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Proteolytic processes in cheese analogues ripening

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Abstract. The research relevance is predefined by the theoretic basics of their production processes not being sufficiently studied, including the proteolysis despite the wide production of cheese analogues incorporating vegetable substances. The research aims to determine the effect of the content of cheese analogues, namely milk fat substitutes, soy protein isolates, and lactobacilli species *Lactococcus lactis ssp. lactis* and *L. lactis ssp. cremoris*, *L. lactis ssp. lactis* *bv. diacetylactis* as well as *Leuconostoc mesenteroides* by the physical, chemical, and sensorial traits, and by amino acids content of cheese analogues. Determination of the physical and chemical parameters was fulfilled according to the standards: active acidity (pH) – DSTU 8550:2015; solids content (by weight) – DSTU 8552:2015 and ISO 5534:2004; non-casein nitrogen content (by weight) – ISO 17997-1:2004; total protein (by weight) – ISO 8968-1:2014 and ISO 8968-5:2001; casein content (by weight) – ISO 17997-1:2004. The fractional composition of proteins was determined using the Polyacrylamide Gel Electrophoresis. While in the cheese dough after analysing the differences in the spectrum of free amino acids between the variants were insignificant, in ripe cheese analogues the accumulation was significantly less than in the control cheeses, only one ripe cheese analogue showed the total amount of free amino acids and was almost equal to the control. During ripening, the content of these compounds increased in cheese analogues from 2.2 times to 3.3 times, in the control the content of free amino acids increased by 2.5 times, and individual free amino acids accumulated from 3-7 to 30-40 times. Active proteolytic processes led to the appearance of all ripe products of such essential amino acids as methionine and isoleucine, there was also an increase in the level of glycine. The practical significance of the study is determined by substantiating rationale proteolytic processes in the manufacture of cheese analogues to ensure consumer quality close to the quality of cheeses made of milk

Keywords dairy industry; dairy products; milk fat substitute; physical and chemical properties; amino acids

Introduction

G. Mangia *et al.* (2022) state that milk and dairy products are of inevitable necessity for human nutrition the said products provide numerous advantages for the health of consumers. Medicine studies, for example, S.M. Vanderhout *et al.* (2020), postulate that milk product help minimizes the probability of obesity, low bone weight, heart attacks and several cancer types. Cheese occupies a special place among dairy products. It is a protein-fat concentrate that retains its properties for several months and even years. According to an evaluation made by L. Lebid (2021), on average, Ukrainian consumes 3.5-4 kg of hard cheeses per year which is significantly less than in the EU, where the average yearly consumption of cheese is 20 kg pro capita. It is predicted that world cheese

production will reach 27 million tons by the end of 2030. R. Kamath *et al.* (2022) state that many enterprises in the dairy industry, along with the traditional assortment, produce analogue products with a combined composition. An analogue cheese can be defined as a cheese-like product obtained by partially or completely replacing components such as milk, milk fat or milk protein, and incorporating vegetable substances, as well as additives such as emulsifying salts, hydrocolloids, preservatives, acidifiers and sometimes flavours (sodium chloride, cottage cheese flavour, etc.) (Dairy Industries, 2020).

According to B.N. Esen *et al.* (2020), cheese-like products (analogue/imitation cheeses) are generally defined as food products produced from a mixture of non-milk fats, milk proteins

or vegetable proteins in certain proportions. Cheese-like products can be categorized as milk-based, partially milk-based, and non-dairy-based. The category of a cheese-like product depends on the source of the protein and/or fat content. The part that uses vegetable protein and vegetable oil in its composition is expressed as milk-based or non-milk-based. Following R. Kamath *et al.* (2022) soy is a highly nutritious food material containing well-balanced amino acids and desirable fatty acids. It plays an important role as a source of protein for many people around the world. In addition, it is necessary to note that the cost of producing cheese analogues may be less than products obtained only from animal proteins. Various food formulations include soy proteins for various purposes, usually related to health benefits or used soy for dairy fortification to alleviate milk availability problems.

