Influence of the maturation process on the sheep's milk of Camembert cheese fatty acid profile change

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Abstract

The aim of this research was to study the fatty acid composition of Camembert cheese fat phase. The object of the research was the sheep’s milk Camembert soft cheese. The Camembert soft cheese was made from sheep’s milk pasteurized at 63°C for 30 minutes before production, using cultures Penicillium camemberti, Geotrichum candidum, as well as Lactococcus lactis, Lactococcus cremoris, Lactococcus diacetylactis, Leuconostoc mesenteroides ssp. cremoris. The study of the cheese fatty acid composition during maturation was carried out using the gas chromatography method in accordance with the state industry standard of the Russian Federation 32915-2014 "Milk and dairy products. The determination of fatty acid composition of the fat phase by gas chromatography". The fatty acids profile in the process of cheese maturation changed significantly. There was an increase in the short chain fatty acids concentration: oil (C4:0), kapron (C6:0), capryl (C8:0). A change in the lauric acid content (C12: 0) and myristic acid (C14: 0) showed a similar trend but with less dynamics. On the 14th day of maturation, the concentration of lauric acid (C12:0) increased by 30%, myristic acid (C14:0) -- by 13%. At the beginning of the maturation period, the C18:1n9t isomer consisted about 70% of the total fatty acid trans-isomers. During maturation, the concentration of C18:1n9t decreased by 98%. It was found that, regardless of the maturation period, fatty acids C10: 0, C14: 0, C16: 0, C18: 0, C18: 1 t11 and C18: 1c9 consisted about 73% of the total fatty acids. There was a decrease in the concentration of w-6-polyunsaturated fatty acids with a simultaneous increase in the concentration of w-3 polyunsaturated fatty acids. The results can serve as a basis for comparative analysis development tools and strategies aimed at improving the nutritional characteristics of sheep’s milk cheese.

Keywords: cheese, technology, lipids, fatty acids, maturation

1. Introduction

Milk fat is a major component of most varieties of cheese, and many consumers limit their consumption of cheese, as more than 60% of the total fatty acids in milk fat are saturated fatty acids. In recent years, consumption of low-fat dairy products has become...
increasingly popular among consumers who care about their health, which has led to the development of new dairy products with improved fatty acid composition and increased biological value [1-10]. However, fat reduction has been proved to be a challenge, as fat is important for the dairy products texture and taste, especially for cheese. Fat reduction in cheeses leads to undesirable texture, lack of characteristic taste or the presence of foreign flavors. Therefore, it is important to study the technological processes that form the sensory qualities of cheeses. The white thin crust with surface mold of Penicillium camemberti, in cheeses such as Camembert, forms a complex ecosystem. Penicillium camemberti, Geotrichum candidum are used for the production of Camembert cheese from pasteurized milk. Soft cheeses maturing with the participation of surface microflora form a complex ecosystem that is not studied well. Furthermore, the lipolytic activity of the surface microflora leads to typical cheese sensory properties. Short-chain fatty acids contribute directly to the organoleptic characteristics of cheeses [11–13]. In the scientific literature there are results of researches devoted to the study of fatty acids concentration in cow's milk cheeses. However there is practically no information about the fatty acid profile change in sheep's milk cheeses during maturation [13–15]. Thus, the purpose of this research was to study the fatty acid composition of the fat phase of cheeses such as sheep's milk Camembert during maturation. The results can serve as a basis for the development of comparative analysis tools and strategies aimed at improving the nutritional characteristics of sheep's milk cheese.

2. Methods and Equipment

2.1. Methods

2.1.1. Diagrammatic representation

The object of the research was the sheep's milk Camembert soft cheese. The Camembert soft cheese was made from sheep's milk pasteurized at 63°C for 30 minutes before production, using cultures Penicillium camemberti, Geotrichum candidum, as well as Lactococcus lactis, Lactococcus cremoris, Lactococcus diacetylactis, Leuconostoc mesenteroides ssp. cremoris. After the application of calcium chloride and rennet, in a dose providing the total duration of coagulation lasted for 30-45 minutes. Before molding, the clot was cut into 1-1.5cm cubes. Then the cheese grain was dewatered and stirred. Then the molding, self-pressing and were carried out. After that maturation was performed at a temperature of 8±2 °C for 14 days.
The study of the cheese fatty acid composition during maturation was carried out using the gas chromatography method in accordance with the state industry standard of the Russian Federation 32915-2014 "Milk and dairy products". Determination of fatty acid composition of the fat phase by gas chromatography.

