [17] modified by Smykov I. T. [18]. For this, a sample of butter and butter pastes was frozen with liquid nitrogen at minus 190 °C at a rate of up to 300 °/s. The frozen sample was placed in a vacuum chamber, where a vacuum of 10^{-6} mm Hg was created. Then the sample was split, and the plane of its split was sputtered with platinum 4–6 nm thick at an angle of 30 °. Then a carbon layer with thickness of 20–30 nm was deposited orthogonally onto the split plane. After deposition, the sample with the replica was removed from the vacuum chamber; the replica was taken onto the liquid surface and washed with a hypochlorite solution. The resulting replica was analyzed using an electron microscope at an accelerating voltage of 80 thousand watts and a magnification of 6600 times.

3. Results and discussion

The results of microstructure studies (Figure 1, left row) illustrate that with a decrease in the mass fraction of fat in butter and butter pastes, an increase in the size of plasma droplets was observed with a simultaneous increase in the unevenness of their distribution. However, due to the high moisture-binding capacity of the introduced components (milk-protein additives, stabilizers), milk plasma was well retained in low-fat products. No release of free moisture droplets was observed on their cut. Considering the nature of the distribution of milk plasma, it can be concluded that from normalized mixtures with skimmed milk powder, as well as from mixtures with stabilizers and emulsifiers, it is possible to obtain low-fat butter and its analogues with a structure similar to that of butter with fat content of 72.5%, i. e. "water-in-oil" type. However, the pictures show that both the prototypes with an increased content of MSNF (Figure 1, a and c) and the samples with stabilizers and emulsifiers (Figure 1, b and d) differed from the control samples (Figure 1, e) by an increased amount and less dispersion of plasma drops. If the average diameter of plasma droplets in butter with a mass fraction of fat of 72.5% under the conditions of the experiment was 2.8 μm, then in experimental samples of butter pastes with fat content of 45% it was 4.9 μm (for a product with an increased content of MSNF) and 5.4 μm (for product with structure stabilizers). The average diameter of plasma droplets in butter with a fat/MSNF ratio of 55.0%/7.5% was 3.0 μm, and butter of similar fat content with structure stabilizers — 3.3 μm (Table 1).

The nature of the distribution in the studied products of the main structural elements of the crystalline framework — fat globules — can be traced both in the photographs with a magnification of 6600 times (Figure 1, right row), and according to Table 1.

Comparison of the ultramicrostructure of experimental samples of butter pastes of 45% of fat and butter of 55% of fat with the addition of skimmed milk powder, stabilizers and emulsifiers with control samples of 72.5% of fat made it possible to reveal both the similarity of their structure and some differences. Thus, in the control butter (Figure 1, e), against the background of a continuous fatty phase, which is both in a crystalline and in a gel-like state, individual fat globules are seen, which make up the main structural framework of the product; there are individual fat globules that are not destroyed in the process of thermomechanical action on the original cream in the butter, as well as fragments of the shells of fat globules and milk plasma in the form of small droplets and interlayers, which is consistent with the results of studies of the microstructure of butter products carried out by other researchers [15,19,20].

The presented images demonstrate that in the test samples of products (both with an increased content of MSNF, and with stabilizers and emulsifiers), fat globules are larger and less evenly dispersed in the volume of the product, which causes a weakening of the structural bonds between them. In these products the weakening of the structural bonds between the fatty components and the increased role of the non-fatty component, which is milk plasma with an increased content of MSNF, can be traced by the change in their structural, mechanical and rheological parameters relative to the control sample of butter, described in [16]. It reflects in a decrease in the hardness and recoverability of the structure, complex shear modulus, elastic modulus and dynamic viscosity of butter and butter pastes made with the addition of MSNF. The weakening of structural bonds between fatty components in products with a high content of MSNF is comparable to that observed in samples of butter and butter pastes of similar fat content, developed with structure stabilizers.

Of great importance in the formation of the structure of low-fat butter and butter pastes with the addition of MSNF is the non-fat phase, which forms less rigid structural bonds than those formed mainly by fat components in butter of 72.5% of fat. The increased content of MSNF in the product contributes to an increase in heat resistance and emulsified fat content, noted in [16]. It should be noted that if the differences in the structural, mechanical and rheological parameters of butter pastes made with MSNF and structure stabilizers are insignificant, then they are present in butter samples: butter with an increased content of MSNF is characterized by higher values of rheological parameters, including hardness.

The depth of penetration of a fat-soluble dye (Sudan) into the structure of control samples of butter under the experimental conditions was 12 mm, in experimental samples of low-fat butter and butter pastes it was 1.1–1.2 times lower. Methylene blue penetrated into the butter of control and experimental samples in trace amounts. The water-soluble dye penetrated into butter pastes with fat content of 45% to a depth of 1 mm, which characterizes the lower closure of plasma drops in products of this composition compared to butter and indicates a greater development of thin milk plasma capillaries in it, which is consistent with the data of other authors [16]. At the same time, the

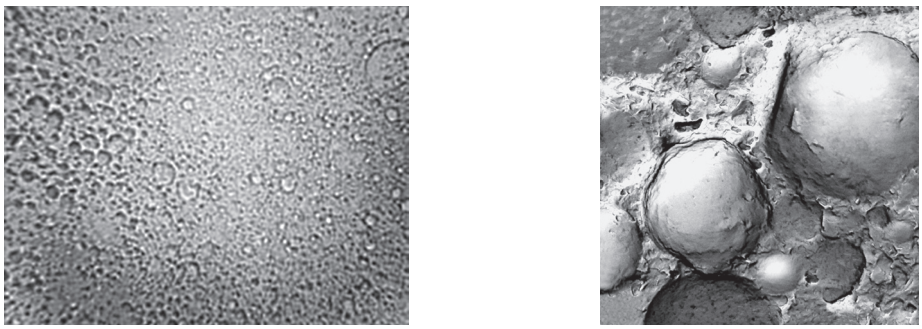
Table 1

Parameters of distributions of plasma droplets and fat globules by size

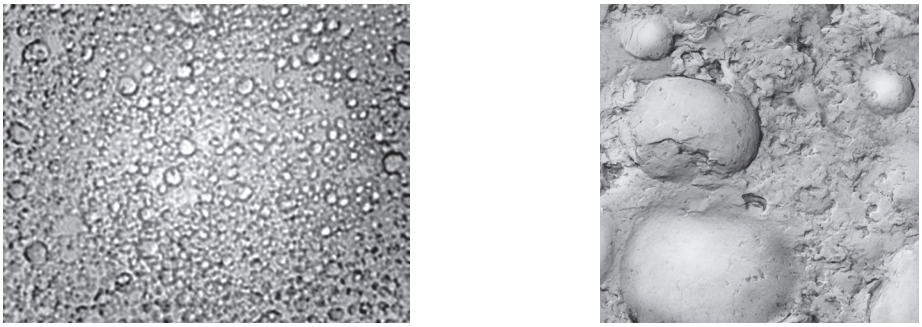
Name indicator	Indicator value for a product sample, μm ± standard deviation				
	Butter with m. f. f.* of 72.5%	Butter with m. f. f.* of 55.0% MSNF of 7.5%	Butter with m. f. f.* of 55.0% with stabilizers and emulsifiers	Butter paste with m. f. f.* of 45% MSNF of 15%	Butter paste with m. f. f.* of 45% with stabilizers and emulsifiers
Average diameter of milk plasma droplets, μm	2.8 ± 0.9	3.0 ± 1.1	3.3 ± 1.3	4.9 ± 1.8	5.4 ± 2.3
Average diameter of fat globules, μm	5.0 ± 1.9	5.4 ± 2.1	5.6 ± 2.2	7.4 ± 3.1	6.0 ± 2.4

* mass fraction of fat

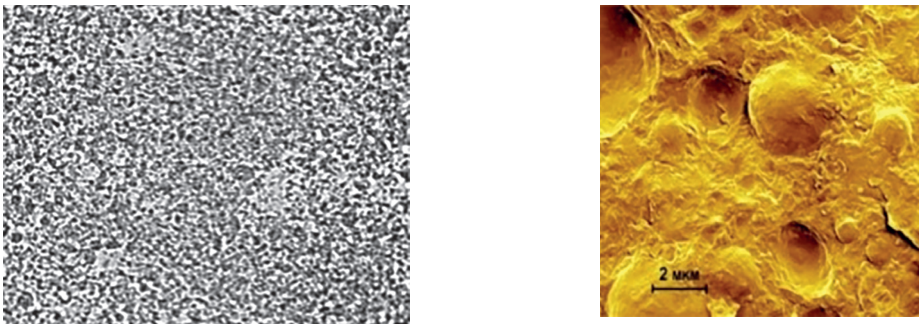
Left row: 280 x magnification Right row: 6,600 x magnification



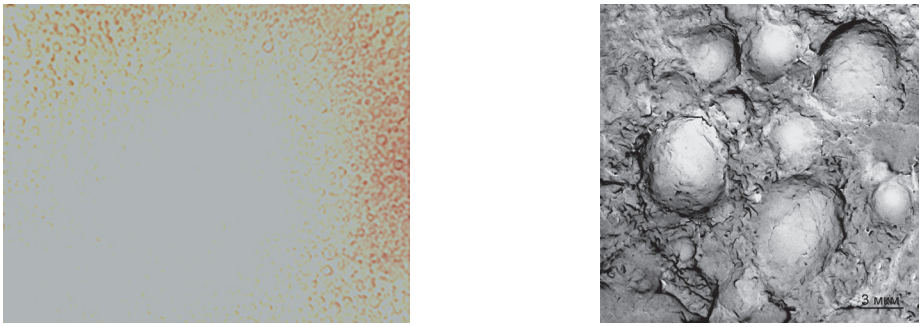
a) butter paste with a mass fraction of fat of 45%, MSNF of 15%;



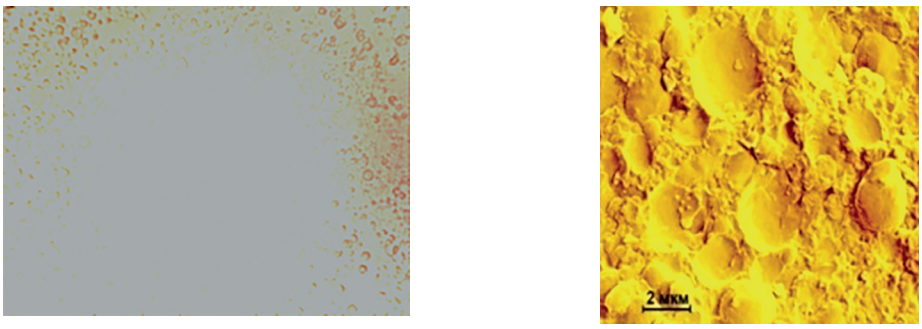
b) butter paste (mass fraction of fat of 45.0% with stabilizers and emulsifiers);



c) butter with a mass fraction of fat of 55%, MSNF of 7.5%;



d) butter with a mass fraction of fat of 55% with stabilizers and emulsifiers;



e) butter with a mass fraction of fat of 72.5% (control).

Figure 1. Microstructure (left row, 280^{x} magnification) and ultramicrostructure (right row, 6600^{x} magnification) of products of low fat content

prevailing penetration of the fat-soluble dye into the product over the water-soluble one confirms the nature of the structure, close to the structure of butter with fat content of 72.5%. Low-fat butter and butter paste are "water-in-oil" products, which allows predicting good storage properties of these products due to the predominant moisture content in the encapsulated form. At the same time, moisture in these products is partially associated with proteins and stabilizers, therefore, its activity will be significantly reduced in comparison with the moisture found in products without adding water-binding ingredients.

4. Conclusion

Based on the results of the studies, it was found that the use of a milk-protein supplement in the form of skimmed milk powder, as well as food additives in the form of stabilizers and emulsifiers in the production of low-fat butter and butter pastes with a mass fraction of fat of 55% and 45%, respectively, makes it possible to obtain a product with the type and nature of the structure close to butter of 72.5% of fat, made without using these additives. Due to the higher plasma content, its more significant effect on the structure of the product appears, which consists in the appearance of additional structural elements (channels and ducts) between its drops, dispersed in the fat phase of the product, which affects the structure of the finished product and the depth of penetration of the water-soluble dye into the product. The structural features of low-fat butter and butter pastes are also the less fine distribution of milk plasma droplets and the presence of larger fat globules involved in the formation of the fat matrix of these products.

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Original scientific paper

METHODOLOGICAL ASPECTS OF THE USE OF DRY COMPONENTS OF CHICKEN EGGS FOR FEEDING CHILDREN WITH PHENYLKETONURIA

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KEY WORDS:
phenylketonuria, dietary nutrition, low-protein pasta, dry egg products (melange, protein, yolk)

ABSTRACT
Currently, one of the most important tasks facing science and production is the creation of functional product technologies for use in different diets of the population in order to preserve and improve health, as well as reduce the risks and consequences of various diseases, including hereditary ones, such as phenylketonuria (PKU). The All-Russian Research Institute of Starch Products develops technologies for the production of low-protein starch-based products/semi-products enriched with functional ingredients and intended for therapeutic nutrition of patients with PKU. As part of the pilot production, the production of these products is organized. Purpose of work:- to justify the possibility of using dry components of chicken eggs (melange, protein, yolk) to enrich low-protein starch products (noodles, vermichel, «spider») intended for feeding children over 3 years old with phenylketonuria;- evaluate organoleptic properties and efficiency of low-protein starch products enriched in hypophenylalanine diet of patients with phenylketonuria older than 3 years.

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1. Introduction

In recent years, studies have been carried out confirming the possibility of using dry ingredients of chicken eggs (protein, yolk, melange) to enrich low-protein starch products [1,2,3]. An important factor determining the feasibility of using enriched starch products in the nutrition of children with phenylketonuria is the low content of protein and, accordingly, phenylalanine. It has been established that the inclusion of new ingredients in small quantities in the composition of dry semi-products allows them to be used in low-protein dietary therapeutic diets of patients with PKU, primarily in the nutrition of children.

Two fundamentally new technological techniques have been developed for introducing dry ingredients of chicken eggs into the starch base: the first is the moisture-thermal treatment of a mixture of corn starch and egg products on a roller dryer, the second is the mechanical mixing of the added ingredients and swellable corn starch.

It was found that the physicochemical properties (swelling, solubility, conditional viscosity) of products obtained using various technological methods are close to each other.

A comparison of the two methods of producing new starch mixtures in combination with dry egg products has shown that it is preferable to use a mechanical mixing method because it is simpler and more economical.

2. Materials and methods

The main ingredients for the production of pasta were: corn starch (GOST 32159–2013) and extrusion corn starch of domestic production «Extramil» (TU9187–001–18020121–2001).

To enrich pasta, dry egg yolk (GOST 30363–2013) and Vetoron Children's Solution (TU9197–033–58693373–06 with changes 1, 2) were used.

According to the results of previous studies [4.5], it has been found that in the development of the formulation of low-protein products, all three components of chicken eggs can be used as a functional additive, but it is preferable to use dry yolk, which is more suitable in quality composition for baby food. Compared to egg melange and protein, the yolk has a higher energy value and the smallest amount of protein, and therefore phenylalanine. In addition, it surpasses melange and protein in terms of organoleptic properties, since it has a pronounced taste and smell characteristic of a chicken egg.