A. Pua *et al.* (2022) consider that the sensorial properties of the analogues made based on the technological procedures similar to the real products made of milk are not satisfactory as the analogues produce specific off-flavours while their texture was also significantly different. A special processing technique is necessary to prepare plant-based ingredients for the fermentation to be done. To make sensorial parameters, including texture, the plant ingredients of analogue milk products undergo bacterial, yeast or fungal fermentation. R.M. Caldeira

et al. (2022) studied the sensorial properties of analogue ice cream made with olive oil. Aroma, flavour, and several other properties appeared to correspond to the milk-based ice cream.

According to C.M. Galanakis (2021), the use of high-quality milk fat substitutes in combination with valuable nutritional supplements makes it possible to regulate the composition of the product, and therefore its properties, obtaining products with a balanced fatty acid composition that meets the principles of healthy nutrition. Competently balanced cheese analogues allow, in addition to expanding the range, to obtain products of improved quality in terms of nutritional value, shelf life and sensorial perception.

The above considerations predefine the research aim, which is to analyse the effect of the content of cheese analogues namely milk fat substitutes, soy protein isolates and lactobacilli species upon the physical, chemical, and sensorial traits, and amino acids content of these products.

Materials and Methods

The principal idea while planning the research its results being presented herewith was the closest possible simulation of the traditional manufacturing process of hard cheeses but with the above-mentioned substitution of raw materials. The objects of research are specified in Table 1.

Table 1. Composition and characteristic traits of the objects of research

Object of research	Composition and characteristic traits
Fermenting bacterial concentrate for hard rennet cheese with a low temperature of the second heating (FBC)	Contains cultures of lactobacilli species <i>Lactococcus lactis ssp. lactis</i> and <i>L. lactis ssp. cremoris</i> , which provided the necessary level of acid formation, as well as aroma-forming species <i>L. lactis ssp. lactis</i> <i>bv. diacetilactis</i> and <i>Leuconostoc mesenteroides</i> ; soy protein isolate (protein content – 90%, odourless, neutral taste, no dietary fibre)
MFS1	Melting point – 32-34°C, hardness according to Kaminsky - 140-180 g/cm
MFS2	Melting point – 32-34°C, hardness according to Kaminsky – 80-120 g/cm
MFS3	Melting point – 32-36°C, hardness according to Kaminsky – 120-140 g/cm
MFS4	Melting point – 32-34°C, hardness according to Kaminsky – 120-140 g/cm

Source: author's development

Cheese and cheese analogues with combined protein and fat phases were produced according to the technology of hard cheese with a low temperature of the second heating (DSTU 4421:2005 (2007). Basic technical parameters of cheese production:

Fat content in dry matter (by weight) – 45%.

Water content (by weight):

➤ after pressing – 44-46%;

➤ ripe cheese – 40-41%.

NaCl content (by weight) – 1.5-1.9%.

the pH of cheese:

➤ after pressing – 5.4-5.5;

➤ ripe cheese – 5.3-5.4.

Second heating temperature – 38-41°C.

Ripening time – 45 days.

Ripening temperature – 10-12°C.

The cheese was shaped like a low cylinder with a slightly convex side surface and rounded edges. The top and bottom surfaces may be slightly convex, the height of the curd head was 8-9 cm, diameter 24-25 cm, weight 3.5-3.7. The cheese had a moderately mild taste and aroma with a slightly spicy aftertaste and slight acidity. The dough is soft and plastic. On the cut, the cheese had a pattern consisting of holes of a round or slightly flattened shape. The skin of the cheese was thin, even, without damage and a thick subcortical layer, the coating was paraffin wax, and the colour of the dough was from white to slightly yellow.

When developing analogue products with a combined protein phase, soy isolate, which by mass fraction was 30% of the total protein content, was dissolved in cow milk and the mixture was pasteurized for 10 minutes at a temperature of (75±3)°C.

For the products with a combined fat phase, the milk mixture was prepared as follows: whole cow's milk was pasteurized for 10 minutes at a temperature of (75±3)°C. Part of the milk was separated to extract the cream. Cream and MFS were used to normalize the initial milk formula for fat (up to 3%), while MFS was added in an amount of 30% of the total fat content in milk.

The resulting milk mixture was subjected to homogenization under pressure (12.5±2.5) MPa at a temperature of (60±3)°C.