To assess the quality of lipids in the fat phase of Camembert cheese, the atherogenicity index (AI) and thrombogenic index (TI) were calculated according to the formulas [17]:

\[
AI = \frac{[12 : 0(4 \cdot 14 : 0) + 16 : 0]}{\omega - 3 PUFA + \omega - 6 PUFA + MUFA}
\]  
(1)

\[
TI = \frac{(14 : 0 + 16 : 0 + 18 : 0)}{0.5 \cdot MUFA + 0.5 \cdot \omega - 6 PUFA + 3 \cdot \omega - 3 PUFA + \omega - 6 PUFA}
\]  
(2)

where PUFA stands for polyunsaturated fatty acids, MUFA stands for monounsaturated fatty acids.

3. Results

Lipids in foodstuff can undergo hydrolytic or oxidative degradation. However, in cheese, oxidative changes are very limited due to the low redox potential. However, triglycerides in all varieties of cheese undergo hydrolysis under the action of endogenous or exogenous lipases, which leads to the release of fatty acids in the cheese during maturation. Ruminant animals' milk fat triglycerides are rich in short-chain fatty acids, which when released have low taste thresholds that contribute significantly to the flavor of many varieties of cheese.

The sensory characteristics acceptability of cheese depends largely on the taste that is formed during maturation. Two important compounds classes that contribute to the flavor are volatile sulfur compounds and fatty acids. Free fatty acids contribute to the cheese taste and aroma formation to a large extent.

Lipolysis is one of the main biochemical processes that contribute to the development of taste during cheese maturation. The characteristic taste of soft cheeses such as Camembert, especially made from sheep's milk, is formed largely as a result of the fat phase exposure to mold Penicillium camemberti. We studied the influence of starter cultures Lactococcus lactis, Lactococcus cremoris, Lactococcus diacetylactis, Leuconostoc mesenteroides ssp. cremoris and Penicillium camemberti, Geotrichum candidum on fatty acid profile change in Camembert cheese. The profile of fatty acids in the process of cheese maturation changed significantly (Figure 1).
Figure 1: change of fatty acid profile of soft sheep's milk Camembert-like cheese in the process of maturation: a) without maturation; b) maturation period-14 days.

Short-chain free fatty acids contribute to the final taste characteristics of cheese. Table 1 shows the dynamics of changes in the composition of fatty acids during maturation. There was an increase in the short chain fatty acids concentration: oil (C4:0) kapron (C6:0), capryl (C8:0). The change in the lauric acid (C12:0) and myristic acid (C14:0) content was characterized by a similar trend. On the 14th day of maturation, the concentration of lauric acid (C12:0) increased by 30%, myristic acid (C14:0) -- by 13%. The concentration of palmitic acid (C16:0) and stearic acid (C18:0) reduces as well.

| Fatty acid | The cheese maturation period |
|------------|-----------------------------|
|            | no maturation | 7 days | 14 days |
| C4:0       | 2.532         | 3.459  | 5.227   |
| C6:0       | 2.836         | 3.767  | 5.806   |
| C8:0       | 2.842         | 2.913  | 4.824   |
| C10:0      | 7.272         | 6.230  | 10.439  |
| C11:0      | 0.158         | 0.291  | 0.354   |
| C12:0      | 3.539         | 3.642  | 4.811   |
| C13:0      | 0.070         | 0.083  | 0.092   |
| C14:0      | 8.323         | 8.768  | 9.445   |
| C15:0      | 0.918         | 1.001  | 1.068   |
| C16:0      | 22.688        | 24.715 | 18.285  |
| C17:0      | 1.092         | 0.612  | 0.662   |
| C18:0      | 13.804        | 10.239 | 11.72   |
| C20:0      | 0.007         | 0.005  | 0.001   |
| C21:0      | 0.609         | 0.740  | 0.862   |
| C22:0      | 0.041         | 0.069  | 0.117   |
| C23:0      | 0.009         | 0.009  | 0.022   |
| C24:0      | 0.002         | 0.047  | 0.019   |
| ∑ saturated fatty acids | 66.742 | 66.590 | 73.206 |

