

PHYSICOCHEMICAL COMPOSITION AND FATTY ACID PROFILE OF WHITE BRINED CHEESE OF RHODOPE TSIGAY EWE'S, REARED IN THE MOUNTAIN REGION OF MIDDLE RHODOPE

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Abstract

The objective of present study was to investigate the changes in the physicochemical composition of the white brined cheese from Rhodope Tsigay breed. The white brined cheese produced from ewe's milk during the lactation is characterized by constant quantities of moisture (57.02 ± 2.93 %) and dry matter (42.98 ± 2.93 %). With advance of lactation the ash content increased from 6.93% to 7.33%, the protein level decreased slightly from 13.30 to 12.80% and the fat content varied during subperiod from 16.10 to 17.00%. The content of saturated fatty acids in cheese made from the milk of Rhodope Tsigay breed remains unchanged from 66.22 g / 100g fat to 67.54 g / 100g fat. The content of monounsaturated fatty acids increased insignificant from May to July in the white brined cheese and reached at the end of the lactation period to 27.67 g / 100g fat. From May to July polyunsaturated fatty acids in the sheep cheese of Rhodope Tsigay breed decreased by 26% (from 8.04 to 5.94 g / 100g fat). The amount of CLA (conjugated linoleic acids) in the tested samples decreased significant from May to July by 41% (from 3.56 to 2.11 g / 100g fat). Similar results were obtained for the amount of trans fatty acids in the cheese during the whole period (from 7.51 to 5.94 g / 100g fat).

Key words: white brined cheese, physicochemical parameters, fatty acid composition

Introduction

Publications on the nutritional value of white brined cheese on the ewe's milk basis often give only the main composition without detailed identification of more specific unique components of benefit for human nutrition, although trade magazines contain many report, that are waiting for scientific evaluation. In the rural and mountain regions the milk collected for human consumption has an essential food value for maintaining the dietary balance of the population. The ewe's milk composition is variable and depends on the diet, lactation period, season, breed, geographic region and other factors.

The production of dairy products with an increased content of biologically active substances depends primarily on the botanical diversity and the vegetation stage of the individual plant species (Mihailova et al., 2004, Kafedjiev and Mihailova, 1998, Collomb et al. 2002, Tsvetkova and Angelov, 2013). The green forages used in the feeding of sheep can contribute to the formation of specific biologically active substances in the final product, as well as to influence positively the taste and sensory characteristics of the dairy products (Pirisi et al.,

2004, Cabiddu et al. 2003 a, b; Alizadeh et al., 2013; Oliveira et al., 2012). Milk with high somatic cell content reduces the cheese output twice, because of the low casein content. The presence of proteolytic and lipolytic enzymes increased the possibility of a quick change of smell and deterioration in the taste of dairy products (Mijačević and Bulajić, 2008).

The content of the short chain fatty acids (caproic, caprylic, capric) in the ewe's milk is 1.5-2 times higher than that in the cow's milk (Fenyvessy and Szakaly, 1995). The fatty acids composition in the ewe's milk fat is of major importance to cheese industry. Factors affecting the cheese quality are the milk composition and cheese making conditions (Shahab Lavasani, 2014).

The concentrations of casein and fat in the milk determine the cheese yield. Selection of milk of high protein content leads to improved rennet coagulation properties and increase cheese yield (Shahab, 2014). During ripening of cheese the proteolytic changes contribute to the formation of properties of texture and flavour (Tsuda et al., 1994).

The developments on the production of cheddar and fontanel have shown that adding of MCT fatty acids (olive oil) improves the taste of the cheese. Based on the above information, the proposed study is essential for enrichment of the fatty acids spectrum of cheese (Alizadeh et al., 2013).

