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Original scientific paper

FATTY ACID COMPOSITION OF LIVNO CHEESE[1*]

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Abstract: During its 132-year production, the characteristics of Livno cheese changed, first because of the transition from sheep to cow's milk, or their mixture. Livno cheese is specific primarly due to the presence of a specific plant cover in mountain area, climatic conditions and milk of autochthonous sheep. The purpose of this work is to determine the fatty acid composition of Livno cheese, with special reference to the contenct of bioactive components that have a positive effect on human health, and to tracking any changes in their content depending on the sampling or feeding period. For the production of Livno cheese, which was sampled after 90 days of ripening at ambient conditions, sheep's milk was used, mentioning that the cow's milk was added in proportion (80:20), which is commonly used in the traditional production of this cheese. Samples were analyzed by gas chromatography in As Vitas laboratory in Oslo Innovation Center, according to the procedure described in Luna and al. (2005). A total of 24 fatty acids were determined during the three sampling periods (July, August and September) In cheese samples, saturated fatty acid content (SFAs) was higher in relation to monounsaturated(MUFAs) and polyunsaturated (PUFAs) fatty acids. The fatty acid composition of the tested cheese samples is specific, because it contains fatty acids which have been proven to have an extremely beneficial effect on human health.

Key words: *sheep, milk, chees, fatty acid, health*

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INTRODUCTION

Much has been written about the beneficial ingredients of milk and dairy products, but some nutrition experts claim that dairy fat is harmful due to the high content of saturated fatty acids and trans fatty acids. Cheese, as a concentrated dairy product, has enormous popularity in nutrition, due to its taste, functionality and nutritional properties. Cheese is an important source of high-quality proteins, calcium and many other important nutrients, which give a valuable contribution to quality nutrition.

Cheese is a significant source of fat in human nutrition and contains a number of fatty acids. Fat in cheese currently has a negative image, mainly due to the perception of consumers about effects of saturated and trans fatty acids on cardiovascular system. Despite the presence of significant amounts of such fatty acids, there is no clear evidence that cheese consumption negatively affects any disease. Moreover, cheese also contains other fatty acids, e.g. conjugated linoleic acid and oleic acid, which have the potential to improve long-term health. The production of quality sheep cheese depends primarily on the quality of the milk for cheese making, or on its physical-chemical, and especially hygienic properties. Raw milk from sheep that are kept in extensive farming is used in the production of autochthonous cheeses. They produce less milk and its chemical composition is characterized by a high share of certain ingredients (Matutinović et al., 2007) Raw milk cheeses mature faster and have a more intense taste than industrially produced cheeses (Savić, 2010). Bearing in mind the high quality of sheep's milk, characterized by low allergenic activity, high concentration of nutraceutical compounds and a relatively high price of sheep milk cheese, there is a significant potential for the world market when it comes to this industry. Because large quantities of sheep's milk are turned into cheese, milk quality is evaluated in terms of its technological and coagulative properties, which depend primarily on the fat and protein content. However, the increasing attention of consumers to the nutritional and health aspects of food, has directed dairy producers in the direction of achieving the appropriate composition of the milk lipids (Nuda et al., 2014). For a long time, consumer perception of animal fats was associated with an increased risk of cardiovascular disease, especially due to the large amount of SFAs. The fatty acid composition and concentration in milk depend on two main factors: animal health status, including breed, age, stage of lactation, environmental factors, and feeding of animals with particular emphasis on the type of feed mixture (Falchero et al., 2010). The main targeted changes in nutrition are the reduction of saturated-unsaturated fatty acid ratio and the increase in conjugated levels of linoleic acid and omega-3 polyunsaturated fatty

acids. Animal nutrition is a major factor regulating the composition of milk fat, and food manipulation with different types of fat is considered a key strategy for achieving a desirable fatty acid profile.