Analysis of the chemical composition of new pasta showed that their protein content per 100 g of dry product is less than 1.0%, fat 3.3–3.6%, ash up to 0.14%, carbohydrates no more than 88.0%, energy value ranges from 1614.1–1617.8 kJ (386.14–387.04 kcal).

The results of the analytical evaluation confirmed that the ingredient composition of these products fully meets the requirements for food products of this class (specialized low-protein products) used in the therapeutic and dietary nutrition of patients with phenylketonuria [6,7,8].

Clinical studies of the safety and effectiveness of these low-protein pasta used for the preparation of low-protein dishes were carried out on the basis of the Advisory and Diagnostic Center of the Federal State Research Institute for Children's Health of the Ministry of Health of the Russian Federation.

Evaluation of the effectiveness of the use of pasta of low-protein starch enriched for dietary therapeutic nutrition for children over 3 years of age: Vermichel, Noodles, Spider was an incomparable, prospective, single-center, uncontrolled study.

Parents (legal representatives) were informed in detail about the goals and tasks of the work, followed by obtaining written informed consent to participate in the study. Children who met the

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inclusion criteria and had no contraindications to prescribing the above low-protein products were randomly selected for follow-up.

Inclusion criteria:

- Children of both sexes between the ages of 3 and 18 years with classical phenylketonuria confirmed at neonatal screening on a hypophenylalanine diet;
- having written consent of parents/legal representatives to participate in the study.

Exclusion criteria:

- acute infectious disease.

The study design is shown in Figure 1.

Under supervisionthere were 10 patients with phenylketonuria, of which 4 boys and 6 girls, aged 3 years 8 months to 11 years 4 months, the average age of patients was 8 years 4 months ± 2 years 8 months.

The study used a clinical method that included the collection of history data, physical examination with an assessment of growth and body weight, the overall health of the child at the beginning and at the end of the study, an assessment of the tolerability and organoleptic qualities of low-protein products according to the 5 points system, which was carried out using telephone and online contacts. All data obtained were recorded in an individual registration card.

Laboratory research methods included a biochemical (fluorimetric) method for determining the level of phenylalanine in blood serum before prescribing the test products and against the background of their intake 1–2 days before the end of the study.

Statistical processing of the obtained data was carried out using the STATISTICA 13.0 package (StatSoftInc., USA). Median (Me) values as well as 5th and 95th percentiles were used to describe the test indicators. The differences between the two dependent groups were assessed using a non-parametric Wilcoxon test. The probability of error (p) was considered statistically significant at values < 0.01

3. Results and discussion

In the course of experimental studies, optimal ratios of starch and dry ingredients of chicken eggs were determined, which is very important for maintaining such a quota of protein, which at the output of the production cycle will be the permissible concentration for a low-protein product. So, to prepare a mixture of starch: dry egg white, the content of the latter should be no more than 5%, for the mixture starch: melange — 10%, and starch: yolk — 15%.

With the addition of egg products and an increase in their quota, the content of amino acids and trace elements in the end product also increases compared to the starting starch, while the amount of phenylalanine varies slightly.

It should be noted that the moisture-thermal treatment of the starch protein mixture can be used to produce an artificial egg substitute as a separate semi-product. The food composition obtained by mechanical mixing of swellable starch and dry egg products can be used as an additive for enriching already existing low-protein products, as well as a necessary ingredient for

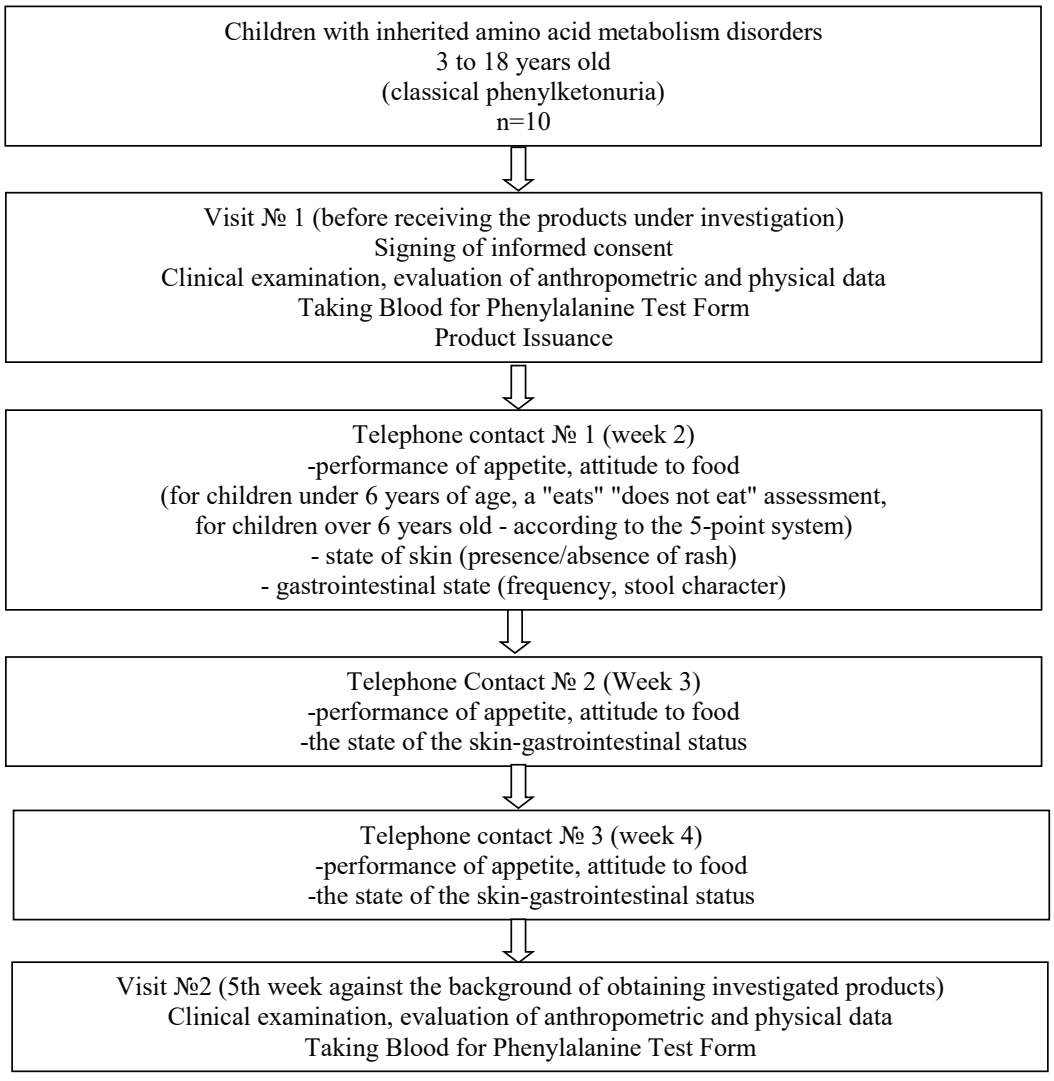

Figure 1. Study Design

baking bread and a filler in various bakery and vegetable dishes (tins, vegetable balls, cutlets, etc.).

In order to increase the nutritional value of low-protein pasta (noodles, vermichel, "spider") widely used in medicinal nutrition of patients with PKU, dry egg products were introduced into their composition.

3.1. Product Assignment Procedure

After the initial examination and assessment of nutritive status (anthropometric indicators), each child was assigned all 3 types of the studied products as part of the low-protein menu throughout the follow-up. The products were given home for 30 days in the form of semi-finished products at the rate of 1 bag (1.0 kg) of each product for use as an independent dish (pasta, vermichel, noodles) or a component for the preparation of other dishes (soup, stew, casserole, etc.).

The investigated products were introduced into the diets of children with phenylketonuria in the form of ready-made dishes instead of previously used similar products of other manufacturers in accordance with clinical recommendations for providing medical care to children with PKU [9] and the fundamental principles of hypophenylalanine diet organization [10,11]. The duration of each product intake was 10 days, in order to avoid uniformity of diet, the products alternated throughout the study.

Organoleptic properties (taste, consistency), as well as the appearance of new low-protein mixtures of starch enriched "Vermichel," "Noodles," "Spider," intended for dietary therapeutic nutrition for children over 3 years old, all children rated "good" and "excellent." Most of them (7 out of 10 children) noted higher palatability compared to previous versions of these products. Three children noted the best appearance, consistency and taste of vermichel, the rest of the children did not single out any separate product. Results of assessment of organoleptic properties of low-protein products are given in Table 1.

Table 1

Assessment of organoleptic properties of dishes from new low-protein mixtures of starch enriched "Vermichel," "Noodles," "Spider"

Parameters	Indicators	Average Score Scores		
		Vermichel	Noodles	Spider
appearance	M±m	4.9±0.18	4.4±0.41	4.8±0.32
	Min	4.0	4.0	4.0
	Max	5.0	5.0	5.0
consistency	M±m	4.5±0.5	4.45±0.45	4.45±0.45
	Min	4.0	4.0	4.0
	Max	5.0	5.0	5.0
taste	M±m	5.0	4.8±0.32	4.9±0.2
	Min	5.0	4.0	4.0
	Max	5.0	5.0	5.0

Physical development indicators before the study began in 8 children corresponded to average age values, one child had mild malnutrition, and another child had excess body weight. Against the background of the intake of the studied products, body weight indicators tended to increase, but remained within the age norm in 8 children, in a child with overweight during the observation period, the increase was only 100 g, in a child with malnutrition, the body weight increased to the lower limit of the average age indicators.

The main criterion for the effectiveness of the hypophenylalanine diet, which necessarily uses low-protein products, is the concentration of phenylalanine in the blood serum of patients. According to clinical recommendations, the therapeutic range of phenylalanine in the blood is 2–6 mg% for patients of all ages, however, for adolescents with PKU over 12 years old, it is allowed to increase to 10 mg% in the absence of neurological clinical symptoms. At the time of inclusion in the study, two of our patients had elevated levels of phenylalanine in the blood (7.1 mg% and 11.4mg%) due to periodic diet impairment, during the use of new low-protein products, phenylalanine in the blood decreased in these children, remaining only slightly elevated (6.8 mg%) in a teenager of 11.5 years due to increased control over compliance with the diet. In all other children, the serum content of phenylalanine was not increased and remained in the range of 2 to 6 mg%. The dynamics of the serum phenylalanine values before and at the end of the study are shown in Table 2.

Table 2

Dynamics of parameters of body weight, growth and content of phenylalanine in blood serum in children with classical phenylketonuria against the background of taking the analysed products

indicators	Me (DI 5;95%)		p*
	Prior to assignment of investigated products	At using the products under investigation	
Bodyweight (kg)	26.6 (14.8;51)	27.55 (15;51)	0.0001
Height (cm)	130.3 (103.5;162)	130.8 (103.5;167)	0.0001
Phenylalanine in the bloodserum (mg%)	4.45 (2.4;12.5)	2.65 (2.0;8,7)	0.0001

* Wilcoxon criteria

4. Conclusion

Thus, the clinical testing of new pasta low-protein products "Vermichel," "Noodles," "Spider," produced using modern technologies enriched with dry yolk and carotene ("Vetoron" — children's solution) showed high organoleptic qualities and effectiveness in maintaining the stability of the permissible level of phenylalanine in the blood of children with phenylketonuria Use of the above products allows to expand the range of hypophenylalanine diet and increase its nutritional value.

The positive results of the study indicate the possibility of widespread use of new enriched low-protein starch products in the practice of therapeutic nutrition in diseases requiring a low-protein diet.

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Review paper

PROTEIN-POLYSACCHARIDE INTERACTIONS IN DAIRY PRODUCTION

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KEY WORDS:
milk proteins, polysaccharides, biocomposites, dairy products, functional foods, nanostructure

ABSTRACT
The review article examines the main global trends in the development of scientific research in the field of increasing the efficiency of dairy products production using polysaccharides of various origins and purposes. It has been shown that non-traditional polysaccharides of plant origin are increasingly involved in industrial production, including polysaccharides of aquatic organisms, which have both enhanced technological properties — emulsifying, gel-forming, texturizing, etc., and innovative nutraceutical properties that make it possible to create food products with new properties and attractive to consumers. It is noted that the nature of protein-polysaccharide interactions, depending on the types of proteins and polysaccharides used in various combinations and conditions of their interactions, can be completely different, which directly affects the organoleptic properties of the finished product. Modern research confirms that the properties of a food product are largely laid down at the molecular — nanoscale, and the development of research on protein-polysaccharide interactions, with the aim of their practical use in the production of dairy products, should be aimed at finding basic patterns in these interactions.

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1. Introduction

Dairy products have been in continued demand from the population throughout the history of humanity. This is due both to the unique properties of milk, inherent in nature, and to the possibility of producing from it a variety of products that have a high storage capacity and attractive taste. Numerous studies confirm the role of dairy products as an important component of a healthy diet. Modern trends in the development and production of new dairy products are aimed at increasing their functionality, reducing the loss of raw materials and meeting the needs of various segments of the population. One of the promising directions in the development of new dairy products is the use of polysaccharides of various origins in them. For example, in the review [1] devoted to the analysis of modern trends in the use of food polysaccharides, mainly of plant origin, it was shown that there are promising results for their use as food additives to control the organoleptic properties of products, as well as sources of biologically active compounds for functional dairy products. This approach not only allows taking a step forward in reducing waste in the food chain, but also offers new ways to diversify dairy production, creating the opportunity to occupy a market niche based on new functional products.

Milk is the best known and most widespread natural hydrocolloid. However, there is a wide variety of other hydrocolloids, which are hydrophilic biopolymers of plants, animals, and microorganisms that can be used in the food industry. Review work [2] is devoted to the analysis of the use of known and potential natural hydrocolloids based on proteins and polysaccharides in the food industry. This review reflects the most recent concepts for the use of hydrocolloids to meet the requirements of consumers and the food industry.

Hydrogels formed from hydrocolloids are three-dimensional networks of hydrophilic biopolymers that can absorb significant amounts of water without dissolving or losing their structural integrity. The use of natural hydrogels from food biopolymers has become widespread in recent decades. In work [3], the basic principles of designing food gels are considered, aimed at changing the rheological and tribological properties of food products, modifying them while maintaining sensory perception and targeted delivery of drugs and biologically active substances into the gastrointestinal tract.

Hydrogels can be formed from hydrocolloids of protein and polysaccharide origin, as well as their composites. Protein-polysaccharide composites, as is known [4], have a wide range of applications in various areas of food production. These composites can have various physical forms, such as gels, films, fibers and individual particles, depending on their components and the particular application. Subsequent processing and use of these composites contribute to a targeted and beneficial change in their structure and properties.

Thus, the use of protein-polysaccharide compositions provides many opportunities for improving organoleptic properties, biological functions, and technological processes for the production of dairy products. The study of protein-polysaccharide interactions opens up opportunities for the development of new ingredients and biopolymer complexes with application in various areas of the food industry.

The purpose of this work is to give an idea of the elements of protein-polysaccharide compositions, how they are formed and how they are used in modern dairy production.