Table 2 shows the change in the monounsaturated fatty acids composition of sheep's milk Camembert cheese during maturation. The most common C18: 1 trans-isomer
is C18:1n9t, consisting up to 60 to 80% of the total fatty acids trans-isomers. During maturation, the concentration of C18:1n9t decreases by 98%.

**TABLE 2:** Maturation process effect on the of monounsaturated fatty acids content in sheep’s milk Camembert-like cheese, %.

| Fatty acid   | The cheese maturation period |
|--------------|----------------------------|
|              | no maturation | 7 days | 14 days |
| C14:1n5-c9   | 0.449         | 0.676  | 0.562   |
| C15:1n6-c9   | 0.333         | 0.312  | 0.316   |
| C16:1n7-c9   | 0.523         | 1.080  | 0.643   |
| C17:1n8-c9   | 0.379         | 0.235  | 0.236   |
| C18:1n9t     | 2.055         | 1.029  | 0.023   |
| C18:1n9c     | 23.836        | 23.407 | 19.461  |
| C20:1        | 1.033         | 0.512  | 0.990   |
| C22:1n9-C13  | 0.007         | 0.018  | 0.031   |
| C24:1        | 0.036         | 0.306  | 0.608   |
| ∑MUFA (monounsaturated) | 28.651 | 27.575 | 22.870 |

Based on the table 2 data analysis result, we can conclude that there is a decrease in the monounsaturated fatty acids concentration during maturation. The concentration of the CIS-isomer of oleic acid (C18:1n9c) decreased by 18%.

**TABLE 3:** Maturation process effect on the of polyunsaturated fatty acids content in sheep’s milk Camembert-like cheese, %.

| Fatty acid   | The cheese maturation period |
|--------------|----------------------------|
|              | no maturation | 7 days | 14 days |
| C18:2n6      | 0.256         | 0.326  | 0.457   |
| C18:2n6c     | 3.453         | 4.328  | 1.910   |
| C18:3n3-t-9, t-12, t-15 | 0.025 | 0.034  | 0.034   |
| C18:3n6-C6, C9, C12 | 0.003 | 0.069  | 0.133   |
| C18:3n3-t-9, t-12, c-15 | -     | -      | -       |
| C18:3n3-c-9,t-12,t-15 + C18:3n3-t-9, c-12, c-15 | 0.398 | 0.360  | 0.410   |
| C18:3n3-c-9, t-12, c15 | 0.015 | -      | 0.026   |
| C18:3n6-C9, C12, C15 | -     | 0.132  | 0.132   |
| C20:2        | 0.075         | 0.068  | 0.135   |
| C20:3n6-C8, C11, C14 | 0.050 | 0.027  | 0.040   |
| C20:3n3-C8, C11, C14, | 0.224 | 0.332  | 0.499   |
| C20:4n6-C8, C11, C14, C17 | 0.022 | 0.029  | 0.031   |
| C22:2n6-C13, 16 | 0.014 | 0.049  | 0.075   |
| C20:5n3-C5, C8, C11, C14, 17 | 0.006 | 0.032  | 0.018   |
| C22:6n3-C4, C7, 10, 13, 16, 19 | 0.066 | 0.049  | 0.024   |
| ∑ polyunsaturated fatty acids | 4.607 | 5.835  | 3.924   |
By the end of the maturation period, the polyunsaturated fatty acids concentration decreases by 14%. However, it is necessary to note the increase in the linoleic acid concentration.

As a result of the analysis of the fatty acid profile changes during the Camembert cheese maturation, it was found that, regardless of the maturation period, the fatty acids C10: 0, C14: 0, C16: 0, C18: 0, C18: 1t9 and C18: 1c9 consist about 73% of the sum of all fatty acids. Figure 2 presents data on changes in the C10 fatty acid profile: 0, C14: 0, C16: 0, C18: 0, C18: 1 t11 and C18: 1c9 in the process of cheese maturation.