The control cheeses were made from whole cow's milk. The ripening duration of cheese analogues and control cheeses was 45 days at a temperature of 10-12°C. The following research methods were used in the work:

➤ determination of active acidity (pH) – potentiometrically according to (DSTU 8550:2015 (2017);

➤ determination of solids content (by weight) – according to (DSTU 8552:2015 (2017); ISO 5534:2004 (2004) and on a water content analyser MA 30 Sartorius;

➤ determination of non-casein nitrogen content (by weight) – by the Kjeldahl method according to (ISO 17997-1: 2004 (2004);

➤ determination of total protein (by weight) – by the Kjeldahl method according to (ISO 8968-1:2014.(2014);ISO 8968-5:2001(2001).

➤ determination of casein content (by weight) – according to (ISO 17997-1: 2004 (2004);

➤ determination of the fractional composition of proteins – by the Polyacrylamide Gel Electrophoresis (PAAG) (Laemmli, 1970).

For the correct analysis of the data obtained by splitting the proteins the value of the sum of caseins (α_s -casein+ β -casein) was taken in the products at the post-pressing stage as 100% and further calculations were performed based on the said provision.

Analysis of the fractional composition of the proteins of the studied samples was fulfilled by denaturing electrophoresis in 12.5% polyacrylamide gel in the presence of sodium dodecyl sulfate using an electrophoretic chamber, at a constant current strength and a voltage of 55 V and 130 V, for 2 hours. A marker consisting of 11 preparations-standards with molecular weights of 250, 150, 100, 70, 50, 40, 30, 20, 15, 10, and 5 kDa (Thremo, USA) was used as a standard solution. Qualitative and quantitative determination of the protein composition of the samples was carried out after densitometry

of the obtained electropherograms with their subsequent processing using the specialized computer software ImagePro v3.

Characterization of the quantitative composition of free amino acids was carried out on the amino acid composition of the samples and was studied on the analyser LC-2000 (Biotronik). A sample of the product (20 g) was placed in 50 cm³ beakers, and 10 cm³ of a trichloroacetic acid solution with a mass fraction of 60% was added to precipitate protein fractions. After 20 min, the precipitated proteins were filtered off on a paper filter and washed with 10 cm³ of a trichloroacetic acid solution with a mass fraction of 5%. 1 cm³ of concentrated sulfuric acid and 6 cm³ of a solution of phosphotungstic acid with a mass fraction of 25% were added to the filtrate. The precipitate formed after 24 h was filtered off on a paper filter and washed with 15 cm³ of H₂SO₄ solution with a mass fraction of 5%. The filtrate was used to determine the content of free amino acids.

The obtained results and graphical representation of the experimental data were carried out using standard Microsoft Excel 2010 statistical processing programs. The accuracy of the obtained results was ensured by three or five repetitions of the studies. Graphical dependencies were built, and the table shows the arithmetic results of parallel measurements with a value of $p=0.05$.

Results

Commercially available products were used to ensure the practical significance of the study. The bacterial concentrate chosen is a mesophilic starter used in the production of cheeses with a low heating temperature. It is suitable for all soft cheeses, semi-hard cheeses as well as hard cheeses such as Swiss cheese and Parmesan. The manufacturers guarantee a high yield of the finished product, good water-holding capacity, and facilitate easy separation of whey. The bacterial concentrate gives the cheese a classic sour-milk taste. Another important trait of the bacterial concentrate chosen was the possibility to apply it not only to whole milk cheeses but also to the cheese analogues with the milk fat replaced by substitutes. All the products used in the formulations of cheese analogues produced in the study were specialized milk fat substitutes manufactured from plant raw materials and guaranteeing proper trans isomers content as well as proper nutritional value and reliable technological characteristics.

Even though the basic conditions for the manufacture of cheese analogues were the same (temperature regimes, bacterial concentrate), the experimental variants of cheese analogues differed in terms of active acidity and moisture content (Table 2).

