

## **Fatty acids profile and lipids health indices of white brined cheese produced from Lacaune sheep milk**

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### **Abstract**

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The objective of the study was to determine the composition of fatty acids in the milk of Lacaune sheep and produced cheese from it. The study was performed with ewe's milk of Lacaune breed, reared in the herd of the private farm in a village of Yambol municipality. Milk samples were collected in the morning and the evening, proportionally to the milk yield, according to rules for milk sampling. To perform an analysis of the fatty acid composition of the Lacaune sheep milk, three milk samples were collected at three different times from April to June 2017. From the milk samples have been made Bulgarian white cheese. The fatty acid composition of raw milk and cheese samples was determined at 60 day of producing. The most abundant fatty acids in milk and cheese were saturated fatty acids (82.37% and 77.42% in the milk and cheese, respectively). Monounsaturated fatty acids (14.31% for raw milk and 16.54% for white brined cheese) were the most numerous in terms of isomers, but mostly in low concentration. The atherogenic index was calculated on the obtained values for lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids and the unsaturated fatty acids. The data for the raw sheep milk was – 2.16 and for produced white brined cheese – 1.63. Omega 6/Omega 3 ratio varies from 1.33 for raw milk and 1.00 for white brined cheese, which is within the range of the optimal values for healthy nutrition.

*Keywords:* Lacaune breeds; sheep milk; fatty acids; profile; lipids; health indices

*Abbreviations:* SFA – saturated fatty acids, USFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, IA – index of atherogenicity, IT – index of thrombogenicity

### **Introduction**

For many years, fatty acids in milk and dairy products had a bad image because they were associated with the increase in serum cholesterol, weight gain, and a number of other diseases. As a result, a common attitude has been created that foods containing animal fats simply have to be avoided. Today, it is known that not only the quantity but also the structure of fatty acids play an important role in determining the state of health (Talpur, 2007). Milk fat contains relatively high amounts of saturated fatty acids, which are a risk factor associated with cardiovascu-

lar diseases (Astrup et al., 2011; Baum et al., 2012), while the low-volume monounsaturated and polyunsaturated fatty acids have a protective effect on of this risk (Lichtenstein et al., 1999).

Early studies were focused only on the content of saturated, monounsaturated and polyunsaturated fatty acids in the diet. Today, the different biological effects of individual saturated fatty acids that have a positive effect on health are known (Walther et al., 2008). Short-chain fatty acids, for example acetate (C2:0), propionate (C3:0) and butyrate (C4:0), are tested for their possible positive effects on colorectal cancer (Hijova & Chmelarova, 2007).

It should be borne in mind that some fatty acids pose a greater risk than others – C18:0, for example, have a relatively neutral effect, whereas C12:0, C14:0 and C16:0 lead to increased cholesterol concentrations (Gibson, 2011).

Consumers are becoming more aware of the link between nutrition and health. Milk fat is considered “bad” by many consumers and scientists are asked to clarify the role of nutrients in chronic diseases (Bauman & Lock, 2010; Grantham et al., 2012). This has led to the development of several indicators – lipid indexes that can be used to evaluate the preventive qualities of foods (Ulbricht & Southgate, 1991; Chilliard et al., 2003).

The purpose of the study was to establish the fatty acid composition and related health indexes of raw sheep milk obtained from the Lacaune breed and produced white brined cheese.

## Material and Methods

### Milk

The raw sheep milk from Lacaune breeds was obtained from a local farm in Vodenichene village, Yambol municipality. Milk samples were obtained in the morning and the evening, proportionally to the milk yield, according to rules

$$IT = \frac{C14:0 + C16:0 + C18:0}{0.5 \times C18:1 + 0.5 \times \sum MUFA + 0.5 \times \sum PUFA - n6 + 3 \times PUFA - n3 + (PUFA - n3 / PUFA - n6)}$$

for milk sampling. The ewe’s were kept under identical conditions of feeding and management. They fed on pasture and received additional a commercial concentrate and alfalfa hay. The amounts concentrate and alfalfa hay were calculated according to the nutritional requirements for sheep depending on the animal’s ages and production status. To perform an analysis of the fatty acid composition of the Lacaune sheep milk, three milk samples were collected at three different times from April to June 2017. From the milk samples have been made Bulgarian white cheese. The fatty acid composition of raw milk and cheese samples was determined at 60 day of producing.

