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Original scientific paper

IMPROVEMENT OF ORGANOLEPTIC INDICATORS OF CHEESE PRODUCTS BY CORRECTING FATTY ACID COMPOSITION OF FAT PHASE

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ABSTRACT

The aim of the study was to establish the possibility of improving the organoleptic characteristics of cheese products by introducing butyric acid into the composition of the used vegetable fat composition. Cheese products made using two fat compositions consisting of refined, deodorized vegetable fats, including those modified by interesterification, were studied. The experimental fat composition contained in its composition additional butyric acid added to the composition of triglycerides. The study of cheese products was carried out in the process of ripening and storage for 120 days in comparison with cheese, the fat phase of which is represented by milk fat. It was found that the degree of proteolysis, estimated by the ratio of total water-soluble nitrogen to total nitrogen, does not depend on the nature of the fat in the protein matrix. Milk fat in cheeses is more subjected to lipolysis than vegetable fats in cheese products. The addition of butyric acid to the vegetable composition in an amount of 0.8% increased the acidity of the fat phase by 0.1 mmol/100 g. According to the assessment of organoleptic characteristics, cheese with milk fat had the most pronounced cheese flavor and aroma, cheese product without butyric acid in fat phase had the least pronounced ones. A cheese product with a fat composition containing added butyric acid was close to cheese with milk fat in terms of the severity of cheese taste, rheological characteristics and the spectrum of volatile flavoring substances. It is concluded that the taste and aroma of cheese products have a positive effect on adjusting the fatty acid composition of vegetable fat compositions by introducing butyric acid into their composition.

1. Introduction

The formation of the taste and aroma of cheeses is associated with the course of numerous biochemical processes during their ripening, the main of which are: 1) proteolysis and catabolism of amino acids, 2) lipolysis and metabolism of fatty acids, 3) metabolism of residual lactose, lactates and citrates. The primary products of cheese ripening (peptides, amino acids and fatty acids) are further metabolized into volatile flavoring compounds, the combination and interaction of which creates a unique flavoring bouquet for each type of cheese [1,2].

It is believed that the proteolysis of caseins to a number of peptides of small and medium size and free amino acids contributes to the creation of the background taste of most types of cheese [3,4]. The primary role in the formation of taste and aroma is played by lipolysis, leading directly to the formation of aromatic compounds by the release of free fatty acids, especially with short and medium chains ($C_4:0 - C_{10}:0$). Free fatty acids also act as precursor molecules for a number of catabolic reactions leading to the formation of flavoring and aromatic compounds, such as methyl ketones, lactones, esters, alkanes and secondary alcohols [5,6].

Among the many taste and aromatic components of cheese, researchers distinguish low molecular weight volatile fatty acids with a short carbon chain (up to $C_4:0 - C_6:0$), which have a low perception threshold and have a noticeable effect on the taste and smell of cheese [7]. Among them, butyric acid ($C_4H_8O_2$) occupies a special place. It is part of milk fat and is bound in the composition of fatty acid acylglycerides in the original milk. Free butyric acid is formed during the ripening of cheese as a result of lipolysis. Its amount increases with ripening in parallel with an increase in the severity and intensity of cheese taste and aroma [8,9,10].

The accumulation of butyric acid in cheeses depends on the composition of the used starter microflora, its lipolytic activity and the specificity of the produced lipases and esterases [11,12]. Given the importance of lipolysis in the formation of the taste and aroma

of cheeses, in modern cheesemaking, the use of lipolytic enzymes is sometimes practiced, which accelerate the process of hydrolysis of milk fat and the accumulation of its products, including free fatty acids, including butyric [12,13,14]. Free butyric acid is always present in cheeses in small concentrations and is an important component of their taste and aroma.

One of the trends in the development of cheesemaking is the use of vegetable fats, replacing milk fat in the product structure. The production of cheese with vegetable fats is widespread in countries experiencing a constant or seasonal shortage of dairy raw materials [15,16,17]. Cheeses with vegetable fats constitute a separate group of cheesemaking products, attractive both for the poor and for healthy food adherents who consider milk fat as a source of saturated fatty acids and cholesterol that negatively affect their health [17,18,19,20,21,22].