Table 2. Physical and chemical indicators in the production of cheese analogues

Variant	Active acidity, pH units		Water content (by weight), %	
	after pressing	ripened for 45 days	after pressing	ripened for 45 days
Control	5.58 ± 0.05	5.15 ± 0.03	44.39 ± 0.12	38.21 ± 0.62
Cheese analogues				
with MFS1	5.16 ± 0.04*	5.10 ± 0.04	48.15 ± 0.67*	41.81 ± 0.75*
with MFS2	5.26 ± 0.01	5.03 ± 0.01*	55.09 ± 1.06*	47.83 ± 0.17*
with MFS3	5.17 ± 0.01*	5.18 ± 0.02	46.01 ± 0.75	41.90 ± 0.34*
with MFS4	5.26 ± 0.02	5.17 ± 0.01	45.03 ± 0.17	39.86 ± 0.25
with soy isolate	5.64 ± 0.02	5.13 ± 0.01	48.84 ± 0.05*	39.89 ± 0.13

Note: * – the difference with the control is significant at $P \leq 0.05$

Source: author's development

After pressing the cheese dough of the experimental variants with MFS a lower active acidity and higher moisture content in comparison to the control cheeses was highlighted. The variant of the cheese analogue with soy isolate was similar in terms of active acidity to the control, but its moisture content was also higher.

During ripening, the active acidity of the curd dough in all experimental variants, except for the variant with MFS2, became similar to the control cheese, however, the water content remained higher. Figures 1 and 2 present the fractional composition of proteins determined according to the method specified above.

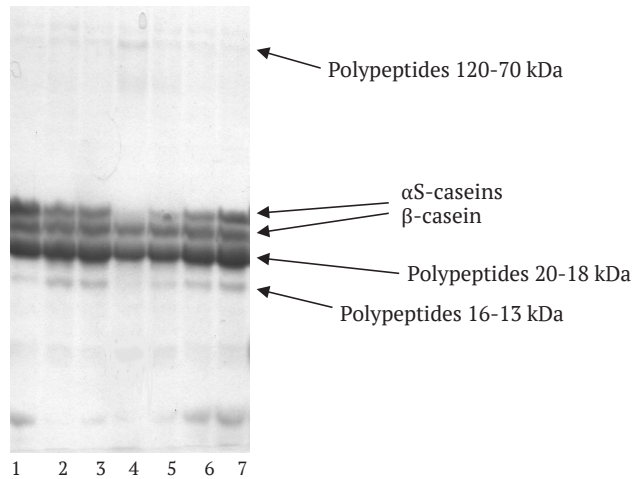


Figure 1. Electrophoretic separation of proteins of analogue cheeses with MFS. 1 – cheese after pressing; 2,3 – ripened control cheeses; ripened analogue cheese products: 4 – with MFS1; 5 – with MFS2; 6 – with MFS3; 7 – with MFS4

Source: author's development

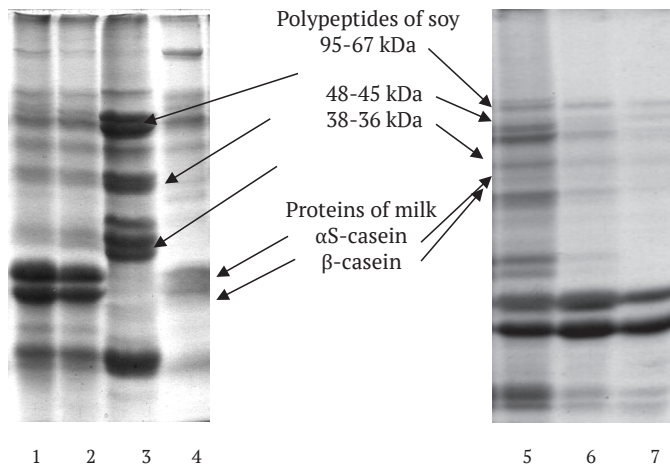


Figure 2. Typical protein electropherograms: 1,2 – cheese analogues with soy isolate after pressing; 3 – soy isolate; 4 – cheese after pressing from cow's milk; 5 – ripe cheese analogue with soy isolate, partially washed from caseins; 6 – cheese analogue with soy isolates on the 45th day of ripening; 7 – ripe cheese (control)

Source: author's development

After pressing, the content of individual fractions of proteins and polypeptides was similar in all variants of cheese analogues MFS and the control, in the variant with soy isolate, the analysis of the protein composition showed the presence of clear bands of characteristic soy proteins with molecular masses of 48 – 45 kDa and 38 – 3 kDa (Fig. 2). During further ripening, proteolytic processes proceeded with different intensity and had different directions in experimental versions of cheese analogues and controls. The α_{s1} -casein fraction, especially in the version with MFS2, was subjected to the strongest splitting during the ripening of the cheese dough, α_{s2} -casein was much weaker, and β -casein was hydrolysed to the least degree. A decrease in the level of caseins was accompanied by the formation of polypeptides of different molecular weights.