The industry for production of cheese on the ewe's milk basis is one of the most traditional and major industry in Bulgaria. Important place in the researches, concerning the biological function of fatty acids profile, take the special physiological effects of conjugated linoleic acids (CLA) for the animal and human organism (Cabiddu et al., 2005). The conjugated linoleic acids (CLA) as part of the unsaturated fatty acids spectrum with biological function take an important place in the investigation during the last 15 years (Abd El-Salam, 2012). The CLA is formed mainly via isomerization of linoleic acid using the anaerobe *Butyrivibrio fibrisolvens* in the rumen. Comparative studies reported the following beneficial effects of conjugated fatty acids: immune modulation, reduction of body fat, normalization of impaired glucose tolerance (Ha, 1987; Jahreis et al., 1999; 2000). The CLA-concentration in the raw milk and milk products varies in dependence on different factors (breed, season, diet, nutrition). The greatest differences in the CLA content were measured in the sheep milk in comparison to the other animal species. New investigations are necessary to clear the mechanism for CLA-transfer into raw milk and white brined cheese.

The unique content of about 25% MCT and 3-5% CLA in the sheep's milk fat fraction and possible quantitative modifications by diet were neither used nor commercially investigated. The novel approach based on investigations on the entire food chain in endemic mountain regions will be of general interest for the necessary answers on the opportunity for “long-term live” through the healthy nutrition. Developing new dairy products with higher CLA content is needed in the near future.

The purpose of this study is to examine the dynamic changes in the composition of white brined cheese of ewe's milk and its fatty acid profile as well as the content of biologically active and anticarcinogenic substances in the white brined cheese.

Material and methods

• Physico-chemical analysis of white brined cheese

From May to July (01.05 - 01.06 - 01.07.) the milk samples of six sheep from the Karakachan breed were taken and 12 samples of white brined cheese according to BDS were prepared. The cheeses have been studied for the basic physico-chemical parameters (proteins, fats, mineral content, humidity, dry matter). The analysis were performed using standard methods:

- Bulgarian white brined cheese - BCS 15-2010
- Humidity - BCS 1109:1989, ISO 9622
- Protein - BCS EN ISO 8968-1:2014
- Fat - BCS EN ISO 1211:2010,
- Ash - BCS 6154:1974

• Fatty acid analyze

The extraction of total lipids in the cheese samples carried out by Roesse-Gottlieb (1973), by means of diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa , Merck, Darmstadt) and dehydration with $\text{NaHSO}_4 \cdot \text{H}_2\text{O}$.

Fatty acid methyl esters (FAME) were analyzed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with flame ionization detector and an automatic injection system (AOC-2010i). The analysis was performed on capillary column CP7420 (100 m x 0.25 mm i.d., 0.2 m, Varian Inc., Palo Alto, CA). Hydrogen is used as carrier gas, and as make-up gas - nitrogen. Four-step furnace mode is programmed - the initial column temperature is 80°C / min, maintained for 15 minutes, then increased by 12°C / min to 170°C and maintained for 20 minutes, followed by a new 4°C / min increase to 186°C for 19 minutes and up to 220°C with 4°C / min until the process is complete.

Results and discussion

• Properties of cheese

In the investigated cheese samples no significant differences in the fat, protein and dry matter content during the different sub-periods have been observed (Table 1).

Table 1. Physical and chemical characteristics of white brined cheese from bulk ewe's milk of Rhodope Tsigay breed during the lactation period

Period		Humidity %	DM %	Ash %	Protein %	Fat %
01. May	x	59.51	40.50	6.93	13.33	16.80
	sd	2.95	2.95	0.62	0.45	2.69
01. June	x	57.62	42.38	7.33	12.80	16.10
	sd	1.50	1.50	0.01	0.78	3.96
01. July	x	53.95	46.06	7.11	13.30	17.00
	sd	0.25	0.25	0.37	0.14	0.28
Whole period	x	57.02	42.98	7.12	13.14	16.63
	sd	2.93	2.93	0.37	0.49	2.19

The protein content in the cheese changed during the experimental period and varied in a narrow range from 12.80% to 13.33%. The total mineral content increased from Mai to June by 6% (6.93 to 7.33 %). The DM-content in the cheese was in the BDS-norm in July and depends on the mineral substances, the protein and fat content in the dairy product.

- **Fatty acids composition of white brined cheese**

The fatty acid composition of white brined cheese is presented in the Table 2. The content of saturated fatty acids in cheese produced from the milk of Rhodope Tsiday breed remains unchanged from 66.22 g / 100g fat to 67.54 g / 100g fat.