### Cheese production

White brined cheese was prepared from sheep milk. The milk was pasteurized at 68 °C for 10 min, and cooled to 32 to 35 °C. *Streptococcus salivarius subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* were used as starter. The cheese was made as described by Peichevski et al. (1988).

### Fatty acids extraction and analysis

The extraction of milk fat was done by the method of Rose-Gottlieb using diethyl ether and petroleum ether (Methodenbuch, Bd. VI VDLUFA-Verlag, Darmstadt, 1985). After that the solvents were evaporated on a vacuum-rotary evaporator. Sodium methylate (CH<sub>3</sub>ONa) was used for obtaining methyl esters of the fatty acids. The fatty acid composition of raw milk and cheese was determined by gas chromatography “Clarus 500” with flame ionization detector and column ThermoScientific, 60 m, ID 0.25 mm, Film: 0,25 µm.

From the data on the fatty acid composition, the following were calculated:

- Index of atherogenicity (IA) – indicating the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated, the former being considered proatherogenic and the latter anti-atherogenic (Ulbricht & Southgate, 1991):

$$IA = \frac{4 \times C14:0 + C16:0 + C18:0}{\sum (MUFA + PUFA)}$$

- Index of thrombogenicity (IT) – showing the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic fatty acids (saturated) and the antithrombogenic fatty acids (Ulbricht & Southgate, 1991):

### Statistics

Statistical software (Statistica 6.0) was used for statistical data analysis. Fisher test was used for mean comparison

## Results and Discussion

The fatty acid content of milk fat in raw milk and mature cheese is presented in Table 1.

The fat content of the mature cheese was similar to that of the milk used to produce it. The content of almost all essential fatty acids, except lauric (C12:0), myristic (C14:0) and stearic (C18:0), decreased.

The high concentration of caproic (C6:0), caprylic (C8:0) and capric (C10:0) fatty acids in sheep and goat milk, compared to cow milk, causes a specific flavor in the milk of these small ruminants (Strzałkowska et al., 2009; Tudisco et al., 2010). In addition, these fatty acids can have beneficial effects on human health by inhibiting bacterial and viral growth as well as dissolving cholesterol deposits (Markiewicz-Kęszycka et al., 2013).

**Table 1. Fatty acid composition of processed milk and ripened cheese, %**

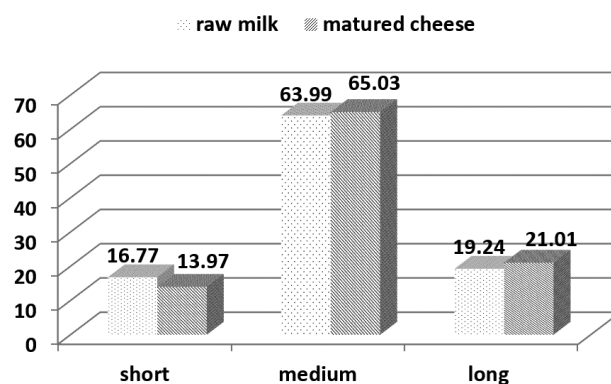
Fatty acids, %	Raw milk		Matured cheese	
	Mean	SD	Mean	SD
C 4:0	2.72	0.02	0.96	0.03
C 6:0	2.83	0.09	2.24	0.01
C 8:0	10.82	0.68	10.06	0.21
C 10:0	0.40	0.09	0.72	0.03
C 12:0	11.19	0.63	12.24	0.60
C 13:0	0.17	0.001	–	–
C 14:0	12.88	1.60	15.54	0.48
C 14:1	0.32	0.01	0.36	0.05
C 14:2	0.31	0.01	0.18	0.01
C 15:0	1.80	0.02	1.60	0.07
C 15:1	0.17	0.003	0.18	0.03
C 16:0	32.51	0.76	27.93	0.22
C 16:1	1.35	0.01	1.18	0.07
C 17:0	3.31	0.50	2.05	0.03
C17:1	–	–	3.79	0.07
C 18:0	3.75	0.05	4.10	0.65
C 18:1	12.48	0.24	14.83	0.81
C 18:2	1.72	0.07	1.06	0.01
C 18:3	1.29	0.01	1.02	0.07