2. Main part

2.1. Casein is the main protein in milk

Milk proteins are the basis for the production of most dairy products. First of all, this concerns casein, which is the main protein of milk and is represented by fractions of α-, β- and κ-caseins. Casein molecules do not have a definite quaternary structure and therefore belong to the group of metamorphic proteins. The property of caseins to change their quaternary structure under the influence of external factors is decisive in the milk processing. In milk, casein is in the form of spherical micelles with a diameter of 50 to 300 nanometers (Figure 1,

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hereinafter, the author's illustrations), which include all casein fractions, and the fractional composition of micelles is different depending on their size [5].

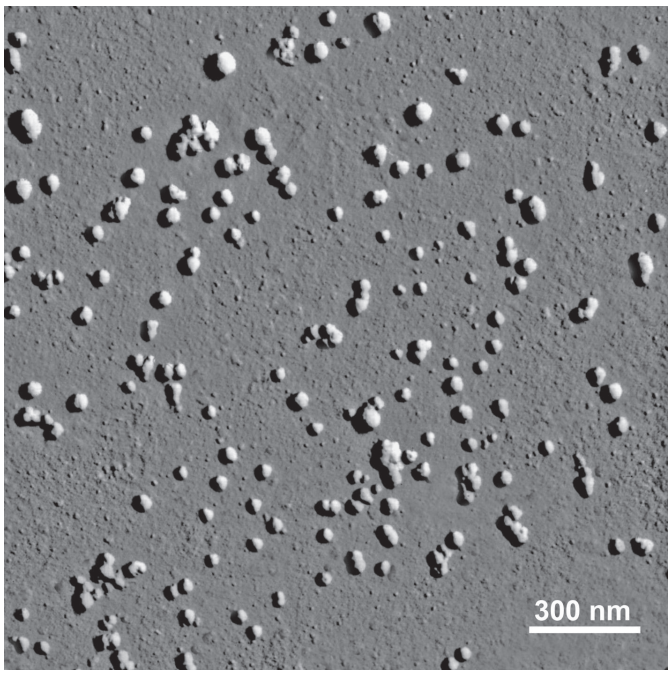

Figure 1. Casein micelles in milk

The composition of a micelle can include from several hundred to tens of thousands of molecules of various caseins, and the κ-casein molecules are predominantly located on the surface of the micelle. A distinctive feature of κ-casein is the presence of a terminal hydrophilic glycomacropeptide with a diameter of 2 and a length of about 10 nanometers [6]. The aggregate of glycomacropeptides on the surface of the micelle forms a hydrophilic «hairy» layer, which determines the hydrophilicity of the micelle and the thermodynamic stability of milk as a whole. At the same time, casein micelle has a loose internal structure containing up to 70% water, i. e. each casein micelle in milk is a stable gel particle capable of subsequent deployment under certain external influences. In addition, a hydrophilic hairy layer covers the surface of a micelle by 90–95%, which, in some cases, allows micelles to carry out hydrophobic interactions [7]. This structure of micelles predetermines their interaction with the environment and determines the possibility of implementing certain technological methods in the production of dairy products.

Most of these technological methods are based on destabilization of casein micelles, which ensures the deployment of casein molecules, their activation and removal of water from micelles.

2.2. Whey proteins

Whey proteins are a group of different globular proteins that differ from each other in structure and properties, and, despite their small amount, are the physiologically most valuable components of milk. Whey proteins, primarily, include β-lactoglobulin (55–60% of all whey proteins) and α-lactalbumin (20–25%); their molecules usually form dimers, tetramers and octamers in milk. The rest of the serum proteins are serum albumin, immunoglobulins, lactoferrin and other minor proteins [8]. β-lactoglobulin and α-lactalbumin are of the greatest importance in the technological processes of the production of dairy products. For example, high temperatures denature whey proteins, which releases the hydrophobic regions of the molecules and triggers hydrophobic interactions with other proteins and, in some cases, the formation of protein gel. These proteins largely affect the organoleptic properties of the finished product by binding to caseins and other components or forming their own macrostructures (Figure 2).

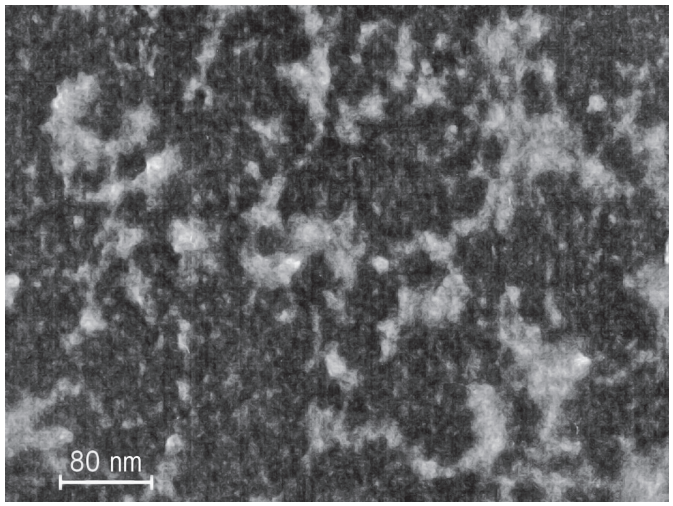

Figure 2. Nanoparticles of α-lactalbumin

Various types of whey proteins, as well as products from them, such as whey protein isolate and whey protein concentrate, are widely used in various areas of food technology, including stabilization of foam and fat emulsions, gel formation, etc. Each specific application of whey proteins may require modification of their functional properties. For example, using enzymatic hydrolysis, thermally induced polymerization, high-pressure treatment or chemical functionalization [9].

2.3. Vegetable proteins

Vegetable proteins are increasingly used in the production of dairy products. Since the range of vegetable proteins is very wide and their properties are very diverse, they are used to achieve different goals. There are several types of classification of vegetable proteins by their properties and fields of application (by origin, by solubility, by amino acid composition by structure, etc.) [10]. In nature, vegetable proteins mainly exist in a bound form, and therefore, for use in dairy products, it is necessary to first extract them from vegetable raw materials, purify and grind them. Depending on the results obtained of extraction, vegetable proteins can be added to dairy products to achieve various goals — structuring, enrichment, prevention, etc., attractive to potential consumers [11]. At the same time, vegetable proteins cannot completely replace milk proteins, neither in terms of amino acid composition, nor in terms of processing, nor in terms of organoleptic properties.

There are many attempts to create a milk-like food product. These milk drinks are obtained using vegetable proteins and various technological operations so that their components have dimensions close to those of natural milk components. However, this is difficult to achieve and, as a rule, electron microscopy can reveal the differences. As an example, Figure 3 shows an electron microscopic photograph of the dispersed phase of soy «milk». Comparing this photograph with Figure 1 makes it easy to notice significant differences in the structure of dispersed phases and to recognize such a structure in any way corresponding to the structure of natural milk is hardly possible. However, this and similar dairy drinks generally do not carry potential health risks and can be sought after by the consumer. However, there is a potential risk of the possibility of adulteration of a natural product.

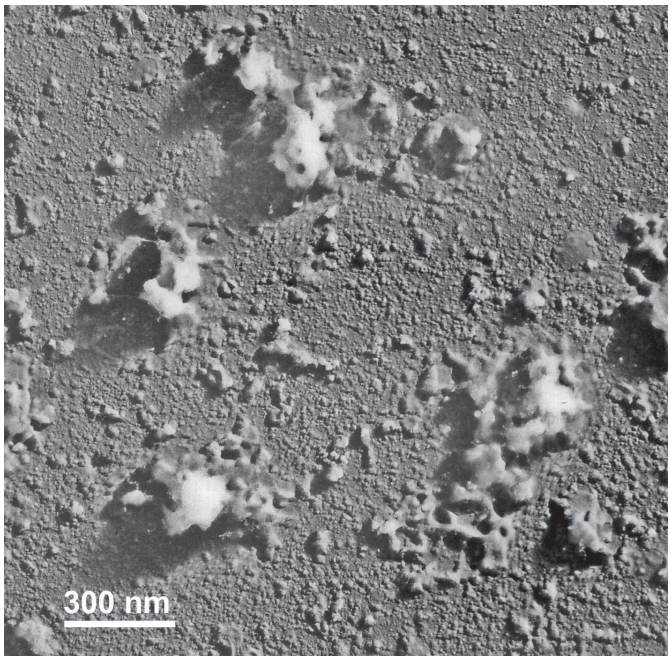

Figure 3. Particles of vegetable protein in a soy drink

2.4. Polysaccharides

Among natural biopolymers, polysaccharides are ideal candidates for widespread use in the food industry. They are non-toxic, biocompatible, biodegradable, hydrophilic, and have high biomimetic and physicochemical properties. Polysaccharides quantitatively represent the most important group of nutrients in food. For these reasons, in recent years, more and more attention has been paid to the non-traditional use of polysaccharides in the production of dairy products.

Polysaccharides are macromolecules of monosaccharides linked by glycosidic bonds. There are a huge number of polysaccharides with different composition and structure, with different physicochemical properties and specific functional use. All polysaccharides can be classified into groups depending on their origin: natural, semi-synthetic, synthetic [12].

Natural polysaccharides are biopolymers (Figure 4) of plant, animal, and microbial origin, for example, starch, chitin, pectin, xanthan, cellulose, etc.

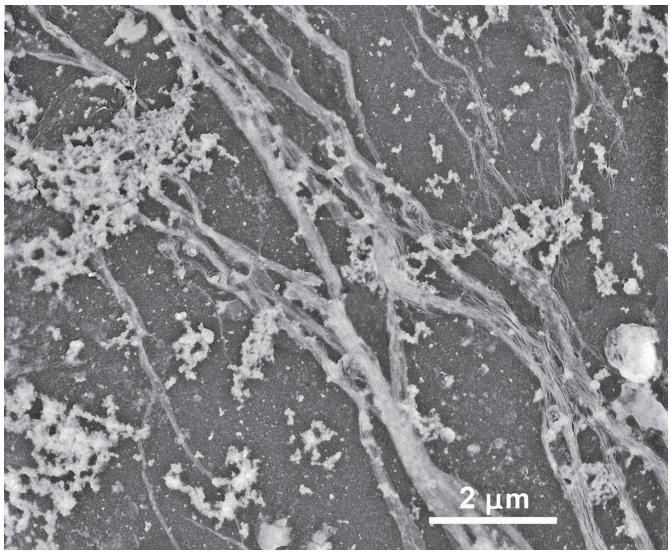

Figure 4. Polysaccharide fiber in a dairy product

Semi-synthetic polysaccharides are synthesized by modifying natural polysaccharides [13]. Starch and cellulose derivatives such as carboxymethyl cellulose and phosphorylated starch are examples of semi-synthetic polysaccharides. Semi-synthetic polysaccharides have enhanced specific properties, for example, emulsifying, non-toxic and less susceptible to microbiological deterioration.

Synthetic polysaccharides are completely produced by the chemical industry, starting from the basic components obtained during the processing of natural hydrocarbons — oil, gas. The result of the synthesis of these polysaccharides is a product with structure and properties corresponding to natural polysaccharides. Synthetic polymerization has made it possible to create analogues of natural polysaccharides such as cellulose, xylan, chitin, hyaluronan, and chondroitin, as well as unnatural polysaccharides such as a cellulose-chitin hybrid, a hyaluronan-chondroitin hybrid, and others [14]. Synthetic polysaccharides have very high rates of specific properties, do not support the development of microorganisms and can be very effective for the food industry, but their possible toxicity has not yet been fully studied, and the cost remains exorbitant.

In modern conditions, the use of natural polysaccharides in the food industry, in comparison with synthetic and semi-synthetic, is preferable due to the following advantages: economy; availability; biodegradability; biocompatibility; the ability to physical and chemical modifications; non-toxicity; environmental friendliness; public acceptance. On the other hand, the growing demand for processed foods and the increasing public awareness of the importance of fiber in food has increased the consumption of foods high in polysaccharides. However, natural polysaccharides also have a number of disadvantages associated with low efficiency (compared to semi-synthetic or synthetic) and sensitivity to microorganisms. With all these advantages and disadvantages, natural polysaccharides are gradually being replaced, and further will be replaced by semi-synthetic and synthetic ones.

According to the classification based on their chemical structure, the following main structural groups of food polysaccharides are distinguished [15]: galactomannans (guar gum, tara gum), glucans (starch, curdlan), fructans (inulin, levan), xylan, ramnan, glucomannans (alginate, konjac), arabinoxylan (flaxseed gum), galactans (agar, carrageenan), arabinogalactan (gum arabic), galacturonans (pectin), glycano-rhamnogalacturonan, glycano-glycosaminoglycans, glucosamine polymers (chitin, chitosan).

The demand and supply for environmentally friendly food ingredients with natural technological (structuring, texturizing, stabilizing) and functional potentials is constantly growing. Plant seed mucilage, which is a polysaccharide hydrocolloid with a certain physicochemical and structural conformational diversity, provides a wide range of technological and functional aspects. The review [16] examines recent advances in the extraction of mucilage from plant seeds, their characteristics, and their use as alternative hydrocolloids for use in the food industry. It has been shown that food intake with mucilage from plant seeds through the oro-gastrointestinal pathway provides modulation of postprandial glycemic and insulinic responses, counteraction of hyperlipidemia, increased satiety, and regulation of the intestinal microbiota function. In addition to their important physiological role, plant seed mucilage has interesting technological, dietary, and functional properties comparable to common commercial polysaccharide hydrocolloids. Their physicochemical and structural diversification correlates with their techno-functionality, for example, gel formation, texturing, interfacial adsorption capacity, etc.

Microbial polysaccharides are released into the environment by numerous types of microorganisms in the course of their vital activity, and therefore they are collectively called exopolysac-

charides. Due to their unique structure and physical properties, exopolysaccharides are widely used as emulsifiers, stabilizers, thickeners, texturizers, film formers and gelling agents. The most commonly used exopolysaccharides are xanthan gum, gellan gum, dextran, and pullulan [17].

Exopolysaccharides produced by lactic acid bacteria are used as natural stabilizers in fermented milk products. Figure 5 shows a photograph illustrating the interaction of thermophilic streptococci with casein micelles in yogurt and their release of exopolysaccharide (light clouds around bacteria). The ability of exopolysaccharides to regulate viscosity largely depends not only on their concentration, but also on their structure and ability to interact with other dairy compounds. The aim of the study [18] was to compare the effect of exopolysaccharides secreted by different strains of *Streptococcus thermophilus* on the formation of milk gel and rheological / physical properties (density, apparent viscosity, elastic modulus, syneresis) of the finished product. This work showed that gel formation and rheological / physical properties of fermented milk products are determined by the structural characteristics of exopolysaccharides, especially the magnitude of its negative charge, structural flexibility, degree of branching and molecular weight.