In this paper, we examined the fatty acid composition of Livno cheese, which is one of the most famous cheeses from the territory of the former Yugoslavia. It is a hard, full-fat cheese, which has a moderately salty, spicy flavor typical for sheep cheeses. Livno cheese is specific primarly due to the presence of a specific plant cover in mountain area, climatic conditions and milk of autochthonous sheep.Today, especially in periods when there is no sheep's milk, Livno cheese is made in the same way, but in different sheep's and cow's milk ratios, sometimes only from cow's milk, which is indicated on the label.

MATERIAL AND METHODS

For the research, the determining factor was the traditional production technology. Tests were carried out on pramenka sheep. The animals were labeled with the appropriate number of ear tags and samples were taken from the same animals throughout different time periods. During the sampling, sheep feeding was based on grazing. The study involved taking fresh sheep's milk during manual milknig in the morning and for the production of the Livno cheese, we used sheep's milk which had previously been sampled, noting that the cow's milk was added in proportion (80:20), which is commonly used in the traditional production of this cheese. Samples were taken after 90 days of ripening in original ambient conditions. Using the method of gas chromatography (GC), the fatty acid composition in the cheese samples was determined : butyric (C4: 0), hexanoic (C6: 0), caprylic (C8: 0), decanoic (C10: 0), lauric (C12: 0), myristic (C14: 0), pentadecanoic (C15: 0), palmitic (16: 0), margaric (C17: 0), stearic (C18: 0), arachidic (C20: 0), myristoleic (C14:1cis-9), palmitoleic (C16:1 cis-9), oleic (C18:1 cis-9), C18:1 cis-11, elaidic (C18:1 trans-9), C18:1 trans-10, vaccenic (C18:1 trans-11), arachidonic (C20:4c5,c8,c11,c14), eicosapentaenoic (C20:5c5,c8,c11,c14,c17), docosahexaenoic (C22:6 c7,c10,c13,c16,c19), linoleic (C18:2 n-6), α linolenic (C18:3 n-3), rumenic (C18:2 cis-9 trans-11 CLA).

The samples were sent in frozen state on dry ice and analyzed in ''As Vitas'' laboratory, Oslo Innovation Center, Norway. Cheese samples were defrosted at room temperature and homogenized prior to analysis. The preparation of the samples was carried out according to the procedure described in Luna et al. (2005), which includes the separation of milk fat by centrifugation and methylation of fatty acids, resulting in methyl esters of fatty acids (FAME), which are analyzed by chromatography. A high resolution GC method has been applied, which provides a very good

separation of FAME. The Select FAME column was 200 mm long and the isothermal separation peak 18: 1 was used. The analysis was performed on the Agilent 689N GC instrument with a split / splitless inlet, a 7683B autosempler and a flame ionization detector (Agilent Technologies, Palo Alto, CA). Separation was performed using a fused silica capillary column (Varian Inc.) CP-SELECT CB FOR FAME (200 mm long, 0.25 mm inner diameter and 0.25 μm film thickness). The following temperature program was applied: the initial temperature of 700C was maintained for 4 minutes, then heated at 200C / minute to 1600C, maintained for 80 minutes and then heated at 30C / min to 2200C, which was maintained for 28 minute. As a carrier gas, hydrogen was used at a pressure of 314 kPa. Fatty acid analysis (C4: 0-C22: 6) was carried out by autoinjection of 1 μl of sample in a split ratio 70: 1, a hydrogen flow of 151 ml / min and a temperature of 2800C. The flame-ionization detector temperature was 2900C at a hydrogen flow rate of 40 ml / min, air flow rate 450 ml / min air and nitrogen flow rate (as a make-up gas) of 45 ml / min. The sampling frequency on the chromatogram was 10 Hz (reading 10 times per second), and the recording time of one chromatogram was 136 minutes. The results obtained are expressed in grams of individual fatty acids per 100 g of total fatty acids (g / 100 g FA).