Along with the positive aspects of the use of vegetable fats in cheesemaking, there is a possibility of organoleptic deterioration in the cheese products made with them in comparison with cheeses, the fat phase of which is represented by natural milk fat. Depending on the type of used vegetable fat, taste, aroma and texture change, which is associated with a change in the composition and structure of the fat phase [15,16,17,22,23,24]. Some authors noted a smaller amount of volatile fatty acids in the fat phase of cheeses with vegetable fats [25], which was considered as one of the reasons for the deterioration of organoleptic indicators. A change in the ratio of fatty acids when replacing milk fat with vegetable one affects the nature and intensity of biochemical and physicochemical processes that occur during the manufacture, ripening and storage of cheese products, and therefore the formation of their organoleptic characteristics [26]. This applies both to taste and to texture; because the inclusion of emulsified vegetable fats in the cheese structure changes the type and distribution of fat droplets in the protein matrix, causing changes in the microstructure that affect the texture [27,28].

A significant difference between vegetable fats and milk fat is the absence of butyric acid in their fatty acid composition. This fact underlies the methods of identification of milk fat in determining the falsification of milk products with vegetable fats, as well as the quantitative determination of milk fat in mixed fats by the amount of butyric acid determined in them [29,30].

Considering the important role of butyric acid in flavoring, manufacturers of food products with vegetable fats used butyric acid to improve the taste and aroma, which was introduced into the fat phase of the product. This technique was first tested with a positive result on margarine [31].

Research experience on the inclusion of short-chain fatty acids ($C_4 - C_{10}$) in the composition of vegetable (high-oleic sunflower) oil, which improved the taste of cheese products made with such milk fat substitute using Swiss cheese technology [22,25,32] is known. Modification of the composition of vegetable oil provided for the synthesis of short-chain triglycerides by esterification of fatty acids with glycerol. The short chain triglycerides, obtained in this way, had a bitter taste, but after interesterification with high oleic sunflower oil and deodorization, it disappeared. The obtained milk fat replacer was close to milk fat in fatty acid composition and had a mild, acceptable taste [25]. All cheese products made using Swiss cheese technology with full replacement of milk fat with modified high oleic sunflower oil did not differ significantly from control cheeses with milk fat in terms of taste and aroma typical of this cheese [32].

Thus, the targeted introduction into the vegetable fat composition of low molecular weight fatty acids, in particular butyric acid, will improve the severity of the taste and aroma of cheese products. One of the Russian companies has laid this ideology as the basis for the creation of new specialized fat compositions for cheese products. To achieve the objectivity in assessing this assumption, researches have been carried out with the purpose to study the possibility of improving the quality of products made using semi-hard cheese technology by using a vegetable fat composition containing butyric acid.

2. Materials and methods

2.1. Study of vegetable fat compositions

In the manufacture of cheese products, fat compositions of the EFKO company (Russia), consisting of refined, deodorized vegetable fats, including those modified by interesterification, were used. A composition of TF 1403–35 EC brand and an experimental composition, which, in contrast to the indicated one, contained butyric acid additionally included in the composition of triglycerides, were used. Both compositions are intended for the production of cheese products and are selected with a focus on the closest possible approximation to milk fat in terms of physicochemical and rheological properties.

The fatty acid composition of vegetable fat compositions was evaluated on Chromos GX-1000 chromatographic complex with a flame ionization detector (Chromos LLC, Russia) and CP 88 Silica for FAME100 $m \times 0.25 \text{ mm} \times 0.2 \text{ }\mu\text{m}$ quartz capillary column («Agilent Technologies», USA). Chromatography modes were: volume of injected sample — 1 mm^3 ; injector temperature — $220 \text{ }^\circ\text{C}$; temperature program of the thermostat: 1) $100 \text{ }^\circ\text{C}$ — 4 min, then a temperature increase of $5 \text{ }^\circ\text{C}$ for 20 min; 2) $170 \text{ }^\circ\text{C}$ — 20 min, a temperature increase of $5 \text{ }^\circ\text{C}$ for 9 min; 3) $215 \text{ }^\circ\text{C}$ — 30 min; carrier gas — nitrogen (pressure in front of the column — 2.7 kgf/cm^2). A standard mixture of fatty acid methyl esters of Supelko 37 Component FAME Mix (Supelko, USA) was used as an identification mixture. Processing of the obtained data was carried out by the method of internal normalization using the Chromos program.