![Figure 2: Fatty acid profile of the fat phase of Camembert-like cheese during maturation: a) without maturation b) maturation period of 14 days.](image)

Figure 3 shows the change in the fatty acids' ratio during the cheese maturation. The amounts of fatty acids in all samples decreased in following order: saturated fatty acids> monounsaturated fatty acids > polyunsaturated fatty acids. The atherogenicity index is closely related to the qualitative and quantitative fatty acids composition. There
is a positive correlation between the variables under consideration. Which means, an increase of myristic (C:14) and palmitic (C:16) acids in milk leads to an increase in the atherogenicity index. There is also a negative correlation between the sum of unsaturated fatty acids with long chains and the atherogenicity index. The atherogenicity index decreases with an increase of unsaturated fatty acids content with long chains ($\sum$ C : 18; C : 20; C: 22) as part of the fat phase of sheep's milk soft cheeses.

![Figure 3: Sum of saturated, monounsaturated, polyunsaturated fatty acids ratio in the Camembert cheese maturation process: 1 - saturated fatty acids; 2 - monounsaturated fatty acids; 3 - polyunsaturated fatty acids.](image)

The main types of W-3 fatty acids used by the body are: α-linolenic acid (C18: 3n-3, αLA), eicosapentaenoic acid (C20: 5n-3), docosapentaenoic acid (C22: 5n-3) and docosahexaenoic acid (C22: 6n-3). Nutritional recommendations are based on different ratios such as w-3 polyunsaturated fatty acids / w-6 polyunsaturated fatty acids and polyunsaturated fatty acids / saturated fatty acids. These values are used to estimate the nutritional value of fat for human consumption. A healthy diet should contain about 4 times more ω-6 fatty acids than ω-3 fatty acids. The $\omega$-6 / $\omega$-3 ratio is an important determinant for reducing the risk of many chronic diseases. Table 4 presents data on the lipids nutritional value in sheep's milk Camembert cheese.

In the process of maturation there is a change in the w-3 and w-6-polyunsaturated fatty acids ratio. There is a w-6-polyunsaturated fatty acids concentration decrease and a simultaneous w-3 polyunsaturated fatty acids concentration increase. The obtained data indicate the possibility of directed regulation of fatty acid profile during the cheese's maturation process.
### Table 4: Fat human consumption biological value Indicators.

| Name of the indicator                              | The cheese maturation period |
|----------------------------------------------------|-----------------------------|
|                                                   | no maturation | 14 days         |
| w-3 polyunsaturated fatty acids                    | 0.734          | 1.011           |
| w-6 polyunsaturated fatty acids                    | 3.864          | 2.777           |
| w-6 / w-3                                          | 5.264          | 2.747           |
| Polyunsaturated fatty acids / saturated fatty acids | 0.069          | 0.054           |
| Atherogenicity index                                | 1.695          | 2.238           |
| Thrombogenic index                                  | 2.66           | 2.795           |

**4. Discussion**

We investigated the regularities characterizing the fatty acid composition transformation process during sheep's milk Camembert cheese maturation. It was found that, regardless of the maturation period, fatty acids C10: 0, C14: 0, C16: 0, C18: 0, C18: 1t11 and C18: 1c9 included about 73% of the total fatty acids. By the end of the maturation period, the polyunsaturated fatty acids concentration decreases by 14%. The increase in hypercholesterolemic and a decrease in hypocholesterolemic fatty acids concentration during the maturation of cheese influenced the increase in the atherogenicity index and thrombogenic index.

**5. Conclusion**

Fatty acids with less than twelve carbon atoms characterize the sheep's milk fat phase fatty acid composition feature and can be used to detect different types of milk mixtures in sheep's milk cheese. The obtained results naturally describe the lipolysis process during the soft cheeses maturation process with the participation of Penicillium camemberti.

Qualitative and quantitative fatty acid sheep's milk composition used for cheese production, technological production features, the symbiotic starter cultures use in the production process are closely connected to the organoleptic cheese characteristics. The data from this study can be used to develop control tools and strategies to improve the nutritional characteristics of sheep cheese.