RESULTS

Table 1 gives an overview of the established values of fatty acids in the milk fat of Livno cheese by the sampling periods. In the SFA class, the highest values determined were for C16: 0, C18: 0 and C14: 0 acids. For C4: 0, C6: 0, C8: 0, C10: 0, C12: 0, C14: 0 and C15: 0, higher values were found in the III sampling period than in I and II sampling (Table 1). From the MUFAs class, the highest value was established for oleic acid in the II period of sampling, while the VA value was the smallest in the III sampling period. In the PUFAs class, C18: 2 n-6 and C18: 3 n-3 dominated.

Table 1. Content of fatty acids in milk fat of Livno cheese by sampling periods

	I sampling	**II sampling**	**III sampling**
Fatty acid (g/100g FA)	**SFA**		
Butyric C4:0	0,96	0,85	1,0
Hexanoic C6:0	0,73	0,59	1,0
Caprylic C8:0	0,67	0,52	1,0
Decanoic C10:0	2,32	1,86	3,32
Lauric C12:0	2,02	1,78	2,80
Myristic C14:0	8,83	8,47	9,92

Pentadecanoic C15:0	1,20	1,17	1,26
Palmitic C16:0	24,97	25,02	24,43
Margaric C17:0	1,04	1,03	0,08
Stearic C18:0	12,19	11,78	11,24
Arachidic C20:0	0,51	0,53	0,43
	MUFA		
Myristoleic C14:1 cis-9	0,26	0,34	0,49
Palmitoleic C16:1 cis-9	0,93	1,18	1,13
Oleic C18:1 cis-9	21,98	23,41	23,08
C18:1 cis-11	0,93	0,97	0,69
Elaidic C18:1 trans-9	0,29	0,73	0,34
C18:1 trans-10	0,41	0,33	0,22
Vaccenic C18:1 trans-11	3,34	3,34	2,49
	PUFA		
Arachidonic C20:4 n-6	0,12	0,14	0,13
Eicosapentaenoic C20:5 n-3 (EPA)	0,11	0,11	0,08
Docosahexaenoic C22:6 n-3 (DHA)	0,07	0,06	0,08
Linoleic C18:2 n-6	2,11	2,25	2,25
α- linolenic C18:3 n-3	1,48	1,07	1,25
Rumenic C18:2 cis-9, trans-11 (CLA)	0,59	0,58	0,60
∑n-3	1,66	1,24	1,41
∑n-6	2,23	2,39	2,38
∑SFA	55,44	53,6	56,48
∑MUFA	28,14	30,30	28,44
∑PUFA	4,48	4,21	4,39
∑UFA	32,62	34,51	32,83
The ratio of fatty acids			
n-6/n-3	1,34	1,92	1,68
SFA/MUFA	1,97	1,76	1,98
SFA/PUFA	12,37	12,73	12,85
MUFA/PUFA	6,28	7,19	6,47
SFA/UFA	1,69	1,55	1,72

UFA/MUFA	1,15	1,13	1,15
UFA/PUFA	7,28	8,19	7,47

SFAs - saturated fatty acids; MUFAs - monounsaturated fatty acids; PUFAs - polyunsaturated fatty acids; UFAs - unsaturated fatty acids. I, II, III-represent sampling periods: July, August and September

DISCUSSION

The chemical composition of products of animal origin, especially the content of certain ingredients such as fatty acids, has attracted the attention of experts for years because of their impact on human health.The fatty acid composition of the cheese is an important marker for determining the characteristic properties of the cheese. The results of these studies show an obvious variation in the content of individual fatty acids in the analyzed Livno cheese samples by sampling periods (Tab.1). The content of SFAs in the analyzed samples of Livno cheese can be a direct consequence of the technological quality of milk, or the content of fatty acids found in milk. The dominant SFA analyzed Liva cheese samples are C14: 0, C16: 0 and C18: 0 (Table 1).