2.2. Production of cheese products

Emulsions were prepared from vegetable fat compositions by dispersing them in skim milk. A mixture was made of milk (whole

and nonfat) and emulsions of vegetable fats based on the calculation of the mass fraction of fat in the mixture of 2.7%. A solution of calcium chloride with a concentration of 30% was calculated on the basis of 30 g/100 kg of the mixture, starter in an amount of 0.7%, which included active acid and aromatizers (*Lc. Lactis*, *Lc. Cremoris*, *Lc. Diacetylactis*, *Leuconostoc*), and milk clotting enzyme preparation of animal origin SP-90 «Extra» were introduced into obtained mixtures. The process of gel preparation and processing of cheese grain was carried out according to the traditional technology of semi-solid cheese with a low temperature of the second heating. After salting, the cheese products were dried for 1 day and sent to the ripening chamber at a temperature of $(13 \pm 1) \text{ }^\circ\text{C}$ and relative humidity of $(80 \pm 2)\%$. After 30 days of ripening, cheese products were stored for three months at a temperature of $(4 \pm 2) \text{ }^\circ\text{C}$. In parallel with the same technology, cheeses were made from a mixture of whole and skim milk, with which experimental cheese products were compared in the process of ripening and storage.

2.3. Cheese product research

The study of cheese products by physicochemical, organoleptic and rheological indicators was carried out after 30, 60, 90, and 120 days from the date of manufacture.

The mass fraction of fat was determined by the Gerber method, the mass fraction of moisture — by the gravimetric method of drying the sample at a temperature of $102 \pm 2 \text{ }^\circ\text{C}$, the mass fraction of salt — by the titrimetric method with silver nitrate, and the active acidity — by the potentiometric method.

The degree of proteolysis was evaluated by the percentage of the total amount of water-soluble nitrogen to the total amount of nitrogen, which was determined by the Kjeldahl method.

The degree of lipolysis was evaluated by the acidity of the fat phase, reflecting the amount of free fatty acids and other substances titrated with alkali, in terms of oleic acid in mmol per 100 g of fat.

Organoleptic evaluation of the experimental and control product images was carried out using the descriptor-profile method [33], taking into account the severity of the main characteristics of taste and aroma (cheese, sour, extraneous, rancid), evaluated on a scale from 0 to 10 points.

The amount of volatile flavoring substances in cheeses and cheese products was determined by gas chromatography on Tsvet-800 gas chromatograph (Russia) with a flame ionization detector and a printed glass column: 2 m in length, internal diameter of 2 mm, nozzle OV-201 on chromatone N-AW-HMD (0.16–0.20 mm). Product samples were thermostated in a closed vessel with subsequent determination and identification in the vapor phase of the individual components of volatile flavoring substances. Analysis conditions were: flame ionization detector; sensitivity on the block of the ionization detector — 10^{10} ; temperature: column thermostat — 50 and $70 \text{ }^\circ\text{C}$, transition chamber — 60 and $80 \text{ }^\circ\text{C}$ and evaporator — 80 and $90 \text{ }^\circ\text{C}$; speed: carrier gas (nitrogen) — $30 \text{ cm}^3/\text{min}$, hydrogen — $30 \text{ cm}^3/\text{min}$; air — $300 \text{ cm}^3/\text{min}$; overpressure at the inlet to the column — 1 kgf/cm^2 ; pressure during sampling — 2 kgf/cm^2 ; the mass of the product for analysis was 3 g; analysis duration — 900 s. The mass fraction of each component in percent was calculated by normalizing the areas of gas chromatographic peaks. To identify substances in the obtained chromatogram, the retention time of hexanol-1 was measured and the relative retention time of each peak was calculated.

A study of the rheological properties of cheese products was carried out on Weissenberg rheogonometer model R-19 from Sangamo Weston Controls Limited (Great Britain) by the method of torsional vibrations with a frequency of 3.16 Hz and an amplitude of angular displacements of the working unit of $1.1 \cdot 10^{-3} \text{ rad}$. The working unit was a cone-plane combination with a diameter of

25 mm and an angle at the apex of the cone of 0.034 rad. Measurement temperature was 21 ± 1 °C.

Mathematical processing of the results was carried out using Microsoft Excel 2010.

3. Results and discussion

Table 1 shows the indicators of the fatty acid composition of the vegetable fat compositions used in the experiment.