Table 2. Fatty acids profile (g/100g fat) in the sheep cheese /Rhodope Tsigay breed/ during the lactation period (01.05. – 01.07.)

Fatty acid profile	May		June		July	
	x	sd	x	sd	x	sd
SFA	66.99	0.67	67.54	0.29	66.22	1.15
MUFA	24.97	0.60	25.09	0.22	27.67	0.97
PUFA	8.04	0.07	7.37	0.07	5.94	0.17
Σ C-18:1Trans-FA	7.51	0.20	5.61	0.08	5.81	0.20
C-18:1tr11	4.13	0.07	3.05	0.04	3.99	0.08
Σ CLA	3.56	0.04	2.95	0.06	2.11	0.07
C-16:0/C-18:1cis9	1.43	0.03	1.36	0.03	1.36	0.07
C-16:0/C-18:1 total	0.98	0.02	1.05	0.01	1.09	0.06
Σ n-3	1.83	0.002	1.84	0.001	2.12	0.03
Σ n-6	3.40	0.03	3.12	0.02	2.37	0.12
n-6/n-3	1.86	0.02	1.70	0.01	1.14	0.02
Σ MCT (C-10>C-14)	23.40	0.41	20.21	0.17	16.64	0.48
Σ SCT (C-4>C-8)	10.57	0.56	9.65	0.26	7.52	0.06
CLA 9c,11t	2.92	0.04	2.36	0.020	1.86	0.07

The content of monounsaturated fatty acids increased insignificant from May to July in the white brined cheese and reached at the end of the lactation period to 27.67 g / 100g fat. From May to July the polyunsaturated fatty acids in the sheep cheese decreased by 26% (from 8.04 to 5.94 g / 100g fat).

Table 3. Content of saturated fatty acid (g/100g fat) in the sheep cheese during the lactation period (01.05. - 01.07.)

SFA - profile	May		June		July	
	x	sd	x	sd	x	sd
C-4:0	3.96	0.27	4.14	0.13	3.08	0.67
C-6:0	3.43	0.16	3.06	0.08	2.26	0.12
C-8:0	3.10	0.13	2.42	0.05	2.18	0.60
C-10:0	8.50	0.26	6.57	0.10	4.24	0.82
C-12:0	4.19	0.09	3.29	0.03	2.30	0.04
C-14:0	9.85	0.05	9.53	0.03	9.55	0.28
C-15:0	0.64	0.01	0.68	0.001	0.56	0.02
C-15:1	1.13	0.003	1.29	0.01	1.18	0.04
C-16:0	0.27	0.001	0.29	0.001	0.26	0.01
C-16:1	21.25	0.07	23.10	0.03	26.48	0.54
C-17:0	0.39	0.002	0.50	0.01	0.46	0.02
C-17:1	0.49	0.01	0.50	0.01	0.47	0.02
C-17:2	0.69	0.01	0.78	0.01	0.69	0.03
C-18:0	7.78	0.15	9.85	0.09	11.09	0.08
C-20:0	0.20	0.01	0.26	0.003	0.31	0.01
C-22:0	0.13	0.01	0.16	0.003	0.20	0.01

The amount of CLA (conjugated linoleic acids) in the tested samples decreased significant from May to July by 41% (from 3.56 to 2.11 g / 100g fat). Similar results were obtained for the amount of trans fatty acids in the cheese during the whole period (from 7.51 to 5.81 g / 100g fat).

A detailed examination of the distribution of saturated fatty acids (SFA) showed that the value of some medium chain fatty acids (C_{8:0} + C_{10:0} + C_{12:0}) decreased significantly in course of lactation from 15.79 to 8.72 g / 100g fat (Table 3). The concentration of myristic acid (C-14:0) remains unchanged throughout the lactation period (9.85 resp. 9.55 g/100 g fat). The important main long fatty acids C16:0 and C18:0 in the cheese increased by 25% and 42.5% (p<0.05; p<0.01) with advance of lactation.

The distribution of monounsaturated fatty acids (MUFA) in the cheese during the different sub-periods shows, that their total content increased insignificantly from 24.97 to 27.67 g /100g fat at the end of the period. These changes are due to changes in the concentrations of oleic acid and vaccenic acid (Table 4).