In the analyzed samples of Livno cheese, the content of C8: 0, C12: 0 and C15: 0 was the highest in the III sampling period. SFAs including C12: 0, taken above energy needs, have hyperlipidemic and hypercholesterolemic effects, but if the diet is balanced in terms of SFA and UFA, then C12: 0, as well as C16: 0 and C18: 0, can mostly be oxidized and without a hyperlipidemic effect. Organic acids contribute to cheese flavor, which is one of the most important qualitative criteria for fresh and mature cheeses. The composition of organic acids in cheese is important for the characterization of cheese. Acids C4: 0, C6: 0 and C8: 0 are the main carriers of the cheese aroma, long-chain acids are present but do not affect smell, while medium-chain acids are responsible for the smell of sheep fats. During the ripening of the cheese there is a change and increase in the composition of organic acids, and the content of organic acids can be taken as the index of cheese ripening (Jerman, 2007) From the MUFA group, the highest concentrations were determined for C18: 1 cis-9 and VA, and the dominant content from the PUFA group was established for C18: 2 n-6 and C18: 3 n-3.

The fatty acid composition is probably a consequence of the conditions of breeding, feeding and production technology. Cheese produced from milk obtained from mountain herds can contain a higher proportion of UFAs, which affects better rheological properties and increased primary proteolysis, compared to cheeses obtained from milk from herds grazing in valley pastures (Matutinović et al.,

2007). The dominant content of C18: 1 cis-9 in the examined cheeses coincides with the literature data (Vilušić et al., 2008). Nudda & Associates (2014) in their research state that seasonal variations affect C18: 1 trans-11 content in the cheese. Seasonal changes are probably related to changes in pasture quality. Acid C18: 2 n-6 as an essential fatty acid can not be synthesized in the human organism.

However, only a small part of this acid comes from immediate food absorption, while most of it originates from the body's reserves, partly compensating for oscillations in food intake (Delaš i sar., 2005). Cheese is a significant source of C18: 2 n-6 and its isomers, which have a potential role in reducing the risk of cancer and cardiovascular disease (Vilušić et al., 2008).

The relationship between SFA, MUFA and PUFA Livno cheese is inversely proportional to the needs of a balanced diet and the impact on human health. ΣSFA in the samples of the examined cheese was higher than ΣMUFA and ΣPUFA, which seems to place this product in a bad position from a nutritionist's point of view. The acids MUFA and PUFA show biological activity (oleinic and its isomers, different linoleic acid isomers, EPA and DHA) (Marenjak et al., 2006). PUFAs show a number of positive effects, but it is important to note that they are very prone to oxidation both in and out of the body, resulting in the formation of highly reactive free radicals and other harmful oxidation products (Kravić, 2010). An anti-carcinogenic action of Livno cheese is studied because it is a good source of potentially anti-cancerous components, conjugated linoleic acid. As a significant source of linoleic acid and its isomers, cheese has a potential role in reducing cancer risk and regulating body weight, or distributing fat deposits. Potential protection against heart disease and hypertension is essential, and an important role is attributed to maintaining good bone health due to its high content of calcium. For over half a century, the concept of healthy eating is based on avoiding the intake of fat and cholesterol, especially saturated fatty acids. In many countries, a diet with low saturated fatty acids is recommended as a part of therapy in people with elevated plasma cholesterol due to a reduction in the risk of cardiovascular disease. By increasing the proportion of polyunsaturated fatty acids in the diet it is possible to reduce blood cholesterol concentration and reduce the buildup of fatty deposits in the arteries which significantly reduces the risk of coronary heart , heart attack and stroke.

CONCLUSION

Relationships of SFA, MUFA and PUFA in Livno cheese are inversely proportional to the needs of balanced nutrition and the impact on human health. ΣSFA in the samples of the examined cheese was higher than ΣMUFA and ΣPUFA, which seems to place this product in a bad position from a nutritionist's point of view. The quality of raw milk can be improved by a particular nutrition strategy, thereby increasing the proportion of biologically active ingredients in raw milk, and in the technological sense, such milk can be an excellent raw material for the processing and marketing of high-quality dairy products such as cheeses.

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