In experimental cheese analogues with soy isolate after pressing, the total content of caseins was 52.89% of the total protein content, and soy proteins – were 29.71%. The weakening or almost complete absence of characteristic bands of soy proteins in the protein spectrum of ripe cheese analogues with soy isolate can be explained by active proteolytic processes that occurred during ripening, proteins of soy isolate accounted for less than 5% of the total protein content in the ripe product.

The total content of caseins in control cheeses and cheese analogues pressing was (83.53±0.20) % of the total protein content. Table 3 shows the relative content of α_s -casein, β -casein and their sum in experimental variants and control cheeses after pressing and after 45 days of ripening.

Table 3. Decomposition of caseins during ripening of cheese analogues

Variant	The relative content of caseins (%)		
	Sum of caseins	α_s -caseins	β -casein
Cheese analogues and control cheeses after pressing	100	56.36	43.64
Control, ripening for 45 days	84.75	43.31	41.44
Ripe cheese analogues			
with MFS1	79.53	37.88	41.65
with MFS2	69.84	30.94	38.90
with MFS3	80.96	38.11	42.85
with MFS4	80.63	39.09	41.54
Cheese analogue with soy isolate after pressing	100	55.85	44.15
Ripe cheese analogue with soy isolate	85.11	42.87	42.24

Source: author's development

During ripening, the content of caseins in the control cheeses decreased by 15.25%, mainly due to the cleavage of α_s -casein.

On the 45th day of ripening, the decomposition of caseins in all variants of cheese analogues with MFS was higher than in the control. If the total level of caseins in control ripe cheese decreased by 15.25%, then in the variants with MFS1 – by 20.47%, with MFS3 – by 19.04%, with

MFS4 – by 19.37%. To the greatest extent, the total level of caseins decreased in the variant with MFS2 – by 30.16%, and, compared to other experimental variants, the decomposition of β -casein was also higher.

Changes in the spectrum of free amino acids at the beginning (after pressing) and at the end of ripening (after 45 days) of the studied cheese analogues with soy isolate are given in Table. 4.

Table 4. Qualitative and quantitative composition of free amino acids in cheese analogues and control cheeses (mg/100 g of product)

Amino acid	Control		Cheese analogue	
	after pressing	ripe	after pressing	ripe
Threonine	-*	27.168**	-	14.085
Valine	-	6.189	0.159	2.883
Methionine	-	0.954	-	0.306
Leucine	0.297	42.063	0.696	20.067
Phenylalanine	0.426	50.619	0.738	28.218
Lysine	8.4	22.932	15.615	16.446
Glutamic acid	20.124	51.156	31.428	35.385
Proline	9.444	16.587	12.327	11.163
Alanine	-	8.43	-	4.581
Aspartic acid	0.945	3.105	0.387	0.411
Tyrosine	6.429	37.281	9.075	28.551
Histidine	2.409	32.217	3.78	16.14
The total amount of free amino acids:	48.474	298.701	74.205	178.236
of which are essential:	9.123	149.925	17.208	82.005

Note: * No compound; ** Measurement error does not exceed 5%

Table 5. Qualitative and quantitative composition of free amino acids in ripe cheese analogues with MFS (mg/100 g of a product) *