Table 1

Fatty acid composition of vegetable fat compositions in comparison with milk fat

Indicators of fatty acid composition	Mass fraction of fatty acids, %		
	Milk fat	ECOLACT TF 1403-35 EK	Experimental fat composition
Sum of saturated fatty acids, %	61.40 ± 6.00	42.70 ± 0.80	45.40 ± 0.90
including butyric acid, %	3.30 ± 0.90	0.01 ± 0.01	0.81 ± 0.01
Sum of unsaturated fatty acids with reference to the isomers, %	38.60 ± 6.00	56.30 ± 0.90	54.60 ± 0.80

Compared to milk fat, vegetable fat compositions have a high content of unsaturated fatty acids and a low content of saturated fatty acids. This confirms the attractiveness of the composition of the fat phase of cheese products from the healthy diet perspective. The experimental fat composition, in contrast to the commercially available composition ECOLACT TF 1403-35 EC, contains butyric acid in the amount of 0.8% (3-5 times less than in milk fat).

The cheese products made by the main physicochemical parameters were identical to cheese with milk fat, which made it possible to establish a reliable effect of vegetable fat compositions on the organoleptic characteristics of cheese products in comparison with cheeses (Table 2).

Table 2

Physicochemical characteristics of the studied products at the age of 30 days

Physicochemical characteristics	Cheese with milk fat	Cheese product with ECOLACT TF 1403-35 EC	Cheese product with experimental fat composition
Mass fraction of fat in dry matter, %	44.7 ± 0.1	44.4 ± 0.3	43.6 ± 0.3
Moisture content, %	42.3 ± 0.3	43.5 ± 0.1	43.3 ± 0.1
Mass fraction of salt, %	1.70 ± 0.03	1.77 ± 0.04	1.82 ± 0.01
Active acidity, units pH	5.13 ± 0.01	5.15 ± 0.02	5.23 ± 0.01

One of the main biochemical processes that occur during the ripening of cheeses and cheese products is proteolysis, as a result of which water-soluble nitrogen-containing substances are formed, which are involved in taste formation. As cheese and cheese products ripen, their amount increases. The ratio of the amount of water-soluble nitrogen-containing substances formed to the amount of all nitrogen-containing substances, expressed as a percentage, is used to judge the degree of proteolysis. From the data presented in Figure 1, it follows that proteolysis does not depend on the nature of the fat in the protein matrix. Both in cheeses with milk fat and in cheese products with vegetable fats at the time of maturity (30 days), the degree of proteolysis was 16%. During subsequent storage for 3 months, the degree of proteolysis increased to 20%.

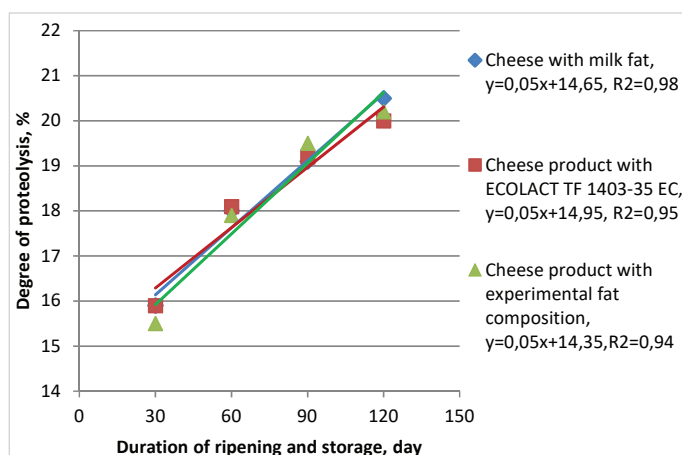


Figure 1. Change in the degree of proteolysis during the ripening and storage of cheeses and cheese products

The results on the absence of an effect on the replacement of milk fat on proteolysis with vegetable fat compositions are consistent with the data of Australian researchers [34], who obtained similar results on Cheddar cheese. In their experiment, the milk fat in the cheese was completely replaced with a vegetable fat composition consisting of cocoa butter, coconut oil and partially hydrogenated cottonseed oil. It was found that cheese made from skim milk homogenized with a mixture of vegetable fats, unlike cheese with milk fat, did not give the characteristic taste of Cheddar even after prolonged ripening for 18 months. Based on the fact that there is no effect on the proteolysis of the nature of fat, the authors conclude that the products of protein hydrolysis, in particular, free amino acids, are not determining factors in the taste formation of cheese. Later studies [3,4] also emphasized that protein hydrolysis products only contribute to the background taste of most types of cheese. The main role is given to milk fat, which, undergoing lipolysis, is a source of key flavor compounds or their precursors.