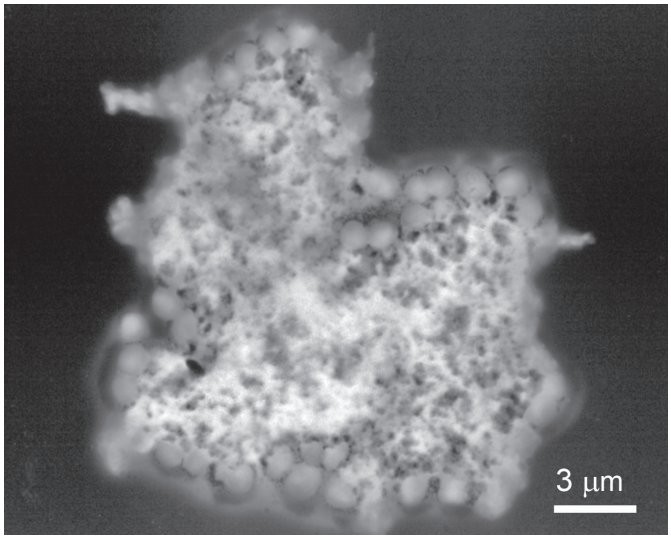

Figure 5. Exopolysaccharide *Streptococcus thermophilus* during milk gel formation

According to the main purpose of use, polysaccharides can be conditionally classified [19, 20, 21] into the following groups:

- Stabilizers that maintain a uniform dispersion of two or more immiscible components in a product;
- Thickeners that increase the texture of the product;
- Emulsifiers that ensure the stability of dispersed systems with different phases such as oil/water or water/oil;
- Gelling agents that cross-link the components of the original dispersed food systems into a single structure with high water retention;
- Texturators that provide solid food products with a given texture;
- Sensitizers that improve the organoleptic properties of products;
- Functional polysaccharides, which serve as biologically active food additives to improve the condition of the human body;
- Technological polysaccharides serving to improve the efficiency of food production.

However, some polysaccharides can simultaneously perform different functions or perform different functions under different conditions of use.

2.5. Protein-polysaccharide interactions

Proteins and polysaccharides are found in many complex multiphase food systems. Considering the fact that in the production of dairy products together with milk proteins, vegetable proteins can be used, the number of varieties of which is quite large, as well as various polysaccharides, the number of which is even greater, the number of paired, triple and more combinations of them is simply immeasurable. Despite the obvious difficulty of describing protein-polysaccharide interactions in such food systems, leading scientists in many countries are actively involved in this problem. Thus, work [22] provides an overview of the types and nature of interactions that can occur between milk proteins and polysaccharide molecules. The extensive research carried out over the past decades describing various protein-polysaccharide interactions and their effect on the structure and functionality of products is discussed.

The review [23] reports on some of the latest advances in this field, demonstrates interesting physicochemical properties of protein-polysaccharide conjugates as stabilizers and emulsifiers, as well as texture modifiers in food products. It also provides an overview of possible interactions between protein and polysaccharide, from the Maillard reaction to enzymatic cross-linking passing through coacervates.

Interactions of casein with various polysaccharides are considered in detail in the review work [24]. It is noted that, in general, these interactions can be associative or segregating. There are two main types of interaction between polysaccharides and proteins: strong association — irreversible binding of proteins with polysaccharides or strong electrostatic complexes; weak association — potentially reversible binding involving non-ionic and weak electrostatic complexes. Electrostatic interactions of proteins with charged sites of polysaccharides play a dominant role. Strongly interacting electrostatic complexes usually form between positively charged proteins and anionic polysaccharides. Weak reversible complexes can form between anionic polysaccharides and proteins that carry almost zero total charge or total negative charge. The distribution and type of charges on the surface of casein micelles create a repulsive barrier that contributes to the stability of the micelles in suspension. This means that if the repulsive and steric stabilizer layer is damaged or destroyed, van der Waals interactions appear and primary aggregation of casein micelles occurs. The polysaccharide macromolecules adsorbed in this process stabilize the dispersed system through steric and electrostatic interactions. The photograph in Figure 6 illustrates the nanostructure of casein-polysaccharide gel.

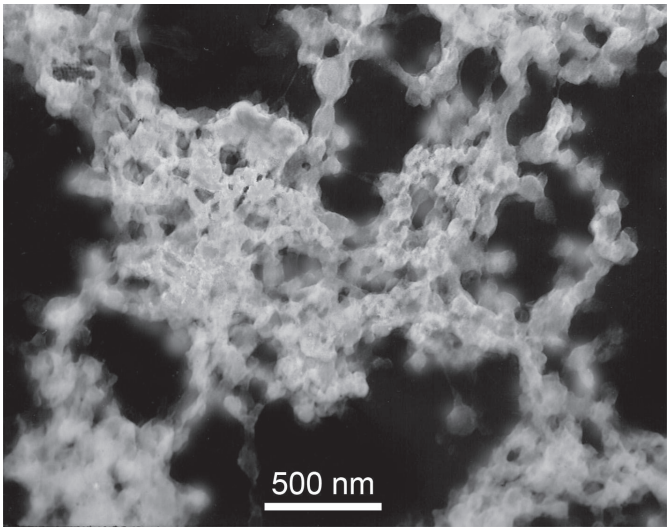

Figure 6. Nanostructure of casein-polysaccharide gel of a dairy product

The aim of the study [25] was to determine the effect of bacterial exopolysaccharide on the milk gel formation. It was determined that the result of the interaction between casein micelles and exopolysaccharide appears in the form of an integral polysaccharide-protein network structure, in which casein micelles were interconnected by exopolysaccharide. The results obtained showed that exopolysaccharides could be used to increase the density of milk gel.

In a study [26], based on the analysis of interfacial adsorption and the microstructure of complexes observed at the air-water interface, it was demonstrated that the intramolecular electrostatic protein-polysaccharide complex, with appropriate stoichiometry, can potentially act as an effective foaming agent in the food industry.

The review [27] outlines the current understanding of the nature of the interaction of casein and pectin at the molecular level in various contexts, that is, acidified milk drinks, oil-in-water emulsions, solid particles, and other colloidal systems. Influencing factors are considered, including pH, ionic strength, concentrations of two biopolymers, processing factors, and others. In addition, current and potential nutritional and pharmaceutical applications of some selected colloidal systems are discussed with illustrative examples. Understanding the mechanisms of casein-pectin interaction makes it possible to develop individual food systems that are of great use for increasing the stability of dairy drinks, encapsulation and protection, as well as for the controlled release of biologically active compounds.

The aim of the study [28] was to assess the ability of biopolymer complexes consisting of whey protein isolate and pectin to form and stabilize nanoemulsions with an interfacial structure. It has been shown that protein-polysaccharide complexes can be formed as a result of electrostatic interactions and can be useful for increasing the stability of nanoemulsions containing short-chain alkanes, which are subject to destabilization upon Ostwald maturation.

In [29], the influence of the acidity of the medium on the formation of stable complexes has been reviewed. It was shown that covalent and non-covalent (mainly electrostatic and hydrophobic) interactions contributed to the formation of stable complexes between caseins and polysaccharides, preventing precipitation near the isoelectric point of pH 4.5.

Biopolymer complexes formed by proteins and polysaccharides may have some specific or innovative functionality. However, little is known about the structural characteristics and mechanisms of molecular interaction of protein-polysaccharide biopolymer complexes. Understanding these interactions is of great interest for the development of new biopolymer complexes. In [30], the structural characteristics and mechanisms of the molecular interaction of lactoferrin and β-glucan were studied. It has been shown that the binding between lactoferrin and β-glucan at 25 °C is a spontaneous process, and electrostatic interactions, hydrogen bonds, and van der Waals interactions promote self-assembly. Self-assembly of lactoferrin-β-glucan provided the formation of physically cross-linked networks at a low concentration of β-glucan, while spherical complexes were formed at its high concentration.

The results of studies [31] have shown that the physical state of whey protein molecules (i. e., native or hydrolyzed) has a significant effect on the rate and degree of protein-polysaccharide conjugation. Low levels of hydrolysis of whey protein molecules resulted in an increase in the rate and extent of their conjugation to polysaccharides with limited associated and improved Maillard reaction products. Native and hydrolyzed solutions of the whey protein-polysaccharide conjugate had increased protein solubility and thermal stability of the solution. It is noted that the conjugation of proteins with polysaccharides represents a potential method for increasing the functionality of hydrolyzed whey proteins in food products.

The review [32] describes in detail the current trends in the creation of functional milk drinks with the addition of plant-based polysaccharides. These include drinks based on whole milk and cream, dairy by-products (whey, buttermilk), and fermented milk drinks with probiotic cultures (kefir, yogurt, etc.).

Functional drinks include health-promoting ingredients including polysaccharides of various origins in excess of the normal nutritional value of the product. The use of such polysaccharide additives as gellan gum, carboxymethyl cellulose, various types of carrageenan and pectin, as well as a number of others in dairy products, has significantly expanded the range of milk-based drinks [33, 34].

The project [35] investigated the effect of methoxylpectin, carboxymethylcellulose, and their mixtures on the stability of acidified beverages from skim and whole milk. The stability and physical properties of acid-induced skim and whole milk have been shown to be markedly transformed in the presence of these polysaccharides. The stability of the drinks improved with increasing amounts of methoxylpectin in the polysaccharide ratio, which underscores the importance of the molecular properties of this polysaccharide. The combination of these polysaccharides has been found to be better suited for stabilizing high-fat milk drinks.

Several studies [36] show that antioxidant effects are the result of a complexation reaction between phenolic compounds and milk proteins after ingestion. The main goal of the study in [37] was to determine the antioxidant and antigenotoxic effect of dairy products, milk and yoghurt after adding 2% of Korean red ginseng extract to them. This study shows that supplementing with red ginseng extract can provide enhanced antioxidant and antigenotoxic effects in dairy products and provide a new functional value-added dairy product to the market.

The work [38] provides an overview of modern knowledge about various polysaccharides of seaweed, their structural compositions, biological activity, and possible nutraceutical applications in food. It has been shown that biologically active components of seaweed polysaccharides have various useful properties, including anticoagulant, anti-inflammatory, antioxidant, anticarcinogenic and antiviral activity and can be successfully used in the development of new food products.

Protein-polysaccharide interactions were in the center of attention of scientists during research, the results of which are presented in [39]. This study aims to improve the antioxidant and hepatoprotective effects of milk proteins and whey protein hydrolyzate through non-covalent interactions with *Psyllium husk* plant mucilage (ispaghula) and Nabq mucilage. The chemical composition, phenolic content and antioxidant activity of milk-protein complexes were studied. The effect of the obtained complexes on liver function, hyperlipidemia and liver histopathology was also investigated. The results showed that the obtained protein-polysaccharide complexes had a significant effect on the normalization of the tested parameters.

The main purpose of the study [40] was to evaluate the physicochemical, rheological and organoleptic properties of ultrafiltered low-fat cheese, when adding various polysaccharides to it. It has been shown that the introduction of galactomannan and novagel into the composition of low-fat (8%) cheese in a concentration (0.1–0.5%) allows obtaining cheese with a texture and organoleptic properties very close to fatty cheeses.

2.6. Probiotics and prebiotics

Research work [41] reports on the prebiotic and antibiofilm potential of proteins and polysaccharides extracted in water from legumes, in particular from red beans (*Phaseolus*

vulgaris L.), when they are introduced into food products. Inhibition of E. coli biofilm formation reaches 79%.

The physicochemical stability of mixtures based on milk and vegetable proteins was investigated in [42]. The results obtained showed that vegetable-milk protein mixtures have rheological properties that are not a simple sum of the properties of solutions of individual proteins. Although the mechanisms underlying these effects are not fully determined in the work, it has been established that such mixtures can exhibit both synergistic and antagonistic properties that affect the physicochemical stability of food products.

The study [43] demonstrated another important effect of using a variety of polysaccharides in fermented dairy products, which significantly affect the development and existence of microorganisms. It has also been shown that different polysaccharides, when ingested, have different effects on the intestinal microbiome. The intestinal microflora respond more quickly to unstructured polysaccharides than to complex polysaccharides. The growing interest and undeniable role of prebiotics in improving the functionality of dairy products, improving their sensory characteristics and extending shelf life by suppressing the development of pathogenic microorganisms allows them to be considered as synbiotic products.

The use of the extract of whole grain quinoa flour (Weissella cibaria) in the fermented milk drink made it possible to significantly increase the viscosity of yoghurt and increase the solubility of milk protein by 54% [44], which increased its bioavailability.

Many of the polysaccharides discussed above have already been used for various purposes in the food and, in particular, the dairy industry. It is obvious that their use must be safe for humans and the environment and, therefore, an appropriate analysis of possible risks must be carried out and it is carried out. For example, the analysis carried out in [45] showed that long-term use of carrageenan in the human diet could jeopardize his health and well-being. Carrageenan over time can cause cumulative effects, manifested in the form of chronic inflammation in obesity, diabetes, and metabolic syndrome.

2.7. Nanotechnology in manufacturing

Despite the seeming simplicity of creating protein-polysaccharide compositions in food, in reality, all changes in food systems initially occur at the molecular and supramolecular levels. In this case, new nanoparticles, nanofibers, nanostructures, nanocapsules, etc. with specific properties can be formed. Micro- and macrostructures are subsequently formed from these nano-objects, which are subsequently perceived organoleptically. Thus, the properties of food are laid down at the nanoscale, and they are manifested at the macrolevel. Therefore, the research of interactions of components in food systems at the nanoscale is currently receiving much attention [46,47].

Among potential nanoparticle systems for the food industry, core-shell nanoparticles based on biopolymers are of particular interest. This interest is mainly due to their unique physical properties, including self-assembly, interfacial properties, binding capacity, as well as their high biocompatibility. In [48], various types of core-shell nanoparticles, modern methods of production, and their use as delivery systems for small molecules, proteins, and nucleic acids were considered. The main problems of their use in the food industry were also identified.

The electron microscope photograph in Figure 7 illustrates the nanostructure of the emulsion shell of a fat globule in the production of a dairy product.

The research results presented in [49] showed that nanoparticles of the ternary complex of soluble curcumin, casein, and soy polysaccharide obtained in the aqueous phase had polysaccharide surfaces, which ensured their hydrophilicity and good solubility. FTIR spectra have shown that hydrogen bonds, hydrophobic and electrostatic interactions provide the formation of ternary complex nanoparticles.

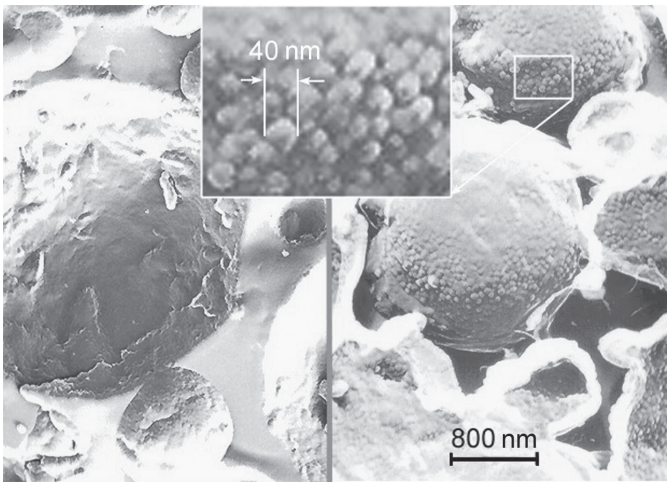

Figure 7. Nanostructure of the emulsion shell of the fat globule

In addition, nanoparticles of the ternary complex were developed and investigated in [50]. Here, a chitosan-caseinate-dextran complex has been obtained through a Schiff base reaction, which has promising properties as a delivery vehicle for oral use of lipophilic bioactive substances. In particular, it is reported that the activity of encapsulated astaxanthin, which is a potential therapeutic agent for the treatment of liver fibrosis, has been significantly improved.