Amino acids	MFS1	MFS2	MFS3	MFS4	Control
Threonine	-	-	10.29	8.69	-
Valine	11.13	8.49	10.23	7.21	11.59
Methionine	1.70	1.76	3.02	1.60	3.01
Ileucine	1.51	1.43	0.77	0.54	1.31
Leucine	29.83	21.03	16.88	15.59	22.99
Lysine	29.54	22.54	21.35	23.95	26.18
Phenylalanine	31.44	24.90	23.95	24.51	31.85
Serene	7.65	4.80	-	-	9.67
Glutamic acid	59.09	46.41	42.89	40.08	50.70
Proline	25.40	59.48	43.80	40.16	50.81
Alanine	7.28	6.18	7.54	6.49	8.76
Tyrosine	39.71	24.54	29.67	26.05	30.72
Histidine	6.67	17.83	17.92	17.39	20.94
Arginine	1.30	1.18	0.69	0.66	-
Glycine	1.64	1.68	1.62	1.47	2.38
Aspartic acid	12.45	9.35	9.42	8.80	-

Note: * Measurement error does not exceed 5%

Source: author's development

From the data presented in Table 5, the content of free amino acids, which is low at the beginning of ripening, significantly increased in ripe products. In the experimental variant with soy isolate by 2.4 times, and in the control variant by 6.2 times compared with the stage after pressing. At the same time, in ripe cheese analogues, the level of free amino acids was 1.7 times lower, and free essential amino acids – were 1.8 times lower compared to control cheeses. Such values of the above said parameters confirm a lower nutritional value of cheese analogues which can be a benefit within special diet plans.

Discussion

Carious cheese analogues were analysed and studied by researchers around the world. Full or partial replacement of milk fat with vegetable analogues provides cost reduction and stabilizes product quality since the production and composition of substitutes are not subject to seasonal fluctuations (Kamath *et al.*, 2022). The possibility of making an analogous cheese product by incorporating and/or substituting, partially, milk fat with olive oil, as well as determining the optimal parameters for its production was studied. The addition of olive oil generated a beneficial effect on the level of cholesterol in the blood, therefore, although the fat content of the product and its caloric level is increased, the detrimental effect of saturated fatty acids is not increased, but it decreases. The lipid content of the whey of the respective preparations was analysed to calculate the amount of fat that passed to the whey (Alonso Degeneffe, 2019). The role of polyunsaturated fatty acids and phospholipids in the prevention and treatment of lipid metabolism disorders, in particular atherosclerosis, has been proven (Sokoła-Wysoczańska *et al.*, 2018). The most important characteristic of fat is the composition of the fatty acids that form it. It should be balanced in terms of the ratio of saturated, monounsaturated, and polyunsaturated fatty acids,

especially in terms of the ratio of polyunsaturated fatty acids of the omega-3 and omega-6 families. At the same time, the undesirability of the use of products containing the trans isomers of fatty acids, and the consumption of products containing a significant amount of saturated fatty acids, has been proven (Dhaka *et al.*, 2011).

According to A. Badem & G. Uçar (2016), the agent starting casein hydrolysis is plasmin. During storage proteolysis increases, but such texture parameters as firmness, viscosity and chewiness tend to decline. No efficiency is shown by non-starter lactic acid bacteria compared to plasmin during casein hydrolysis. However, the said bacteria do form casein-peptide derivatives. Peptidase, dipeptidase, tripeptidase, carboxypeptidase, aminopeptidase and endopeptidase and proteinase are the enzymes *Lactobacillus* has the said enzymes being utilized and the necessary amino acids are thus obtained. The formation of the above-listed enzymes is determined by isolating peptides as well as free amino acids from the casein having undertaken the hydrolysis process. During storage plasmin and microbial enzymes behave in a contradicting way thus maximizing free amino acids content (Mulvihill & McCarthy, 1994).

In the study C.R. Cunha *et al.* (2010), analogues of cheese “Requeijão cremoso” were studied, which were made by replacing 25% and 50% of milk fat with vegetable fat. Replacing part of the milk cream with vegetable fat led to an increase in hardness. Traditional cheese was a homogeneous protein mass, in which numerous small fat particles were dispersed, while in analogues fat globules were present of a large diameter, such changes were observed in samples with an enlarged vegetable fat particle.