Adoption of milk fat correlates positively with the increase of plasma saturated fatty acids (Karimi et al., 2012; Gómez-Cortés et al., 2015) and on this basis it can be expected that there will be a negative correlation between milk fat and cardiovascular disease, but the latest evidence contradicts this assumption. In some studies, the saturated fatty acids C12:0, C14:0 and C16:0 have been shown to be detrimental to health as they increase LDL cholesterol, while other saturated fatty acids neutralize their effect by increasing HDL cholesterol (Gibson, 2011). It has been found that saturated C15:0 and C17:0 have a positive relation to health, which refers to the etiology of several diseases (Khaw et al., 2012; Meikle et al., 2013) – both acids are associated with the reduction of risk of developing multiple sclerosis, as they are supposed to be responsible for increasing membrane fluidity (Holman et al., 1995) to a degree similar to that seen in polyunsaturated fatty acids (Holman et al., 1989). Pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) are also associated with reducing the risk of diabetes and the increase in insulin sensitivity (Nestel et al., 2013; Forouhi et al., 2014). The C15:0/C17:0 ratio is approximately 1: 1.8 for raw sheep milk and 1: 1.2 for mature brined cheese. These data contradict the findings of Fievez et al. (2012) that in milk fat of ruminants this ratio is 2:1 due to the higher production of C16:0 over C18:0 during de novo lipogenesis.

Among the unsaturated fatty acids, oleic (C18:1) and linoleic (C18:2) acid are the most important contributors to enrichment of aromatic components and are considered highly nutritious due to their protective role against cardiovascular disease (Hornstra, 1999).

Oleic acid is the most common monounsaturated fatty acid in milk, so milk and dairy products are the main source of this acid in human nutrition in many countries. It can help reduce the levels of plasma cholesterol and triglycerides. With regard to the oxidation processes in the human body, oleic acid is more resistant than omega-3 and omega-6 fatty acids. During the maturing of the white brined cheese from sheep milk of the Lacaune breed, oleic acid (C18:1) increased by 2.35% and linoleic (C18:2) decreased by 0.7%.

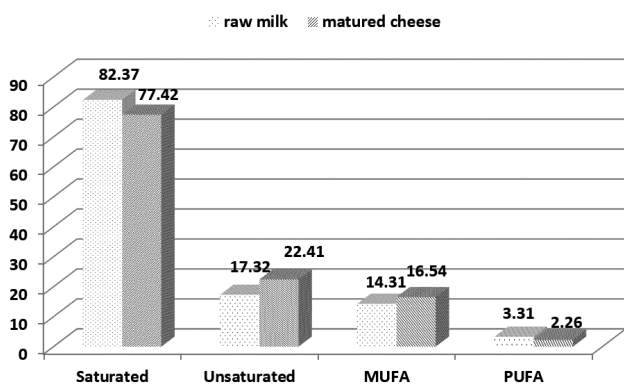
Short-chain fatty acids contained in milk fat have no influence on blood cholesterol elevation, as opposed to medium-chain lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids that have a significant effect on LDL cholesterol (Fuquay et al., 2011). The content of short-chain fatty acids (C4:0 to C10:0) in mature cheese decreased from raw milk by about 3%. (Fig. 1).

**Figure 1. Dynamics of short-, medium- and long-chain fatty acids**

Medium chain fatty acids (C11:0 to C17:0) increased during maturation of white brined cheese by 1% compared to raw milk. These results are similar to those obtained by Dimitrov et al. (2001) for sheep cheese. The same trend was observed in long-chain fatty acids (above C18:0) – compared to raw milk level, they increased during ripening by about 2%.

The amount of saturated fatty acids in the raw milk was 82.37%, decreased during maturation and reached 77.42% (Fig. 2). These results are similar to those obtained by Laskaridis et al. (2013) for fatty acid profile of Feta cheese during ripening.

The trend in the amount of unsaturated fatty acids was opposite. Their content was the lowest in the raw milk and

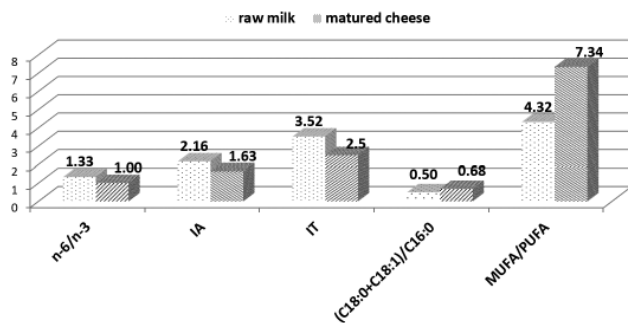


**Figure 2. Saturated and unsaturated fatty acids in raw sheep milk and mature white brined cheese**

increased during maturation by 5.09%, in proportion to the decrease in saturated fatty acids.