2.8. Dietary fiber

The greatest positive effect on human health can be achieved when using synbiotic substances (prebiotics) in food products, which stimulate the growth and activity of microorganisms (probiotics) and improve their adhesion to the walls of the gastrointestinal tract. These properties are common to non-hydrolyzable plant oligo- and polysaccharides such as pectin, inulin, fructooligosaccharides, xylo-oligosaccharides, and starch [51,52]. Dietary fibers of various origins have different physiological effects depending on the chemical composition and microstructure. This leads to the search for new sources of raw materials and a well-defined process for their use to preserve the target functional properties of the final product. Dietary fiber lowers postprandial serum glucose levels in at least three ways. Firstly, dietary fiber increases the viscosity of the juice of the small intestine and prevents the diffusion of glucose; secondly, they bind glucose and reduce the concentration of available glucose in the small intestine; and thirdly, they slow down the action of α-amylase by encapsulating the starch and the enzyme, and can directly inhibit the enzyme.

The photograph in Figure 8 illustrates the nanostructure of dietary fiber in an innovative dairy product.

Work [53] examines current knowledge about the health effects of dietary fiber, prebiotics and dairy products. The beneficial effects of certain dietary fiber on human health — on defecation, lowering postprandial glycemic response, and maintaining normal blood cholesterol levels — are generally accepted, but other probable health benefits of dietary fiber are still debated. Although the concept of prebiotics has evolved significantly over the past two decades, the line between prebiotics and non-probiotic dietary fiber remains unclear.

Studies of the influence of processing technology of dietary fibers on their properties, the results of which are given in [54], showed that the structural modification of fibers that occurs dur-

ing processing could lead to significant changes in fiber properties. The processing steps must be clearly defined to achieve the desired functional properties.

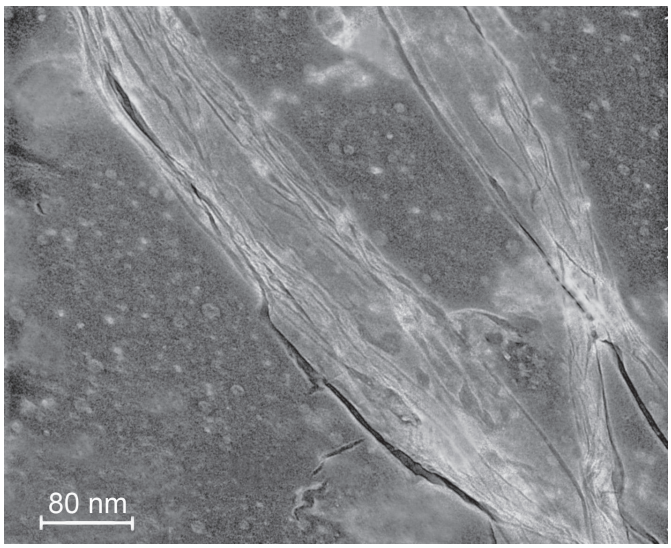

Figure 8. Nanostructure of polysaccharide dietary fiber

The effect of the addition of dietary fiber (acacia gum, inulin, and pectin) on the kinetics of milk coagulation was investigated in [55]. It has been shown that the inclusion of any fiber source significantly alters the kinetics of milk rennet coagulation. The level and significance of these changes depends on the added ingredient. The addition of acacia gum and pectin resulted in a significant reduction in duration of gel formation. The addition of acacia gum or inulin led to an increase in the elastic modulus of the coagulum. While the addition of pectin initially reduced the coagulation time, higher levels of the ingredient resulted in its increase.

The innovative development of food products, including milk proteins and dietary fiber, increases the bioavailability of individual components to meet consumer needs, while improving their sensory properties. A review [56] of significant results in the developing field of dairy protein-ligand interactions may help in planning experiments to create the required products. It is noted that at a fundamental level, it is necessary to understand the kinetics of molecular transport of biologically active compounds, including after heat treatment, from compositions based on protein and dietary fiber in nutraceutical-type products.

Along with the use of dietary fiber in dairy products, attempts to develop and use synthetic dietary fibers continue. For their synthesis, both proteins of various (dairy) origin and polysaccharides are used. Thus, in [57], an electromechanical (electrospinning) method for producing dietary fiber from maltodextrin with whey protein isolate and soy protein isolate is considered. The study demonstrated the successful electrospinning of dietary fibers from aqueous solutions and the dependence of fiber properties on their composition.

2.9. Technological applications

The results of studies of the interaction of casein micelles with κ-carrageenan and λ-carrageenan, carried out using scanning electron microscopy [58], showed that the ability of κ-carrageenan to form gel, in contrast to λ-carrageenan, is explained by the ability of κ-carrageenan to form larger aggregates at a similar concentration of carrageenan. This is achieved by the formation of κ-carrageenan strands, to which casein micelles are attached, which is not observed for λ-carrageenan. It is also suggested that part of the product structure arises from the unfolding of casein micelles upon dehydration.

The effect of κ-carrageenan on acid-induced gelation of whey proteins was studied in [59], where the effect of adding κ-carrageenan on the relationship between the total charge density of proteins and pH was determined and it was shown that an increase in the gelation temperature leads to an increase in its elastic modulus.

Four polysaccharides of plant origin — okra polysaccharide, apple pectin, sodium alginate and konjac glucomannan — were studied in yoghurt to study their effect on gelation characteristics [60]. The results showed that okra polysaccharide, konjac glucomannan and apple pectin increased the water holding capacity, density and elasticity of yoghurt, while sodium alginate showed opposite effects. Research has also shown that the addition of okra polysaccharide and apple pectin reduces the porous structure of the gel and promotes the formation of larger protein clusters, ultimately resulting in a more compact protein network.

The work [61] was devoted to the assessment of the microstructural, rheological, and textural state of aqueous mixtures of sodium caseinate and gum container and their acid gels. Acid gels with different microstructure and texture were obtained, depending on the concentration ratio of both biopolymers, either a continuous network of a protein gel or a water-in-water emulsion was observed.

Rheological and microscopic studies of the stabilizing effect of salecan on the structure of yoghurt were carried out in [62]. Molecules of salecan, a linear, negatively charged polysaccharide representing (1 → 3) -β-D-glucan, formed an additional string structure in yogurt that anchors casein micelles. The new structure provided yoghurt with increased stability, improved rheological

The effect of pullulan on the physicochemical properties of yoghurt was investigated in [63]. It was determined that yoghurt with 1% of pullulan has a weakened milk gel structure, with a reduced initial viscosity and increased syneresis compared to the control. The addition of 2% of pullulan significantly improves product stability by increasing gel strength, higher viscosity, and reduced syneresis. This study demonstrated that the physicochemical properties of yoghurt are largely determined by the concentration of pullulan in yoghurt.

In an experimental study [64], a soluble fraction of polysaccharides was isolated from the fungal fruit of *Pleurotus ostreatus mushrooms* (oyster mushroom), which was used to stabilize a casein-gluconate matrix of kefir with a low lactose content. Research has shown that the soluble fraction of polysaccharides added to milk before fermentation affects the texture of the finished kefir, organoleptically improving its texture.

The aim of the study in [65] was to evaluate the properties of yoghurt powder obtained by drying in a drying cabinet; carrageenan was added to the yoghurt at the initial stage as a stabilizer. It was shown that the optimal concentration of carrageenan was 2%, while the resulting yoghurt powder had the highest bulk density and the best granulometric texture. The water activity in the resulting product was rather low, which ensured a long shelf life. Better recovery of the yoghurt was achieved using warm water, resulting in a stable product with no visible phase separation.

2.10. Whey protein binding

The study of the mechanism of interaction between whey proteins and polysaccharides is the subject of work [66]. The structure-function relationship was determined for mixtures of basil gum and whey protein isolate at the beginning of the formation of a soluble complex, the formation of the most soluble complex and the prevailing thermodynamic incompatibility. Regardless of the ratio of the components in the mixture, all dispersions showed the maximum interaction at pH = 5.0, the beginning of the formation of a soluble complex near pH = 6.0, and the behavior of the interaction of thermodynamic incompatibility at pH = 7.0. Cole-Cole

diagrams based on dynamic rheometry confirmed the Gibbs free energy change of mixtures based on intrinsic viscosity data. The results obtained are important for creating new structures from mixtures of proteins and polysaccharides.

The effect of various extracts of chia and flax seed mucilage on the kinetics of acid gelation of whey proteins was investigated in [67]. Based on a model adapted to the Flory-Stockmeier theory, it has been demonstrated that the presence of mucilage extracts from plant seeds inhibits the cross-linking ability of whey proteins, which leads to the formation of short protein crosslinks and therefore reduces gel hardness. However, it was found that the rate of formation of elastically active structural networks increases with a certain content of mucus extracts, which indicates a synergistic effect of gel structuring. The mucilage type and the protein to polysaccharide ratio mainly determine the structuring and stabilizing properties of seed mucilage extracts in cold-set whey protein gels.

In [68], the results of studies of the surface and gel rheology of whey protein isolate under the influence of hydrophobization, thermal denaturation, and the addition of erythritol were presented. Hydrophobization shortened the linear viscoelastic area and decreased the surface tension modulus. The addition of erythritol to the hydrophobized protein caused a further decrease in the surface tension modulus. Hydrophobization of whey proteins followed by the addition of erythritol to the hydrophobized protein dispersion made it possible to form water-water (W/W) emulsions of proteins and alginate. Modification of whey proteins through hydrophobization can alter the cold-set gelation process and the rheology of the whey protein isolate gel. The cold-set gelling process occurs in two stages. Whey proteins are initially denatured by heat under non-gelation conditions (i. e., under low ionic strength, low protein content, and at pH values far from the protein's isoelectric point, pI), resulting in the formation of soluble whey protein aggregates. The protein dispersion can then be gelled at ambient temperature by adding salt and / or adjusting the pH in the pI direction. The rheology of such gels is determined by the interaction of aggregating protein particles, which strongly depends on the chemical composition of the aggregates [69].

The production of dairy products using high pressure is one of the new technologies for non-thermal milk processing, which makes it possible to obtain mixed protein-polysaccharide gels that can encapsulate and store thermosensitive biologically active compounds. The study [70] studied the properties of mixed gels of β-lactoglobulin and κ-carrageenan, obtained under pressure (0.1–600 MPa) for 30 minutes at 25 °C. The results showed that the pressure required to form these mixed gels was at least 400 MPa. Exposure to high pressure induced hydrophobic interactions between the components of the mixed gel at all pH values, and the gel structure was more compact and uniform at higher pressures.

The work [71] presents the results of systematic studies of the relationship between the electrostatic interaction of whey protein isolate / sodium alginate and the viscosity of the foam, the characteristics of interfacial adsorption, and the microstructure of the complexes observed at the air-water interface. It has been demonstrated that the electrostatic protein / polysaccharide complex can potentially act as an effective foaming agent in the food industry.

The aim of the research was [72] to study the effect of exopolysaccharides produced in situ by bacteria *Leuconostoc pseudomesenteroides* and *Weissella confusa* on the properties of the protein concentrate of common beans. A clear improvement in rheological and textural properties was shown, which was observed in pastes with the addition of sucrose after fermentation.

2.11. Packaging

Proteins and polysaccharides, as well as their compositions, can be used to not only create new dairy products or improve their properties, but also to increase the efficiency of technological processes for their production and storage. For example, the review [73] considers the creation of packaging materials for food products, their mechanical and thermal stability, biodegradability, non-toxicity, and antibacterial activity when using bionanocomposites of various proteins and polysaccharides. It is noted that along with the positive properties of polysaccharides, such as chitosan, carboxymethylcellulose, starch and cellophane, in relation to packaging materials, polysaccharides have some deficiencies, for example, poor mechanical properties and low water resistance. It has been shown that these deficiencies can be eliminated in the development of bionanocomposites based on polysaccharides. An article [74] is also devoted to a review of current advances in research and development of protein-based bionanocomposites for use in food packaging. The interest in protein-based biocomposites is due to their stability, renewability, biodegradability, and low carbon footprint. The inherent deficiencies of protein-based materials for food packaging are their low mechanical strength, poor thermal, barrier, and low physicochemical properties. Bionanocomposites based on the combined basis of proteins and polysaccharides provide an opportunity to overcome these problems and can displace non-biodegradable plastic for food packaging made from petroleum resources.

In [75], it is noted that replacing existing plastics with modern packaging materials obtained from non-renewable sources requires the creation of new environmentally friendly biopolymers. Natural products such as protein-containing waste from dairy plants have significant potential for bioplastics. Rich in casein and whey proteins, material collected from dairy wastewater using a flotation procedure is self-associating and has the potential to form bioplastics, but produces brittle films. The use of additional biopolymers such as polysaccharides to form composites with these proteins has the potential to improve the physical properties of such films.

2.12. Manufacturing applications

Industrial wastewater from dairy plants contains a variety of valuable residual materials, including lactose, milk fat, phospholipids, and milk proteins. It is clear that there are economic, environmental and social benefits to the efficient recovery of these materials from dairy wastewater. Since the volume of this wastewater discharge is enormous and the concentration of materials is low, isoelectric precipitation, salting out and ion exchange adsorption are not cost effective. Work [76] demonstrates the promise of using the flotation method for the extraction of casein from wastewater generated during milk processing. It has been shown that the best results are obtained by using xanthan gum as a foam stabilizer based on the association between a protein and a polysaccharide. Electrostatic interactions between protein and polysaccharide, which is significantly dependent on pH, provide a close relationship between them and have a noticeable effect on surface tension, foaming, foam stability, zeta potential and average particle size of the dispersion. The level of extraction of casein from dairy wastewater using the flotation process reached 86 percent in the experiment.

3. Conclusion

The interaction of milk proteins with polysaccharides of various origins and possessing a wide variety of properties, as well as the use of non-dairy proteins in the production of dairy products, has recently attracted the attention of researchers in many countries of the world. The nature of protein-polysaccharide interactions, depending on the proteins and polysaccharides used in various combinations and the conditions of their interactions can be completely different. Therefore, despite the different

goals pursued by researchers, ultimately all research is aimed at finding basic patterns in these interactions.

Knowledge of the basic patterns of protein-polysaccharide interactions makes it possible to simplify the achievement of the following main goals in food production:

- obtaining balanced food products for functional purposes;
- involvement in the production of new food raw materials;
- improving the quality of products;
- increasing the shelf life of products;
- increasing the efficiency of production processes;
- reduction of production waste;
- creation of biodegradable non-toxic packaging materials.

Modern studies of protein-polysaccharide interactions are based on the use of high-tech analytical equipment, such as electron and confocal microscopy, spectrometry of various types, etc. As these studies have shown, the physicochemical and, accordingly, organoleptic properties of food products are laid down at the nanoscale, and are already manifested at the macro level at the consumer.

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HISTORICAL AND STATISTICAL DATA ON THE DEVELOPMENT OF THE DOMESTIC ALCOHOLIC BEVERAGES INDUSTRY

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alcoholic beverages, statistical data, beverage history, wine, beer, cognac

ABSTRACT
The method of historical and statistical data analysis makes it possible to identify development and characteristic patterns, both temporary and permanent, production criteria for various branches of the food industry. The application of this method made it possible to trace formation of the alcohol industry in Russia and identify critical historical events that influenced its development. The article presents and analyzes statistical data on the production of the main types of alcoholic beverages industry since 1913.