The manufacturing process of a cheese analogue with the second heating is characterized by (Mogutova *et al.*, 2021). The said process consists of three successive stages, which are carried out at a speed of not more than 10...20 revolutions per minute with intensive

kneading. Stage 1 – heating is carried out at a temperature of 38 ... 42°C for 10 ... 15 minutes. Stage 2 – vegetable flour and salt are added to the curd (no more than 2 ... 2.5%). The curd is intensively mixed with an increase in temperature to 50 ... 55°C. Stage 3 – the curd is kept at a temperature of 60 ... 65°C for 10 ... 15 minutes. The product is formed in the form of bars, cylinders, spheres, and other shapes and pressed at a load of 2...3 kg/cm² for 1...3 hours until the cheese product reaches a mass fraction of moisture of 40...60%. Ripening of the curd product is within 12...18 days at a temperature of 5...15°C and a relative air humidity of about 85%. After the ripening period, the cheese analogue has a curd, slightly sour taste, and smell characteristic of prescription components; homogeneous, tender, slightly fragile or brittle, as dense as possible; colour – from white to yellow, uneven colour is allowed. Dough without holes; single cells of irregular shape are allowed. For a cheese product, the shelf life is 14 days from the end date of maturation.

A new type of semi-hard cheese product has been developed using a milk fat substitute. The said cheese product is made from a normalized vegetable-milk mixture, by acid-rennet coagulation of proteins, followed by moulding, self-pressing, pressing, salting, and ageing. Physical and chemical parameters of the raw materials for the cheese product were fat content in dry matter (by mass) – 45.2%, water content – 48%, and pH – 5.5. Compared to typical cheeses with low second heating temperature new type of cheese analogue had higher moisture content. This has been achieved by norming milk during the formation of the mixture, adjusting the temperature of the second heating and the duration of cheese grain processing, as well as reducing the duration of pressing and ripening (Savchenko *et al.*, 2018).

The use of corn oil in the production of light cheese instead of milk fat significantly affected the amount of dry matter, fat and salt in the dry matter, protein and titrated acidity and

pH value of the samples. During the ripening of cheeses, the content of water-soluble nitrogen increased. However, no differences were found between the peptide profiles of all cheese samples (Arslan *et al.*, 2014).

The replacement of milk fat with emulsified olive oil and the production of the Gouda cheese analogue resulted in a lower solids content in cheeses containing fat substitutes than in full and low-fat control cheeses due to the higher water-binding capacity of fat substitutes. The values of free fatty acids were highest in the case of cheese products with reduced fat content (Felfoul *et al.*, 2015).

Reduced-fat cheese analogues are made from partially skimmed cow's milk with the addition of soy protein concentrate. It was found that cheese analogues had higher protein content with an increase in soy protein content, which confirms that part of the soy proteins remained in the casein matrix, and the fat content increased to 22% relative to the control sample. However, foods were classified as low-fat foods and considered functional as an additional source of plant-based protein with a high biological value due to the high content of amino acids and essential fatty acids (Rinaldoni *et al.*, 2014).

Thus, the obtained values of the physical and chemical indicators of cheese analogues do not correspond to the results obtained by (O'Malley *et al.*, 2000): results of pH values measured were from pH 5.03 to pH 5.18 when authors showed pH 7.29 – 7.33 for rennet caseins; pH 6.40 – 6.50 for pilot scale cheese analogue; pH 6.46 – 6.48 for industrial scale cheese analogue. For our samples water content (by weight) was 39.86% – 41.90%, while (O'Malley *et al.*, 2000) showed 50.30% – 50.88% for sample scale cheese analogue and 47.42% – 47.76% for industrial scale cheese analogue. Such a discrepancy seems to originate from the differences in plant raw materials used in the studies compared. However, the electrophoretograms of proteins are the same in both studies.

Comparing our results of pH and water content (by weight) shown in the paragraph above and the data of other scholars: pH 6.10 and water content 48.80% for analogue pizza cheese (Fox *et al.*, 2000; Chavan & Jana, 2007; Gao *et al.*, 2022); pH 5.66 and water content 53.35% for analogue mozzarella cheese (Jana, 1998; Chavan & Jana, 2007) it can be stated that the said results are consistently close.

The results of pH and water content (by weight), according to D.M. Mulvihill & A. McCarthy (1994) were pH 6.17 and 47.21%. The electrophoretograms of proteins do not contradict those made during our studies.