The most significant change was in the content of the monounsaturated fatty acids (MUFA) in mature cheese which increased by 2.23% compared to raw milk. The amount of polyunsaturated fatty acids (PUFA) decreases by about 1% – from 3.31% for raw milk to 2.26% for mature cheese.

The biologically significant ratio of omega-6/omega-3 in the production of white brined cheese from sheep milk ranged from 1.33: 1 in the raw milk to 1: 1 in mature cheese (Fig. 3). For healthy nutrition, the ratio between omega-6 and omega-3 fatty acids must be 1:1 to 5:1. As eating foods that are balanced in the omega-6/omega-3 ratio can reduce the risk of cancer, cardiovascular disease, immune system disorders, allergies, diabetes, obesity, etc. diseases, the balanced intake of omega-6 and omega-3 fatty acids is considered to be extremely important (Simopoulos, 2006). MUFA and omega-6 PUFA reduce cholesterol levels, and omega-3 PUFA, that of triacylglycerols (Van Elswyk et al., 1998). Weill et al. (2002) indicate that according to the nutritional recommendations, the ratio of C18:2 n-6/C18:3 n-3 in human food should be less than 5.



**Figure 3. Lipid indexes of sheep milk and cheese produced in conjunction with cardiovascular diseases**

The quality of milk fat depends on the length of the carbon chain of fatty acids, their degree of (non) saturation and their location in the triglyceride molecule (Křížová et al., 2017). The atherogenic index (IA) proposed by Ulbright & Southgate (1991) is based on information on the effect of different fatty acids on serum cholesterol and on the concentration of low- and high-density lipoproteins in humans. The typical atherogenic index for milk fat is about 2 (Bobe et al., 2003). The atherogenic index obtained for raw milk was slightly higher (2.16) than the above-mentioned and close to that established by Markiewicz-Kęszycka et al. (2013) for sheep milk (2.21). In the production and ripening of white brined cheese, it decreased and reached 1.63 in mature cheese. It should be noted that the lower value of the atherogenicity index, the better the food from the nutritional point of view of the lipid fraction.

The same decreasing tendency was also observed in the thrombogenic index (IT) – it decreased during ripening and from 3.52 in raw milk, it reached 2.50 in mature cheese. These values are similar to those reported by Ulbright & Southgate (1991) as being healthy. The use of foods characterized by a low atherogenic and thrombogenic index may reduce the potential risk of cardiovascular disease (Cuttrignelli et al., 2008; Menezes et al., 2009).

According to Bonanome & Grundy (1988), the ratio (C18:1 + C18:0)/C16:0 can be a better indicator compared to the SFA/USFA ratio, whether the corresponding fat will increase cholesterol. The higher ratio (over 0.4) is considered to be better. In our study, this ratio ranged from 0.5 for raw milk to 0.67 for mature white brined cheese from sheep milk (Fig. 3). These data are similar to those obtained by Mira et al. (1999) for goat milk involving 6% rape in the feed ration.

The MUFA/PUFA ratio in milk and cheese was high – 4.32 and 7.35, respectively, which can be explained by the combined effect of increasing unsaturated and decreasing saturated fatty acids. Various studies have shown that a high mono/polyunsaturated fatty acid diet provides better protection against cardiovascular diseases than foods rich in polyunsaturated acids only (De Lorgeril et al., 1994; Nicolosi, 2004).

## Conclusions

The amount of saturated fatty acids in raw sheep milk from the Lacaune breed decreased during processing and cheese ripening by 5%. The amount of unsaturated fatty acids increased during maturation by the same percentage in proportion to the decrease in saturated fatty acids. The most significant change was in the content of monounsaturated fatty acids in mature cheese, which increased by 2.23% com-

pared to raw milk. The Omega 6/Omega 3 ratio ranged from 1.33:1 for raw milk to 1:1 for white brined cheese produced, which is within the limits of healthy nutrition. The atherogenic index values ranged from 2.16 in raw sheep milk to 1.63 in mature cheese. The same trend was observed in the thrombogenic index – It decreased during cheese maturation from 3.52 to 2.5. Based on the resulting lipid indices and ratios, it can be said that Lacaune's raw sheep milk, as well as its white brined cheese, have a low risk factor for human health.

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