1. Introduction

Every person, every nation at all times had, has and will have its own favorite beverages, which correspond to the national flavor and personal preferences. Therefore, the range of beverages produced in the world is very large. It is also difficult to analyze in detail the history of origin and statistics of the beverages production and consumption. This is due to the fact that not only each kind and type of beverage, but also each brand has its own rich history, covered with legends about the origin and production, which has always aroused the interest of not only specialists, but also a wide range of consumers. When analyzing the available statistical data, a problem arises related to the allocation of individual subgroups of produced beverages, especially in modern times.

To begin with, consider a brief history of the alcoholic beverages' main types origin.

2. Main part

In literary sources, the history of wine is most fully covered, which is associated with its special role in the history of mankind [1,2,3,4,5,6]. Wine along with flour, oil, etc. refer to the first food products obtained by humans as a result of primary raw materials processing [7]. The word wine comes from the Latin word «*vinum*». It is possible that the Latin word «vinum» was transformed into other European languages, and then into Slavic. Another opinion should not be ruled out that it is based on the Indo-European «*voino*», which passed into the Semitic (Hebrew) «*jajin*», the Arabic dialectal «*waynun*», and later — the ancient Greek «*ojnos*». The analysis of the transitions and their sequence allows tracing the ways of spreading the winemaking culture in Europe [1,2,8].

Experts do not have a consensus about when and where winemaking appeared. One can only reliably assert that grapes were cultivated already 5–7 thousand years ago [8].

In 1668, a Benedictine monk of Dom Pierre Perignon (1638–1715), a winegrower and winemaker from the Abbey of Hautevilliers in the center of Champagne, receives a wine with sparkling properties and original taste, which quickly gains worldwide fame after the information publication in 1718 about the *champagne* production technology [7,9].

The first mention of the cognac production in literature is found in 1630. Then winemakers used distillation to produce grape vodka from wine. And for a higher concentration of alcohol, secondary distillation was performed. The new beverage was named after the place of its production — the Cognac city (department of Charente). The production of modern cognac began only at the beginning of the 18th century, when, due to the war between France and England, French winemakers discovered that when stored for a long time in oak barrels, alcohols acquire a special pleasant bouquet and taste.

Cognacs produced outside the Charente wine region are called brandy. The name of the drink comes from the word «*brandwijn*» or «*brandewyn*», which in Dutch means «distilled» or «burnt wine».

The history of whiskey production goes back more than five centuries (the production of whiskey in Scotland in 1494–1495 was founded by the monk John Cor), but a number of experts dispute this statement and believe that whiskey began to be produced in Ireland as early as the X–XI centuries. [10]. Whiskey is a product of the distillation of fermented wort made from rye, corn and barley malt, followed by aging in oak barrels fired from the inside [11].

Beer — belongs to the oldest low-alcohol beverages, the history of which is more than 5 thousand years old. The first mention of beer among the Slavs dates back to 448. According to the geography of prevalence and the variety of trade marks, beer can be considered the most demanded beverage, the production technology of which has been improving for centuries [12].

The homeland of vodka is Russia — this is the opinion of V. V. Pokhlebkin, having studied a large number of historical materials (archives, archaeological data, etc.) [13, 14]. At the same time, there is evidence, which should be treated with a great deal of doubt, that vodka began to be produced in Poland in 1405 [10].

A number of national beverages should also be attributed to vodkas — *kahasa* (Brazilian made from sugarcane), *pulque and tequila* (Mexican made from fermented agave juice), *bamboo* (Indonesian bamboo), *hanshina and maotai* (Chinese wheat and rice), *shochu and sake* (Japanese rice), *plum brandy* (Bulgarian plum vodka), *mulberry oghi* (Armenian mulberry), *chacha* (Georgian grape), *arak* (from fruit and vegetable raw materials, from rice, from a date palm), *grappa* (Italian from grape pomace), etc. [10].

The beginning of the appearance of domestic winemaking and wine can be considered the XVII–XVIII centuries [7,15].

It is assumed that at the beginning of the XVII century Persian merchants brought Transcaucasian vines to Astrakhan and handed them over to a local monk for planting near the monas-

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tery. Viticulture and winemaking in this area began to develop successfully. In 1613, wine began to be supplied for the royal table. According to the surviving documents, in 1658 the governor Romodanovsky sent 41 barrels of church wine to Moscow. From the tsar's charter dated January 17, 1659 addressed to the Astrakhan governors Prince Dmitry Lvov and Nikifor Beklemeshev, it is known that Paskayunos Padavin was in charge of state winemaking, who was ordered to prepare exclusively church wine [2,8,15,16].

In the lower reaches of the Don, winemaking began in the time of Peter I, who in 1706 ordered the planting of vineyards there. In 1794, the Don Cossacks were resettled to the Kuban, and vineyards were also planted there. The region from which vineyards spread to Russia was also Crimea [17, 18].

Wine production in Russia almost until the end of the 19th century was of an artisanal nature [19,20].

At the beginning of the twentieth century, there were few large vineyards in Russia. Winemaking was also technically imperfect. The grapes were processed using primitive techniques and technology.

In 1913, about 3 million dcl of grape wine were produced. Expensive vintage wines were imported from abroad, mainly from France, Germany, Greece, Austria [19].

Sparkling wines prepared according to French technology were first obtained in 1799 in the Crimea, but in the middle of the 19th century this production was practically closed. And it was restored thanks to the efforts of L. S. Golitsyn (1845–1915) at the very end of the XIX — beginning of the XX century. Champagne was used only for domestic Russian consumption, so this production did not receive much development. The highest level of champagne production in Russia was reached in 1913–479 thousand bottles [21,22,23,24].

Brandy production originated in Kizlyar, where the production of grape alcohol already existed. At the end of the XIX century production of cognac began in Russia (Tbilisi, 1888). Cognac production received successful development: by the end of 1913, there were already 43 factories in Russia, which in total produced 435.4 thousand dcl (354 thousand buckets; 1 bucket was considered equal to 12.3 liters) of cognac [10,13,25].

Brewing on the territory of Russia has the longest history, which was formed in ancient times. By 1913, 1,016 breweries were operating in Russia, but beer production was insignificant. In terms of beer production, Russia lagged behind the United States 7 times, Germany — 6.2 times, and England — 5.3 times.

By 1914, brewing industries in Russia had formed into an industry with a fairly high level of production concentration: 85 of the largest breweries with an annual capacity of over 24,600 hl each produced 58.5% of the total beer output, and 822 plants with an annual capacity of up to 12,300 hl produced only 26, 2% of all products of the brewing industry [20,26,27].

In connection with the outbreak of the First World War, the sale of all alcoholic beverages, including beer, was prohibited; all breweries were closed.

In distillery production by 1913 the number of small factories was decreasing, and the share of large and medium-sized distilleries accounted for 86% of the total produced alcohol. Distilleries of an industrial type, in contrast to agricultural distilleries, producing alcohol only from September 1 to May 1, were the largest of the enterprises producing alcohol, better technically equipped, had more advanced technology and production organization.

Before the First World War, Russia took the first place in the production of alcohol in the world (65 million dcl against a world production of 250 million dcl). In Russia, 3.3 liters per capita were produced, of which 89.6% was consumed for drinking, and 10.4% — for technical needs (for comparison: Germany produced 5.4 liters per capita, of which 51.8%, and for technical — 48.2%) [19.28].

Table 1 shows data on the alcoholic beverages production in 1913 [19].

Table 1

Information on the alcoholic beverages production in 1913

Products	Production
Ethyl alcohol, mln dcl	46.7
Vodka and vodka products, mln dcl	118.9
Beer, mln dcl	80.6

It is necessary to note one specific feature characteristic of Russian enterprises at the beginning of the 20th century. Among the enterprises, a special group stood out — qualification enterprises. Qualification enterprises included enterprises that used steam engines or 15 or more workers in the absence of steam engines [7]. Table 2 shows data on qualification enterprises in Russia at the beginning of the 20th century.

Table 2

The structure of qualification food production

Food production	Enterprises		Workers	
	число	% [1]	число	%
Production of kvass, vinegar, mineral waters and sparkling beverages	58	0.73	1000	0.26
Malt	101	1.27	3452	0.90
Distillery, yeast-distillery and alcohol-purifying	2754	34.65	43775	11.42
Vodka and liqueur	64	0.80	2665	0.69
Wine and cognac	68	0.86	964	0.26
Brewing and mead	1139	14.33	23246	6.07
Wine warehouses[2]	342	4.30	39473	7.95

[1] Data on the percentage refer to the total number of food production in the country, including those not included in the list given in column 1 (for example, coffee, tobacco, salt, tea, etc.); therefore, their sum is less than 100%.
[2] In state-owned wine warehouses, alcohol was monopolistically processed into vodka, so the aforementioned vodka and liqueur production has such a small share in total.

The First World War dealt a severe blow to the food industry in Russia. For 1914–1917 according to official data, 1,087 qualification food establishments were closed [29]. The volume of distillery production decreased over the specified period by more than 10 times (it should be noted that the sale of vodka during the war in the country was prohibited).

The 1917 revolution and the ensuing Civil War caused a further decline in the food industry. Measures to food production nationalization could not have an impact on the growth of output, but, to a certain extent, reduced the rate of decline in output. Table 3 shows data on the decline in the production of alcoholic beverages in 1920 compared to the pre-war level.

Table 3

Reduction of alcoholic beverages in 1920 compared to the pre-war level

Production	1920 in% to 1913
Distillery, yeast and vodka	0.5
Beer-mead	0.3

1920 was a year of extreme decline in domestic food production, when, after a four-year world and three-year Civil War, all foundations of the industry were shaken to the ground and in terms of production level, the state of the material and technical and raw material base, it was thrown back many years [20,30].

Only by the end of the 20s of the twentieth century there was a stable growth in food production, but in many areas the results of 1913 were unattainable. So, in 1928, 23.3 million dcl of crude alcohol were produced, which amounted to 50% of its production in 1913 [26.28].

In the first five-year plans (1928–1932 and 1933–1937) 6 new distilleries were put into operation. The planned economy, the dispersal of food enterprises throughout the USSR, the creation of powerful industries in fast-growing cities allowed in 1940 to significantly exceed the indicators of 1913 (Table 4).

The Second World War interrupted the development of the food industry and required a radical restructuring of all its work in relation to wartime conditions. During the war, 649 distilleries were destroyed [28,32].

By the beginning of 1946, 3,223 food enterprises were restored, including distilleries and wineries [32]. Nevertheless, the loss of production capacity and the raw material base was so great that the level of production of the alcoholic beverage industry was thrown back many years as a result of the war.

The main task of the fourth five-year plan (1946–1950) was to restore the affected areas of the country, restore the pre-war level of industry, and then surpass it. Since 1950, the implementation of the fifth five-year plan (1950–1955) began, in which significant emphasis was placed on improving the performance of the food industry in 1940.

A significant increase in the scale of production of alcoholic beverages in the fifth five-year period is evidenced by the indicators of the production of its most important types, shown in Table 5 [28,31].

It should be noted that in 1958 the growth in the production of agricultural raw materials not only provided domestic food production with the necessary raw materials, but also a certain surplus arose, which made it possible to proceed to a further increase in the number of industries producing alcoholic beverages.

The development of the food industry in subsequent years began to experience difficulties, there were signs of stagnation and crisis phenomena (mid-70s), but they did not affect alcoholic beverages (see Table 5).

The next five-year plan (1971–1975) partially retained the inertia of the positive development of the food industry, but by the end of the five-year period, stagnation was clearly evident. The accumulation of manifestations of stagnant phenomena continued in the next two five-year plans (1981–1985 and 1986–1990) [13, 19].

The collapse of the USSR in 1991 led to the creation of modern Russia, but caused colossal damage to the country's economy, including the production of the main types of alcoholic beverages (Table 6) [32].

At the turn of the 21st century, the Russian alcohol industry faced the challenge of survival and preservation. At this time, a modern classification of the main branches of the food industry was introduced [33,34,35,36,37].

Table 7 provides a list of the main sectors and industries (sub-sectors) of the modern alcoholic industry. It should be noted that the production of beer is combined with the production of nonalcoholic beverages [33,36].

Table 4

Production of the main types of alcoholic beverages during the pre-war five-year plans

Products	Years			1937 in% to 1913 г.	1940 in% to	
	1913	1937	1940		1913	1937
Crude alcohol, mln dcl	46.7	76.7	89.9	164.2	192.5	117.2
Vodka and vodka products, mln dcl	118.9	89.7	92.5	75.4	77.7	103.1
Grape wine, thousands of dcl	30000	9015	19695	—	65.6	218.4
Cognacs, thousands of dcl	125	278	268	—	214.4	97.4
Champagne, thousands of bottles	479	556	8019	—	17 times	14.4 times
Beer, thousands of bottles	80.6	89.7	121.3	111.2	150.4	135.3

Table 5

Production of the main types of alcoholic beverages in the 50s – 60s of XX

Products	Years			1955 in% to			Years		1965 in% to 1958	1970 in% to 1965
	1940	1950	1955	1940	1950	1958	1965	1970		
Crude alcohol, mln dcl	89.9	73.0	127.8	142.1	175.0	163.9	236.0	—	143.9	—
Grape wine, mln dcl	19.7	23.8	47.4	239.1	197.9	61.8	133.9	268.0	216.7	200.1
Beer, mln dcl	121.3	130.8	181.4	152.2	141.2	199.1	316.9	419.0	159.2	132.2

Table 6

Production of alcoholic beverages in 1992–2000

Products	Years			2000 in% to 1992
	1992	1995	2000	
	Alcoholic beverages:			
in absolute alcohol, mln dcl	76,3	60,8	74,4	97,5
in kind, mln dcl:				
vodka and alcoholic products	152,0	123,0	123,0	80,9
grape wines	39,8	15,2	24,1	60,5
fruit wines	2,5	7,6	2,8	112,0
champagne and sparkling wines	7,6	8,2	6,8	89,5
cognacs	1,8	0,9	1,7	94,5
beer	279,0	213,0	516,0	184,9

Table 7

List of the main branches of the alcohol industry

No.	Branch	Production
1	Alcoholic and distillery	1. Alcoholic
		2. Distillery
2	Winemaking	1. Production of wine materials and wines from grapes and fruit and berry raw materials (primary winemaking)
		2. Processing and packaging of wines, finishing of finished products (secondary winemaking)
		3. Production of champagne and sparkling wines
		4. Production of cognac distillate and cognacs
		5. Processing of secondary raw materials for winemaking
3	Non-alcoholic beer	1. Malt
		2. Brewing
		3. Non-alcoholic
		4. Kvass
		5. Extraction and packaging of mineral waters
		6. Production of beverage concentrates
		7. Carbon dioxide production

The collapse of political power in 1991, the subsequent sharp change in the social way of life of the people, the disruption of financial and industrial ties — all this could not affect the economic indicators of the country, both in the short and long term. In the period 1991–2000 there was a collapse of industrial production, including the release of food products. The collapse affected both the number of operating enterprises, and, naturally, the volume of production [33,34,35,36,37].

The indicators of the use of sown areas can serve as evidence of overcoming the crisis of 1991–2000. The growth in acreage indicates the confidence of agricultural producers in the future, which is a kind of guarantee for food producers, including alcohol producers (Table 8) [38,39].