The degree of lipolysis in cheese products in comparison with cheese was evaluated by the change in the acidity of the fat phase during ripening and storage. Figure 2 presents the results.

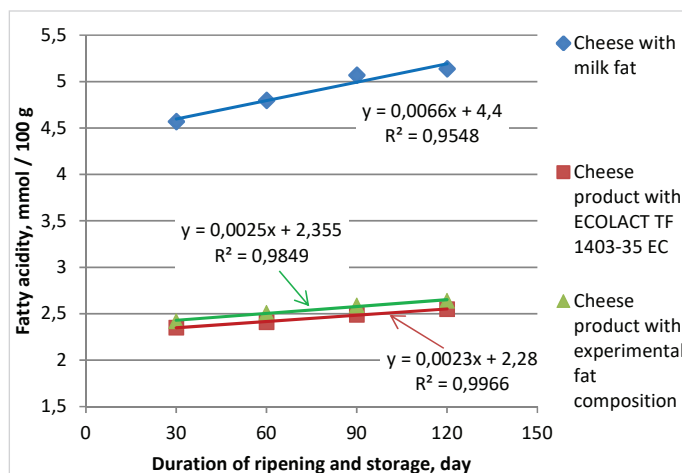
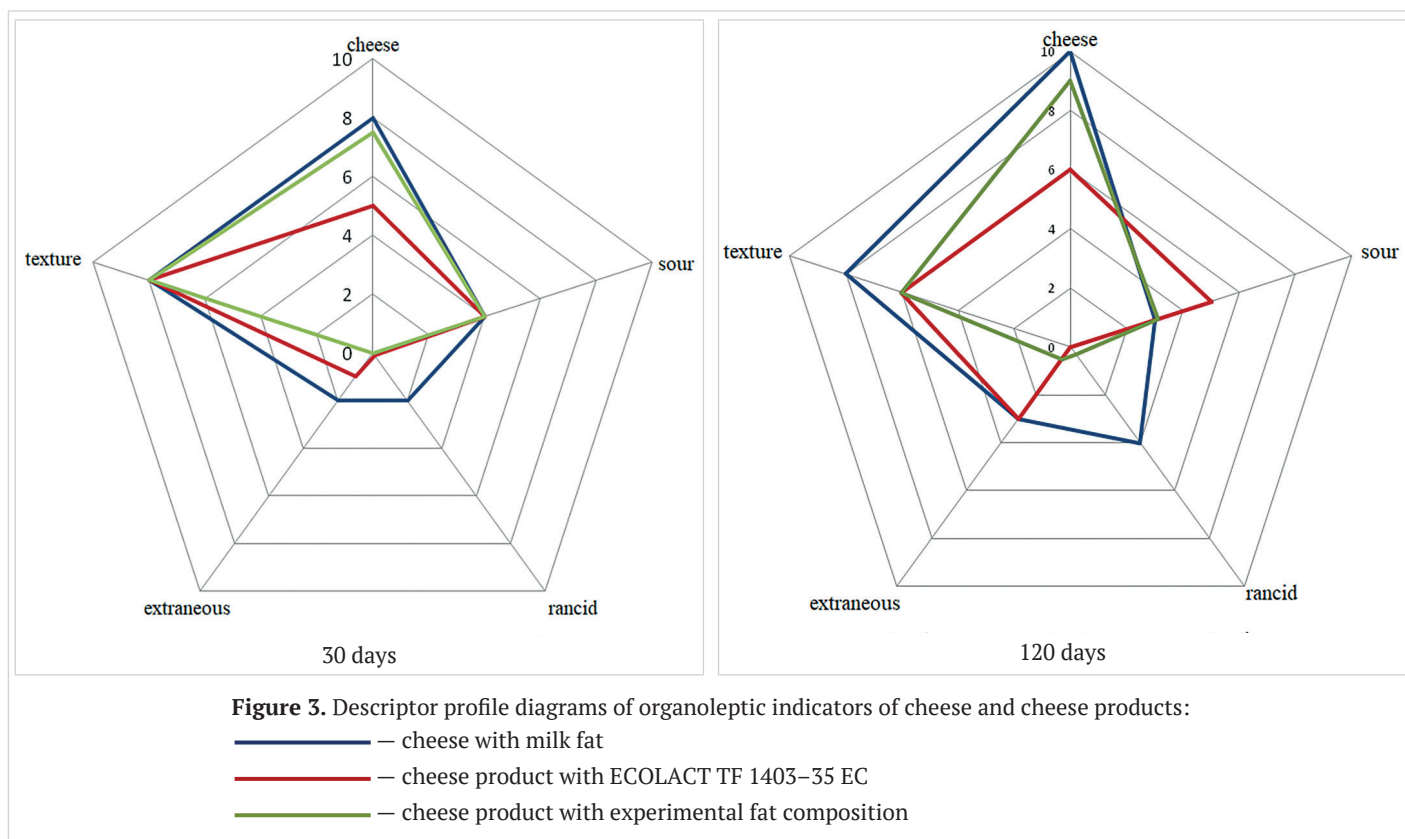