Table 4. Content of mono unsaturated fatty acid (g/100g fat) in the sheep cheese during the lactation period (01.05. - 01.07.)

MUFA profile	May		June		July	
	x	sd	x	sd	x	sd
C-10:1	0.29	0.01	0.24	0.01	0.13	0.002
C-14:1	0.19	0.001	0.20	0.001	0.16	0.01
C-16:1	0.99	0.005	1.04	0.005	1.19	0.05
C-18:1tr4	0.04	0.01	0.06	0.01	0.02	0.01
C-18:1tr5	0.02	0.005	0.04	0.02	0.02	0.004
C-18:1tr6/7	0.28	0.01	0.17	0.01	0.23	0.02
C-18:1tr9	0.33	0.01	0.29	0.01	0.23	0.02
C-18:1tr10	0.44	0.002	0.28	0.002	0.081	0.005
C-18:1tr11	4.13	0.07	3.05	0.04	3.99	0.08
C-18:1tr12	0.52	0.02	0.36	0.03	0.38	0.04
C-18:1tr13	0.66	0.05	0.40	0.01	0.00	0.00
C-18:1tr15	0.42	0.02	0.35	0.06	0.31	0.03
C-18:1tr16	0.67	0.02	0.61	0.01	0.63	0.03
C-18:1cis9	14.83	0.38	16.99	0.38	19.52	0.64
C-18:1cis11	0.45	0.02	0.44	0.05	0.33	0.02
C-18:1cis12	0.20	0.03	0.11	0.003	0.16	0.02
C-18:1cis13	0.21	0.01	0.20	0.02	0.08	0.01
C-18:1cis15	0.22	0.03	0.18	0.02	0.23	0.02

A detailed distribution of mono unsaturated fatty acids (MUFA) in the cheese during the different sub-periods shows, that in the MUFA profile predominat is the spectrum of *cis*- and *trans*- isomers of the oleic acid (C18:1). The *trans*-isomers of C18:1 in the cheese decreased continuously by 22,6% during the whole period, while the *cis*-isomers of C18:1 increased from 15.91 to 20.32 g/100g fat (Table 4)

Oleic acid C18:1cis9 increased from 14.83 (01. May) to 19.52 g /100g fat (01.July). The *trans*-fatty acids content in the cheese samples is from great interest for the science and the human nutrition. Their concentration in the eleven known isomers decreased stepwise from 7,51 to 5,81 g /100g fat (May – July). A particular place in this regard is the vaccenic acid (C18:1trans11). At the beginning of lactation, it took about 55,0% from the *trans* FA-spectrum in the cheese and decreased insignificant from 4.13 in May to 3.05 g /100g fat in June. All *trans* isomers with except of C18:1t11 are considered as "undesirable", due to the presence of carcinogenic effect.

Polyunsaturated fatty acids (PUFA) occupy the last place of the major fatty acid groups (Table 5). In the middle of the investigated period the PUFA content reached to 7.37 g /100 g fat. Their value in the ewe's cheese ranges between 8.04 g/100g fat in May and 5.94 g /100g fat in July. The linoleic acid (C-18:2cis9,12) increased during the lactation by 13,4% and the other isomer C-18:2 trans9,12 decreased during the lactation period from 0,68 g /100g fat to 0,15 g /100g fat.

Table 5. Content of mono unsaturated fatty acid (g/100g fat) in the sheep cheese during the lactation period (01.05. - 01.07.)