The results of the alcohol industry in Russia for 2000–2011 show that the industry overcame the impact of the 2008 crisis and, based on the results of 2011, as a whole reached a stable level of development. The decline in vodka consumption is a positive trend against the background of the population's desire for a healthy lifestyle. The reserve made for the future allowed the continued stable development of the food industry.

Table 8

Production of alcoholic beverages in 2010–2011

Products	Years		Rate,%
	2010	2011	period of 2011 to the period of 2010
Beer, mln dcl	977.4	994.1	101.7
Vodka and alcoholic products, mln dcl	106.3	93.9	88.4
Low alcohol beverages (with an alcohol content not exceeding 9%), mln dcl	30.2	31.9	105.5

The statement that the food industry in Russia has taken the path of formation and consistent development is confirmed by real production data. Considering, that the period under review (2012–2017) includes the time of significant sanctions restrictions and the crisis of 2013, the preservation and growth of indicators of the production activity of the beverage industry suggests that the alcohol industry is in a stable state [28,31,32,33,34,35,36,37,38,39,40,41,42,43,44,45], although there is a decrease in output for certain types of products (Table 9).

Table 10 shows the final results of the alcohol industry for 2018–2019 [46,47].

It should be noted that in a number of cases, a decrease in volumes is due to the fact that new product subgroups are allocated. For example, due to changes in legislation, the classification group "beer" is divided into two — "beer" and "beer beverages", which introduces time complexity in statistical reports.

3. Conclusion

Thus, the historical and statistical collection of information makes it possible to clearly trace the development of the alcohol industry in Russia and the influence of various historical factors on it. The above data confirm that the beverage industry maintains a positive development trend.

Table 9

Beverage production. Release of the main products in 2012–2017

Products	2012	2013	2014	2015	2016	2017	Rate,% period of 2017 to 2012
Beer, excluding brewing waste, mln dcl	948.9	831.0	766.5	730.3	763.2	744.5	78.5
Ethyl alcohol from food raw materials (excluding distillates), mln dcl	48.4	46.2	40.1	42.4	47.0	66.3	137.0
Vodka, mln dcl	97.8	85.7	66.6	63.9	73.2	79.8	81.6
Cognac, mln dcl	9.8	7.4	6.9	7.7	7.6	8.5	86.7
Wine, mln dcl	36.7	33.9	32.1	40.0	36.9	32.0	87.2
Sparkling and champagne wines, mln dcl	0.7	17.2	15.6	16.1	14.7	12.8	1828.6
Low alcohol beverages (with an alcohol content not exceeding 9%), mln dcl	20.5	26.3	17.1	9.5	6.7	6.4	31.2

Table 10

Results of the food industry in 2018–2019

Products	Years		Rate,% period of 2019 to the period of the 2018
	2018	2019	
Beer, excluding brewing waste, mln dcl	774.7	770.7	99.5
Ethyl alcohol from food raw materials (excluding distillates) — total, mln dcl	59.2	55.4	93.5
Vodka, mln dcl	79.8	83.6	104.7
Cognac, mln dcl	8.4	9.2	109.5
Sparkling and champagne wines, mln dcl	12.4	13.2	106.4
Wine, mln dcl	30.4	32.8	107.9
Low alcohol beverages (with an alcohol content not exceeding 9%), mln dcl	6.8	7.6	111.7

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Original scientific paper

RESEARCH ON THE POSSIBILITY OF EXTENDING THE SHELF LIFE OF CHEESE RAW MATERIAL AND HEAT-TREATED CHEESE BY THEIR FREEZING FOR FURTHER USE IN HORECA

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KEY WORDS:
heat-treated cheese, cheese raw material, freezing, low-temperature storage, functional properties, shelf life

ABSTRACT

The article presents the results of a study of the regularities of changes in the functional properties and quality indicators of heat-treated cheeses made from frozen cheese raw material or frozen after thermomechanical processing for further use in HoReCa. The objects of the study were: Caliatta cheese — a semi-hard ripening cheese intended as the main raw material in the production of heat-treated cheese, as well as heat-treated «pizza-cheese», subjected to freezing at temperatures of minus 14 ± 2 °C and minus 55 ± 2 °C and low-temperature storage at a temperature of minus 14 ± 2 °C for 270 days, followed by defrosting at a temperature of 20 ± 2 °C. To confirm the possibility of using the freezing technique in order to increase the shelf life of both the original cheese raw material and heat-treated cheese, their microbiological and physicochemical indicators were determined by standardized methods. Studies of structural and mechanical (rheological) properties were carried out on a Weissenberg rheogoniometer, recording changes in the elastic modulus (G') and dynamic viscosity (η'). The length of the cheese thread, as one of the main functional properties of the «pizza-cheese», was assessed with a fork test after baking. Organoleptic characteristics were assessed by flavor, texture and appearance. Research results have shown that low-temperature storage of frozen cheese can be considered as a way to retard biological and physicochemical changes, which is a safe way to increase shelf life. Freezing cheese raw material increases the length of the cheese thread in proportion to the temperature and duration of the low-temperature storage. When obtaining heat-treated cheese from both unfrozen and frozen cheese raw material, a significant deterioration in the desired functional properties is observed. Thus, the receipt of heat-treated cheese from the original cheese raw material for further use in the production of pizza is justified only by economic feasibility. Freezing «pizza-cheese» at a temperature of minus 55 ± 2 °C, made from unfrozen cheese raw material, ensures the preservation of functional properties and increases the shelf life up to 150 days.

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1. Introduction

Currently, "pizza-cheeses" are the leading cheeses in terms of production growth, thanks to the development of the fast food system. The product, called "pizza-cheese", in its composition and manufacturing technology differs significantly from traditional types of cheese, being between two groups — "cheese" and "processed cheese", and its safety and quality indicators, including functional properties, are defined as raw materials and technological modes of thermomechanical treatment. The functional properties of "pizza-cheeses", including stretchability (formation of a "cheese" thread), are the same mandatory quality indicators as microbiological, organoleptic and physicochemical criteria for their assessment. "Pizza-cheeses" form a special group of heat-treated cheeses (HC) as the main raw materials for which cheeses and other milk processing products, as well as food additives, are used. Heat-treated cheeses are produced by thermomechanical processing of raw materials at a temperature not higher than 72 ± 3 °C, which is the main distinguishing feature of the processed cheese production modes and ensures the formation of specific functional characteristics [1].

In the conditions of the developing cheese market for HoReCa and the seasonality of dairy production, the problem of extending the shelf life of both cheese raw material and heat-treated cheeses while maintaining quality indicators becomes extremely urgent. Domestic and foreign researchers have accumulated experience in extending the shelf life of various cheeses using freezing and further low-temperature storage. In the practice of cheesemaking there are a number of freezing technologies for both traditional and processed cheeses, as well as curd grains. However, there is evidence that, as a result of freezing, the properties of the cheese raw material used for the production of processed cheeses can change significantly [2].

In a number of foreign countries, extensive research has been carried out to develop technology for freezing and further refrigerated storage of traditional types of cheese made from cow, goat and sheep milk. Many researchers are of the opinion about the need for relatively quick and deep freezing of cheeses to preserve organoleptic properties for 6 months at storage temperatures from minus 18 °C to minus 30 °C [2,3,4,5,6,7,8,9].

A. Conte et al. assessed the effect of the freezing speed and two-month low-temperature storage of Mozzarella cheese and found that high freezing speeds have a positive effect on the quality of the cheese. However, the authors noted a deterioration in the cheese texture, including porosity, which may be associated with the formation of ice and further protein dehydration [10].

Domestic authors also carried out research on the development of technologies for freezing and low-temperature storage of cheeses. Thus, studies carried out earlier at VNIIMS found out that freezing natural cheeses both at temperatures of minus 18 °C and minus 184 °C causes profound changes in the structure, which worsens their quality after defrosting. At the same

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time, no significant changes occur in cheeses frozen at minus 18 °C: after defrosting, the structure of the cheese is restored to a satisfactory one. The influence of low-temperature storage of raw rennet cheeses, defrosting modes on the quality of processed cheeses produced from them has been established. In samples of processed cheese made from raw materials stored for 9 months at a temperature of minus 16 °C, a decrease in elastic and viscous properties was noted, which indicates a negative effect of freezing raw materials on the quality of the finished product. At the same time, slow defrosting of raw cheese at 20 °C makes it possible to obtain processed cheeses with satisfactory structural and mechanical properties and organoleptic characteristics [11].

However, until now there are no materials that make it possible to assess the influence of technological methods of freezing, low-temperature storage and defrosting, not only on the microbiological, structural and mechanical, physicochemical and organoleptic characteristics of both cheese raw material and heat-treated cheeses, but also on the functional properties of the finished product, the quality of which is usually assessed after high-temperature processing — baking pizza. Given the relevance of the problem, the purpose of this work is to study the patterns of change in quality indicators, including the functional properties of cheese raw material and heat-treated cheeses, frozen at different temperature conditions and subjected to long-term low-temperature storage, in order to extend their shelf life.

2. Materials and methods

When carrying out the research, samples of cheese raw material of Caliatta cheese and heat-treated cheeses, both industrially produced and produced in the experimental cheesemaking department of VNIIMS, according to previously developed technologies, were used as objects.

Samples packaged with weight of 250–300 g and packed in film were subjected to freezing at temperatures of minus 14 ± 2 °C and minus 55 ± 2 °C and further low-temperature storage at a temperature of minus 14 ± 2 °C for 270 days, followed by defrosting at a temperature of 20 ± 2 °C. Freshly produced cheeses not subjected to freezing and storage were used as control samples.

In the samples under study, standardized methods were used to determine the physicochemical parameters (titratable acidity and mass fraction of moisture), as well as bacterial contamination by the number of viable cells of mesophilic (KMAFanM) and thermophilic (KTAFanM) of aerobic and facultatively anaerobic microorganisms, coliform bacteria, yeast, and spore microorganisms.

Studies of structural and mechanical (rheological) properties were carried out on a Weissenberg rheogoniometer, recording changes in the elastic modulus (G') and dynamic viscosity (η'). Samples of cheese filling the space between the conical surface and the plane were subjected to sinusoidally measured shear strain of known amplitude and frequency. The studies were carried out in the mode of periodic shear deformation with working bodies "cone-plane" with a diameter of 25 mm, with an angle at the apex of the cone of 0.034 radians, the amplitude of angular displacements of working bodies — $1.54 \cdot 10^{-3}$ rad, which achieved the linearity of this mode. The measurements were carried out at frequencies f = 0.316; 0.500; 1.000; 1.990 s^{-1}. The sample was thermostated at 18 ± 3 °C directly in the rheogoniometer. The processing of experimental data was carried out in an automatic mode using a data collection and processing system using a specially developed program [11].

The length of the cheese thread, as one of the main indicators of the functional properties of "cheese for pizza", was assessed with a fork test after baking [12,13,14]. For this, 100 g of chopped cheese was placed on a porcelain flat surface and sent to bake in an oven at 200 °C for 12 minutes. After the baking time, the cheese was cooled at room temperature for 1–3 minutes. Then the fork was immersed in the cheese by 1–3 mm and slowly, at a constant speed, lifted up until all the threads broke. Stretchability was assessed by the average length of cheese threads taken from three different locations in the melted cheese.

The organoleptic assessment of the cheeses, including the assessment of flavor, texture and appearance, was carried out according to a conventional scale.

3. Results and discussion

At the first stage of the research, there was a study of the effect of freezing, followed by low-temperature storage and defrosting of cheese raw material on changes in microbiological, structural and mechanical, physicochemical, organoleptic and functional properties, both of the initial cheese raw material and heat-treated cheeses produced from it.

Microbiological indicators of the safety of cheese raw material and heat-treated cheese, when determining the shelf life, require special attention. Table 1 shows the data on changes in the number of normalized groups of microorganisms in the process of low-temperature storage of frozen samples after defrosting. It should be considered that the inoculation of heat-treated cheese samples was carried out not immediately after production, but after 3–4 days, which provided conditions for the reactivation of cells that received a thermal shock during thermomechanical processing. Analysis of the obtained results shows that when samples are frozen at a temperature of minus 14 ± 2 °C, bacterial contamination (QMAFAnM index) decreases on average by 0.5 orders of magnitude, and at a temperature of minus 55 ± 2 °C — by an order of magnitude, therefore, freezing modes have little effect on the safety of bacterial cells.

Table 1

Safety indicators of cheese raw material in the process of low-temperature storage and heat-treated cheese produced from it

Storage point, days	Freezing mode, °C	Storage mode, °C	QMAFAnM, CFU/cm³		Coliform bacteria		Yeast, mold, CFU/cm³		Number of spores, MPN spore/cm³	
			cheese raw material	HC	cheese raw material	HC	cheese raw material	HC	cheese raw material	HC
before freezing			3.0×10^9	6.6×10^8						
90	minus 14 ± 2		9.8×10^8	1.8×10^8						
	minus 55 ± 2		4.7×10^8	2.5×10^8						
180	minus 14 ± 2	minus 14 ± 2	9.4×10^8	2.3×10^8	absent in 0.1g		absent in 0.1g		absent in 0.1g	
	minus 55 ± 2		4.2×10^8	9.6×10^8						
270	minus 14 ± 2		2.4×10^8	7.9×10^7						
	minus 55 ± 2		2.8×10^8	7.5×10^7						

It was found that under low-temperature storage conditions, as expected, there are no signs of microflora development and microbiological safety indicators do not deteriorate. It can be assumed that when the cheese raw material is frozen, the most viable cells of thermophilic starter microorganisms are preserved, and when heat-treated cheese is obtained and the melting temperature is 72±3 °C, these microorganisms survive and, having come out of the thermal shock, are able to resume their viability.

Figure 1 shows the data on changes in the number of thermophilic starter microorganisms, represented by bacteria of the species Streptococcus thermophilus, during the low-temperature storage of frozen cheese raw material and heat-treated cheese after defrosting.

Microbiological research data show that the cheese raw material was developed using Streptococcus thermophilus starter cultures, therefore the indicators of QMAFAnM and QTAFAnM (Quantity of Thermophilic Aerobic and Facultative Anaerobic Microorganisms) are almost identical. When frozen, the number of viable cells decreases on average by 0,5–1,0 order of magnitude and during further storage remains at the same level for up to 180 days. When receiving heat-treated cheese, the total number of viable cells decreases insignificantly: when receiving a product from unfrozen cheese raw material — on average by 1.0 order of magnitude, and during the production from frozen cheese raw material — by 0.5 order of magnitude.