Figure 2. Change in the acidity of the fat phase of cheese and cheese products during ripening and storage

The acidity of milk fat, which constitutes the fatty phase of cheese, after 30 days of ripening was two times higher than in the compositions of vegetable fats that make up the fatty phase of cheese products. During subsequent storage for 4 months, the amount of free fatty acids in milk fat increased by 0.6 mmol/100 g, in vegetable fat compositions — by 0.2 mmol/100 g. Therefore, milk fat in cheeses is more subjected to lipolysis than vegetable



fats in cheese products. One of the reasons for this may be the insufficient lipolytic activity of the microorganisms that make up the starter cultures in relation to the vegetable fats that make up these compositions.

The addition of 0.8% butyric acid to the herbal composition increased the acidity by 0.1 mmol/100 g. This difference was maintained throughout the entire studied storage period.

A study of organoleptic indices when cheese products reached maturity (in 30 days) showed that the cheese with milk fat had the most pronounced cheese taste (Figure 3).

The cheese product with ECOLACT TF 1403-35 EC was significantly inferior to it in terms of severity of cheese taste, and the cheese product with an experimental fat composition containing added butyric acid in terms of severity of cheese taste was close to cheese with milk fat. After 4 months of storage, the cheese flavor in all objects intensified while maintaining the same tendency for the difference in severity.

The acidic note of taste was the same in all objects by 30 days. By 120 days of storage, it intensified in the cheese product with ECOLACT TF 1403-35 EC. In the cheese with milk fat and cheese product with an experimental fat composition, on the contrary, the severity of the sour taste has become weaker.

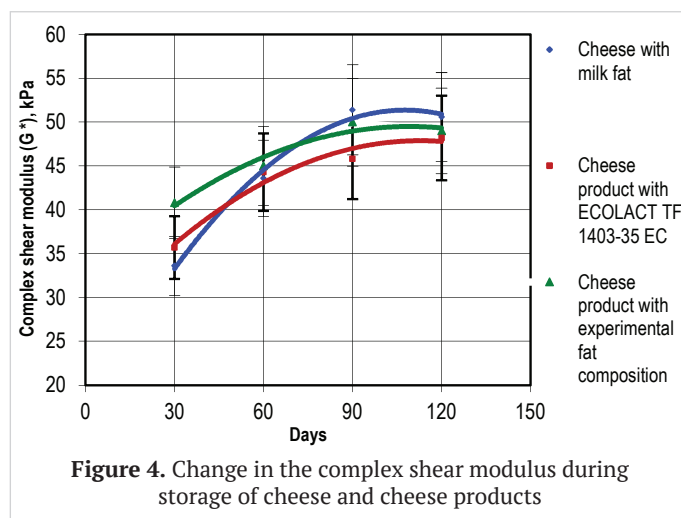
In both cheese products for the entire period of ripening and storage rancidity was not felt. In contrast to them, a slight rancidity was noted in the cheese with milk fat by 30 days, which intensified by 120 days of storage.

A slight extraneous taste was noted in the cheese with milk fat and cheese product with ECOLACT TF 1403-35 EC by 30 days. By 120 days of storage, it became more visible. In the cheese product with an experimental fat composition, an extraneous taste was not observed throughout the entire maturity and storage period.

The texture of all objects was characterized as elastic-plastic by 30 days. By 120 days of storage, it became compact. At the same time, a slight incoherence and friability was felt in cheese products, felt during chewing.