PUFA profile	May		June		July	
	x	sd	x	sd	x	sd
C-18:2tr9,12	0.68	0.026	0.67	0.013	0.08	0.003
C-18:2cis9;12	1.57	0.007	1.60	0.010	1.77	0.067
C-18:3cis6,9,12(γ)	0.17	0.004	0.14	0.010	0.03	0.003
C-18:3cis9,12,15(α)	1.44	0.010	1.46	0.003	1.51	0.036
CLA 9c,11t/8t,10c	2.92	0.043	2.36	0.019	1.86	0.065
CLAc9c11	0.11	0.000	0.09	0.010	0.09	0.005
CLA-Y	0.05	0.003	0.04	0.002	-	-
CLAt11t13	0.08	0.002	0.09	0.005	-	-
CLAt9t11	0.18	0.009	0.20	0.009	-	-
C-20:5n3	0.11	0.009	0.12	0.008	0.15	0.03
C-22:5n3	0.16	0.011	0.13	0.015	0.18	0.005
C-22:6n3	0.07	0.014	0.09	0.003	0.08	0.000

The γ -linolenic acid was relatively low, during the considered period (0.17-0.03) and the α -linolenic acid was relatively constant and increased from 1.44 to 1.51 g /100g fat during the lactation period. The content of conjugated fatty acids (CLA) in the studied cheese samples is influenced by the season, respectively by the quality of meadow grass (Table 5). Their amount ranges from 3.53 to 2.12 g /100g fat. In terms of nutrition, only the configurations - CLA cis9,trans11 and CLA trans9,cis11 - are important as functional nutritional component for prevention of colon and stomach cancer.

Unsaturated long-chain fatty acids (omega-3 and omega-6 fatty acids) occupy an important place in the human nutrition in the treatment of coronary and cardiovascular diseases (Table 6). The ewe's cheese is very poor in omega-3 fatty acids. Their concentration increased from May to July from 1.83 to 2.12 g/100 g fat. In contrary, the omega-6 fatty acids in the cheese samples decreased from 3.40 to 2.37 g/100g fat. The ratio between two groups (ω -6 / ω -3) ranges from 1.86 to 1.14 and indicate that the tested cheese was with low risk factor for human nutrition (raw materials and natural foods whose factor is <5.0 have a low risk factor).

Table 6. The content of ω -3 и ω -6 fatty acids in the white brined cheese (g / 100g fat) during the lactation period (01.05. - 01.07.)

Σ n-3 in cheese	01. May		01. June		01. July	
	x	sd	x	sd	x	sd
C-18:3cis9,12,15 (α)	1.44	0.010	1.46	0.003	1.51	0.036
C-18:4 c6,9,12,15	0.05	0.001	0.04	0.004	0.09	0.002
C-20:5n3	0.11	0.009	0.12	0.008	0.15	0.032
C-22:5n3	0.16	0.011	0.13	0.015	0.18	0.005
C-22:6n3	0.07	0.014	0.09	0.003	0.08	0.001
Σ n-6 in cheese	01. May		01. June		01. July	
	x	sd	x	sd	x	sd
C-18:1tr12	0.52	0.017	0.36	0.027	0.38	0.035
C-18:1cis12	0.20	0.026	0.11	0.003	0.16	0.015
C-18:2tr9,12	0.68	0.026	0.67	0.013	0.08	0.003
C-18:2cis9;12	1.57	0.007	1.60	0.010	1.77	0.067
C-18:3cis6,9,12 (γ)	0.17	0.004	0.14	0.010	0.03	0.003
C-20:3n6	0.02	0.004	0.01	-	-	-
C-20:4n6	0.13	0.001	0.14	0.004	-	-
C-22:4n6	0.09	0.01	0.09	0.001	-	-

Conclusion

The present investigation on the physicochemical properties and fatty acid composition of produced white brined cheese in the region of the Middle Rhodopes showed that with advance of lactation stage, the concentration of the major fatty acid groups changes, resulting in a stepwise increase in the amount of monounsaturated fatty acids by 11.1% compared to polyunsaturated (PUFA), which content decreased by 26.1%.

The trans-isomers of C18:1 in the cheese decreased continuously by 22.6% during the whole period, while the cis-isomers of C18:1 increased by 27.7%.

Dynamic changes in the linoleic acid (C18:2) and alpha-linolenic acid (C18:3), which are a substrate for CLA synthesis (anticarcinogenic activity) in the rumen of ruminants, have been followed. Analyzed cheese samples had a high biological value (high content of CLA – 3.56 g/100 fat and vaccenic acid – 4.13 g/100 fat). Ewe's cheese from the Tsigay breed is an important dairy product with an increased biological value and foodstuff with high anticarcinogenic action.

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