The comparison of the data obtained during the research and the results published by the scholars mentioned shows that results of the determination of physical and chemical traits as well as the described peculiarities of the technological processes applied are not controversial and originate mostly in the great diversity of raw material used.

Conclusions

If in the cheese dough after pressing the differences in the spectrum of free amino acids between the variants were insignificant, in ripe cheese analogues they accumulated significantly less than in the control cheeses: from MFS1 and MFS4 – by 24.4%, from MFS3 – by 26.7%. Only in ripe cheese analogue with MFS1 the total amount of free amino acids was almost equal to the control product. During ripening, the content of these compounds increased in the variants with MFS from 2.2 times (MFS3 and MFS4) to 3.0 (MFS1) and 3.3 times (MFS2), in the control the content of free amino acids increased by 2.5 times.

During ripening, individual free amino acids accumulated from 3-7 to 30-40 times. The ratio between essential and non-essential free amino acids slightly increased in all variants.

The specific cheese taste is known to be determined by significant amounts of free proline, methionine, as well as aspartic and glutamic acids. These amino acids accumulated during ripening in all variants, both experimental and control. Active proteolytic processes led to the appearance of all ripe products of such essential amino acids as methionine and isoleucine, there was also an increase in the level of glycine. The presence of free arginine was characteristic of ripe products with MFS, which in significant quantities can give the product an unpleasant aftertaste of spoilage. In the control cheeses, the presence of this amino acid in the unbound state was not found.

The results obtained and the literature sources reviewed substantiate the possible directions of research the most promising of these being: embracing a wider range of cheese analogues and the ingredients used for their manufacturing, mostly milk fat substitutes as well as complex bacterial concentrates. Series of research on the texture of the said cheese products should be fulfilled embracing the recognized sensorial methods as well as Texture Profile Analysis employing a relevant testing machine.

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Conflict of Interest

None.

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Перебіг протеолітичних процесів при визріванні комбінованих сирних продуктів

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Анотація. Незважаючи на широке виробництво аналогів сирів, до складу яких входять рослинні речовини, теоретичні основи процесів їх виробництва, у тому числі протеоліз, ще недостатньо вивчені, тому тема даної роботи є актуальною. Метою даної роботи є визначення впливу вмісту аналогів сиру, а саме заміників молочного жиру, ізолятів соєвого білка, лактобацил виду *Lactococcus lactis ssp. Lactis*, *L. lactis ssp. cremoris*, *L. lactis ssp. lactis* *bv. diacetilactis*, а також *Leuconostoc mesenteroides* на фізичні, хімічні та органолептичні характеристики, а також на вміст амінокислот в аналогах сиру. Визначення фізико-хімічних показників проводили за нормативами: активної кислотності (рН) – ДСТУ 8550:2015; вміст сухих речовин (за масою) – ДСТУ 8552:2015 та ISO 5534:2004; вміст неказеїнового азоту (за масою) – ISO 17997-1:2004; загальний білок (за масою) – ISO 8968-1:2014 та ISO 8968-5:2001; зміст казеїну (за масою) – ISO 17997-1:2004. Визначення фракційного складу білків проводили методом електрофорезу поліакриламідному гелі. Якщо в сирній масі після пресування відмінності в спектрі вільних амінокислот між варіантами були незначними, то у зрілих аналогах сиру їх накопичувалося менше, ніж у контрольних сирах, тільки в одному аналогу

сиру загальна кількість вільних амінокислот було менше, майже зрівнявся із контролем. У процесі дозрівання вміст цих сполук збільшився в аналогах сиру від 2,2 до 3,3 рази, у контролі вміст вільних амінокислот збільшився у 2,5 рази. При дозріванні накопичення окремих вільних амінокислот становило від 3-7 до 30-40 разів. Активні протеолітичні процеси призвели до появи у всіх стиглих продуктах таких незамінних амінокислот, як метіонін та ізолейцин, також відбулося підвищення рівня гліцину. Практичне значення роботи полягає в обґрунтуванні раціональних протеолітичних процесів при виготовленні аналогів сиру з метою забезпечення споживчої якості, наближеної до якості сирів з молока

Ключові слова: молочна промисловість; молоковмісний замінник молочного жиру; фізико-хімічні властивості; амінокислоти