Studies of rheological indicators, supplementing the organoleptic assessment of texture, showed close G^* values for all objects,

slightly increasing during storage for the first two months with stabilization for 90-120 days (Figure 4).



A significant improvement in the taste and aroma of cheese products with an experimental fat composition is due to the addition of butyric acid, which, along with other volatile flavoring substances, is involved in the formation of their flavor.

Table 3 shows the total content of volatile flavoring substances formed during ripening and storage in the vapor phase of the investigated cheese products in comparison with cheeses.

All objects are characterized by substantial increase in the total amount of flavoring substances in the first 30 days, by 60 days — by three times compared with the level reached in 30 days. Then, in the cheese with milk fat and cheese product with an experimental fat composition, a decrease in the total amount of flavoring substances occurs, probably due to the transfer of their part to non-volatile compounds. The cheese product with ECOLACT TF 1403-35 EC showed a different trend: by 120 days of storage, an increase in the total amount of flavoring substances was noted

in it. Apparently, this can be attributed to a significant difference in the fatty acid composition of the fat phase of this product and the absence of low molecular weight fatty acids in it, which are subjected to the most noticeable transformation.

Table 3

Total content of volatile flavoring substances in the vapor phase of cheeses and cheese products, measured by the total area of the chromatogram

Objects of study	The total content of volatile flavoring substances, nA ^o sec				
	0 days (after pressing)	30 days	60 days	90 days	120 days
Cheese with milk fat	0.277	2.15	6.14	4.20	3.14
Cheese product with ECOLACT TF 1403–35 EC	0.314	2.14	6.28	6.21	7.87
Cheese product with experimental fat composition	0.318	2.00	5.68	4.42	4.94

Table 4 presents the content of individual volatile flavoring substances in the vapor phase of cheeses and cheese products at the age of 60 days, characterized by the maximum amount of flavoring compounds.

Table 4

Content of individual volatile flavoring substances in the vapor phase of cheeses and cheese products

Name	Mass fraction of volatile taste and aromatic substances, %		
	Cheese with milk fat	Cheese product with ECOLACT TF 1403–35 EC	Cheese product with experimental fat composition
propanone	23.0	16.2	5.5
ethanal	64.3	68.9	80.0
butanal	0.005	0.018	1.755
butanone-2	12.2	14.8	12.1
pentanone-2	0.13	—	0.135
butyric acid	0.001	—	0.005

The spectrum of volatile flavoring substances of the vapor phase of cheese with milk fat and cheese product with an experimental fat composition showed the uniformity of the set of volatile

substances, and, consequently, the proximity of the taste profile, but with a lesser degree of severity in the cheese product with an experimental fat composition. The vapor phase of these objects is 99.5% represented by six volatile flavoring substances, including two aldehydes (ethanal, butanal), three ketones (propanone, butanone-2, pentanone-2) and butyric acid. It should be noted that the proportion of butanal, pentanone and butyric acid was low and ranged from 1.75% to 0.001%. However, the detection of these substances even in small concentrations cannot be ignored, since, having a high sensitivity threshold, they can affect the taste and aroma of the finished product.

In the vapor phase of the cheese product with ECOLACT TF 1403–35 EC, only four volatile flavors were identified: ethanal, propanone, butanone-2 and butanal. Their amount is 99.9% of the total amount of flavoring substances. This difference explains the less pronounced cheese taste and aroma of this product compared to the cheese product with an experimental fat composition containing additionally added butyric acid.

4. Conclusion

The formation in the process of ripening and storage of organoleptic characteristics of cheesemaking products depends on the composition of the fat phase, as a result of lipolysis of which flavor compounds are formed. Vegetable fats, which make up the fatty phase of cheese products, are less subjected to lipolysis than milk fat in cheeses. This is the main reason for the mild taste and aroma of cheese products compared to cheeses. Proteolysis makes the same contribution to the total amount of aromatic substances formed, as it does not depend on the nature of the fat found in the protein matrix.

The correction of the fatty acid composition of vegetable fat compositions by including butyric acid in the fat matrix had a positive effect on the organoleptic characteristics of cheese products in the overall perception of flavor. Cheese products made with a modified fat composition were close to cheeses with milk fat in terms of severity of cheese taste and aroma, rheological parameters and the spectrum of volatile flavoring substances.

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