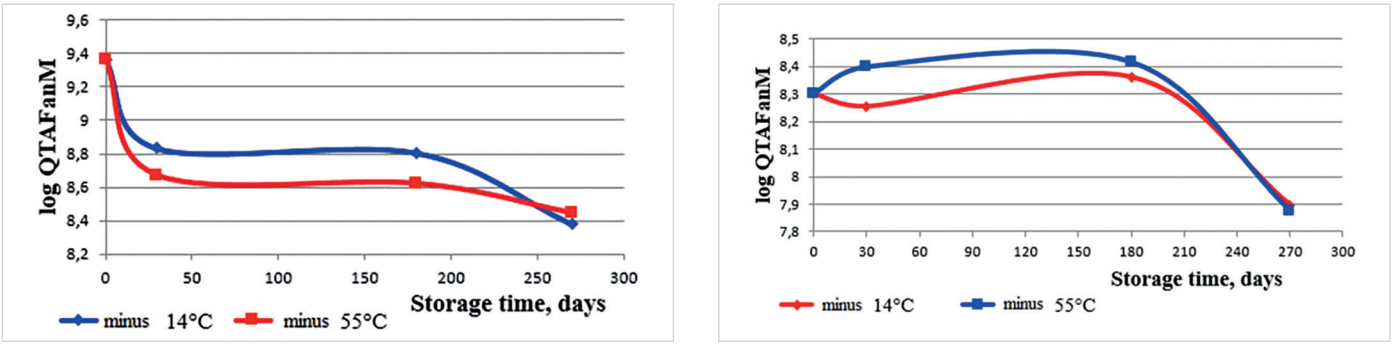
The study of frozen samples of cheese raw material and samples of heat-treated cheeses produced from them did not establish the effect of the depth of freezing and the duration of low-temperature storage on the main physicochemical parameters, such as pH and mass fraction of moisture.

Figure 2 presents the results of studies of the rheological parameters of heat-treated cheeses made from frozen cheese raw material.

When studying the rheological parameters of samples with different periods of low-temperature storage, it was found that during storage, the modulus of elasticity (G') and dynamic viscosity (η') increase in samples produced from cheese raw material stored at low-temperature conditions up to 180 days, and decrease at longer storage. A decrease in the freezing temperature to minus 55±2 °C leads to slightly larger losses of G' and η' values during storage in comparison with the freezing mode of minus 14±2 °C. It can be assumed that with an intense decrease in the freezing temperature, smaller water crystals are formed, which ensures the stability of the protein framework. In heat-treated cheeses made from cheese raw material for 270 days of storage, a slight decrease in elastic and viscous properties was noted. With the organoleptic assessment of the texture of these cheeses, an increase in the "incoherent" and "crumbly" texture and the appearance of new "mealy", "plastic" and "non-layered" defects are noted. It was found that lowering the freezing temperature to minus 55±2 °C and storing frozen cheese raw material for more than 180 days leads to a deterioration in the texture of heat-treated cheeses.

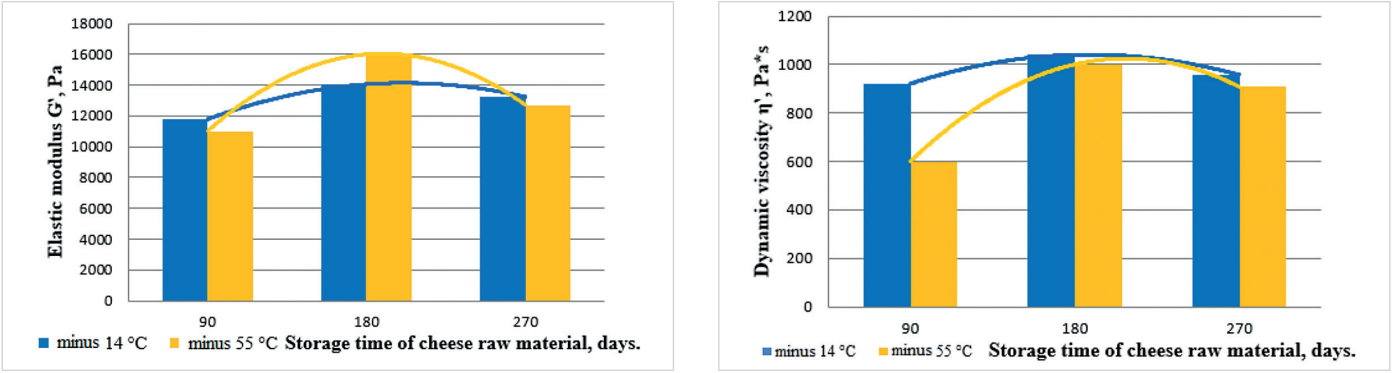
In the course of the organoleptic evaluation, it was found that the change in the flavor of frozen cheese raw material occurs immediately after freezing and further defrosting. By 90 days of low-temperature storage in all samples of cheese raw material, an excessively sour flavor was noted against the fermented milk in the original cheese raw material, the intensity of the cheese flavor weakens, and a slight foreign taste appears. The influence of the temperature regimes of freezing cheese raw material on the flavor indicators of heat-treated cheese was not revealed.

Table 2 presents the results of studies of the effect of freezing, low-temperature storage with further defrosting of cheese raw material on the functional properties of heat-treated cheeses, including the length of the cheese thread after frying.



(a) (b)

Figure 1. Change in QTAFAnM(a) of cheese raw material during low-temperature storage and (b) heat-treated cheese produced from it (point 0 — before freezing)



(a) (b)

Figure 2. Change in modulus of elasticity (a) and viscosity (b) of heat-treated cheese made from frozen cheese raw material

Table 2

Length of cheese thread after frying cheese raw material and heat-treated cheese made from frozen cheese raw material

Storage point, days	Freezing mode, °C	Storage mode, °C	Length of thread of cheese raw material, cm	Length of cheese thread of heat-treated cheese made from cheese raw material, cm
Before freezing			25	12
90	minus 14±2	minus 14±2	40	10
	minus 55±2		40	12
180	minus 14±2		45	10
	minus 55±2		60	15
270	minus 14±2		50	12
	minus 55±2		50	15

From the data presented in Table 2, it follows that the process of freezing cheese raw material increases the length of the cheese thread in comparison with unfrozen cheese and improves this indicator during low-temperature storage for more than 180 days. A comparison of the two freezing temperatures showed that freezing to a lower temperature resulted in a longer cheese thread. With both modes of freezing, an increase in the viscoelastic and plastic properties of the cheese raw material was noted, which led to a more viscous-liquid product during the frying process.

When receiving heat-treated cheese from both unfrozen and frozen cheese raw material, a significant deterioration in functional properties is observed. The length of the cheese thread after baking is reduced by 2.5–5 times. Thus, the receipt of heat-treated cheese from the original cheese raw material for further use in the production of pizza is justified only by economic feasibility.

At the second stage, studies were carried out on the effect of freezing and low-temperature storage of heat-treated cheeses made from unfrozen cheese raw material in order to establish the possibility of increasing the shelf life and further use for HoReCa. Freezing and further low-temperature storage modes were similar to those used in the previous series of experiments.

As a result of the studies carried out, it was found that the mass fraction of moisture in quickly frozen heat-treated cheese at a temperature of minus 55±2 °C and stored at a temperature of minus 14±2 °C for 150 days practically does not change. Slow freezing of pizza-cheese at a temperature of minus 14±2 °C and storage at this temperature results in a moisture loss of 1.5% after defrosting. Freezing speed and low-temperature storage have no effect on pH (Figure 3).

During the freezing and further low-temperature storage of heat-treated pizza-cheeses, the level of bacterial contamination tends to decrease slowly (Figure 4).

Figure 5 shows the dynamics of changes in the structural and mechanical indicators of heat-treated cheeses during low-temperature storage.

During low-temperature storage under both freezing modes, the elastic modulus and dynamic viscosity tend to increase. At the same time, the absolute values of structural and mechanical indicators are higher for samples frozen at a temperature of minus 55±2 °C.

When evaluating organoleptic indicators, it was found that before freezing, heat-treated cheeses had a weakly expressed cheese, slightly acidic flavor and an insufficiently dense, slightly elastic, plastic texture of medium layering. After freezing without low-temperature storage, the sour flavor intensifies. In the samples of heat-treated cheeses, frozen at a temperature of minus 14±2 °C, softness appears, layering significantly decreases and elasticity disappears. During low-temperature storage, the sour flavor is enhanced and a slight off-flavor appears. In samples subjected to freezing at a temperature of minus 55±2 °C, the cheese flavor and original layering are preserved.

When evaluating the functional properties of heat-treated cheese after frying, it was found that the freezing process at a temperature of minus 14±2 °C without storage leads to a decrease in the cheese thread by about 1.5 times, and the freezing mode at a temperature of minus 55±2 °C increases the original length of the cheese thread. In this case, the further low-temperature storage of this cheese at a temperature of minus 14±2 °C in both cases does not affect the length of the cheese thread (Table 3). Freezing "pizza-cheese" at a temperature of minus 55±2 °C, made from unfrozen cheese raw material ensures the preservation of functional properties and increases the shelf life up to 150 days.

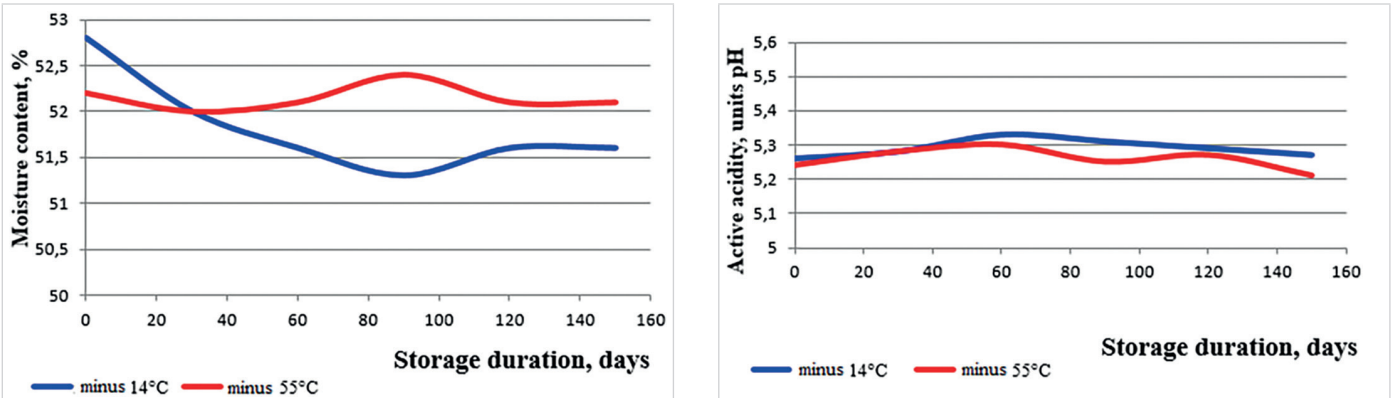
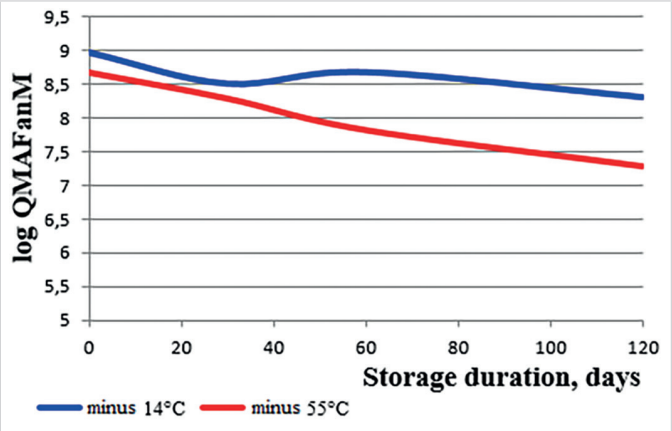


Figure 3. Change in the mass fraction of moisture and active acidity in heat-treated cheeses frozen at temperatures of minus 14±2 °C and minus 55±2 °C and stored at a temperature of minus 14±2 °C



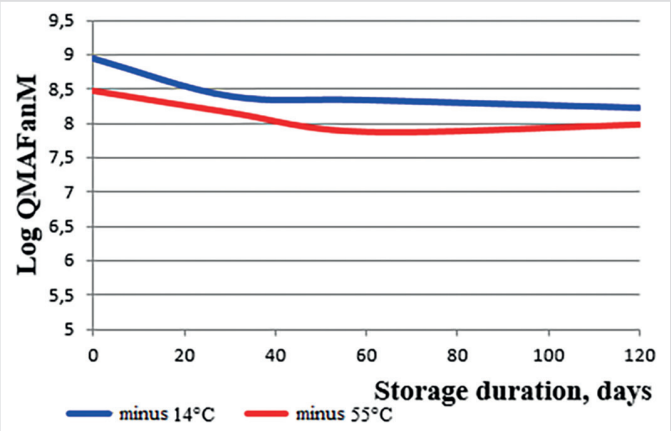
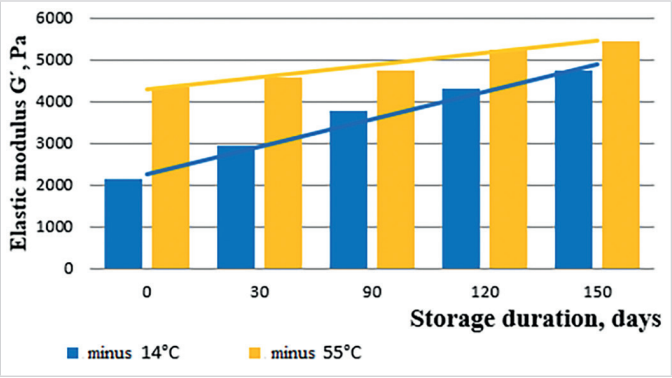


Figure 4. Change in QMAFanM and QTAFanM in heat-treated cheeses frozen at temperatures of minus 14±2 °C and minus 55±2 °C and stored at minus 14±2 °C



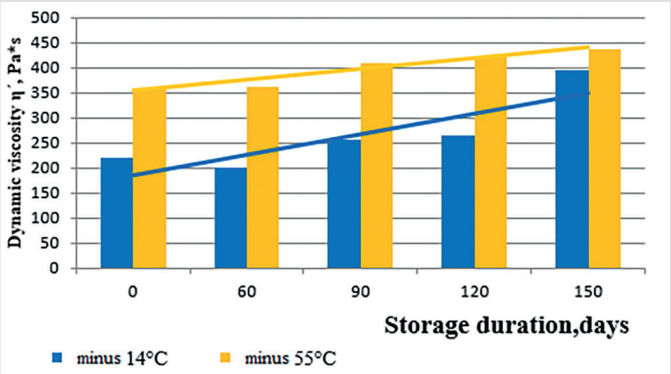


Figure 5. Change in the modulus of elasticity and dynamic viscosity of heat-treated cheeses frozen at temperatures of minus 14±2 °C and minus 55±2 °C and stored at minus 14±2 °C

Table 3

Change in the length of the cheese thread of heat-treated cheeses after freezing and low-temperature storage

Storage point, days	Freezing mode, °C	Storage mode, °C	Length of cheese thread, cm
before freezing			14
0	minus 14±2 °C	minus 14±2 °C	13
30			13
60			11
90			10
120			10
150			9
0	minus 55±2 °C		21
30			21
60			20
90			20
120			21
150			19

4. Conclusions

Thus, as a result of the studies carried out, it was found that the operations of freezing and low-temperature storage, followed by defrosting of both cheese raw material and heat-treated cheeses, affect both the quality indicators and functional properties, and the shelf life. At the same time, the direction and intensity of the influence depend on the selected freezing and low-temperature storage modes, which, in turn, should be determined based on the indicators of microbiological safety and the quality of cheese raw material and heat-treated cheese. Using the freezing technique followed by low-temperature storage, you can increase the shelf life of cheese raw material up to 180 days, and heat-treated cheese for pizza up to 150 days.

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