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Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Prosekov, A. Yu., Dyshlyuk, L. S., Milent’eva, I. S., et al. (2018). Study of the biofunctional properties of cedar and pine oil with the use of in vitro testing cultures. Foods and Raw Materials. vol. 6. (1). pp. 136--143.

[2] Zimina, M. I., F. A. Gazieva, Pozo-Dengra, J., et al. (2017). Determination of the intensity of bacteriocin production by strains of lactic acid bacteria and their effectiveness. Foods and Raw Materials. vol. 5. (1). pp. 108--117.

[3] Novoselova, M. V., Prosekov, A. Yu. (2016). Technological options for the production of lactoferrin. Foods and Raw Materials. vol. 4. (1). pp. 90--101.

[4] Prosekov, A. Yu., Ivanova, S. A. (2016). Providing food security in the existing tendencies of population growth and political and economic instability in the world. Foods and Raw Materials. vol. 4. (2). pp. 201--211.

[5] Zimina, M. I., Sukhih, S. A., Babich, O. O., et al. (2016). Investigating antibiotic activity of the genus bacillus strains and properties of their bacteriocins in order to develop next-generation pharmaceuticals. Foods and Raw Materials. vol. 4. (2). pp. 92--100.

[6] Piskaeva, A. I., Sidorin Yu. Yu., Dyshlyuk L. S., et al. (2014). Research on the influence of silver clusters on decomposer microorganisms and E. coli bacteria. Foods and Raw Materials. vol. 2. (1). Pp. 62--66.

[7] Prosekov, A. Yu. (2014). Theory and practice of prion protein analysis in food products. Foods and Raw Materials. vol. 2. pp. 106--120.

[8] Voblikova, T., Mannino, S., Barybina, L., et al. (2019). Immobilisation of bifidobacteria in biodegradable food-grade microparticles. Foods and Raw Materials. vol. 7. (1). pp.
74–83.

[9] Barybina, L. I., Beloysova, E. V., Voblikova, T. V., et al. (2019). Multicomponent meat products for sports nutrition. *Journal of Hygienic Engineering and Design*. vol. 28. pp. 81–84.

[10] Golubtsova, Yu. V., Prosekov, A. Yu., Moskvina, N. A. (2019). Identification of fruit and berry raw materials in multicomponent food systems. *The dairy industry*. vol. 3. pp. 28–29.

[11] Prosekov, A. Yu., Dyshlyuk, L. S., Belova, D. D. (2019). Biodegradable antimicrobial packaging in cheese making. *Cheesemaking and buttermaking*. vol. 3. pp. 40–42.

[12] Guarrasi, V., Sannino, C., Moschetti, M., et al. (2017). The individual contribution of starter and non-starter lactic acid bacteria to the volatile organic compound composition of Caciocavallo Palermitano cheese. *International Journal of Food Microbiology*. vol. 259. pp. 35–42.

[13] Kim, N. S., Lee, J. H., Han, K. M., et al. (2014). Discrimination of commercial cheeses from fatty acid profiles and phytosterol contents obtained by GC and PCA. *Food Chemistry*. vol. 143. pp. 40–47.

[14] Sádecká, E., Kolek, D., Rich, H., et al. (2014). The Principal volatile odorants and dynamics of their formation during the production of May Bryndza cheese. *JFood Chemistry*. vol. 150. pp. 301–306.

[15] Han R., Zheng N., Zhang Y., et al. (2014). Milk fatty acid profiles in Holstein dairy cows fed diets based on corn stover or mixed forage. *Archives of Animal Nutrition*. vol. 68:1. pp. 63–71.

[16] Akbaridoust G., Plozza, T., Trenerry, V.C., (2015). Influence of paste-based feeding systems on fatty acids, organic acids and volatile organic flavor compounds in yogurt. *The Journal of dairy research*. vol. 82(3). pp. 279–286.

[17] Ulbrich, T.L.V., Southgate D.A.T. (1991). Coronary heart disease seven dietary factors. *Lancet*. vol. 338. pp. 985–992.