Scientific Papers. Series D. Animal Science. Vol. LXV, No. 1, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PHYSICOCHEMICAL COMPOSITION AND FATTY ACIDS IN KEFIR FROM MILK OF “BULGARIAN WHITE DAIRY” GOAT BREED AND ITS CROSSINGS

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Abstract

The physicochemical parameters of kefir on the 3rd and 14th day of the storage process produced from the milk of 'Bulgarian White Dairy' (BWD) goat breed and its crossings with 'Toggenburg' (TG) and ‘Anglo-Nubian’ (AN) were studied. The main groups of fatty acids in kefir on the 14th day of storage were identified and a qualitative assessment of milk fat was made based on lipid indices. The highest content of protein, fat and dry matter in kefir on the 3rd and 14th day was found in BWDxAN breed (5.42%, 5.34%; 4.77%, 4.66% and 15.84%, 15.74%), and the lowest in kefir from BWD (5.07%, 4.99%; 4.14%, 4.05%; 14.92%, 14.15%). Kefir from the milk of BWDxAN has the highest content of saturated fatty acids (SFAs), and monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) predominate in kefir from BWD. The content of SFAs in the studied kefir was from 3.0g/100g to 3.59 g/100 g. Therefore, kefir products are determined as high in content of saturated fatty acids (over 1.5 g/100 g product) and low content of trans fatty acids (0.12-0.17 g/100 g product).

Key words: *fatty acids, goat milk, kefir, lipid indices, physicochemical composition.*

INTRODUCTION

Milk and dairy products are some of the best accepted and widely consumed foods. To obtain them, fermentation technologies are used with the participation of various lactic acid bacteria, thus increasing their dietary potential (Nacheva et al., 2018). Consumption of goat's milk products is associated with health benefits beyond their nutritional value and compared to other types of milk, goat has unique bioactive characteristics such as high digestibility, alkalinity, high buffering capacity and certain therapeutic values associated with healthy feeding (Park & Haenlein, 2007).

Fermented milk, in particular kefir, is an extremely suitable form for the absorption of nutrients in milk on the one hand and for the impact of lactic acid bacteria on various body functions on the other (Slacanac et al., 2010). One of the possible alternatives to avoid and reduce the negative impact of milk proteins in people allergic to cow's milk is to include goat's milk and fermented products in their diet. One of the most useful products for human health in the group of fermented lactic acid foods is kefir. It is a traditional drink originating from the Caucasus region, but is consumed worldwide, the result of two fermentations with kefir grains - lactic acid and alcohol (Guzel-Seydim et al., 2011). It has all the beneficial characteristics of lactic acid drinks, providing the body's needs for calcium, and at the same time is a dietary lactic acid product with high absorption, rich in many beneficial bacteria and suitable for all ages (Roshtunkina, 2010). The starter culture of kefir, which is the kefir grains are small with an irregular shape, yellowish-white, firm granules that resemble miniature cauliflower and are a formation containing a matrix of proteins, fats, sugars and symbiotic association of lactic acid bacteria, such as *Lactobacillus kefiri*, *Leuconostoc*, *Lactococcus* and *Acetobacter* and fermenting and non-fermenting lactose yeasts (*Kluyveromyces marxianus*, *Saccharomyces* *unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*) (Oliveira et al., 2013).

Milk fat also plays an important role in the production of fermented beverages, as interest in various omega-3 and CLA fatty acids, which have a significant effect on metabolism, has recently increased.

The possibilities for including goat's milk as a component in functional products are limited and have not been sufficiently studied. Therefore, the aim of the present study is to produce kefir from goat's milk of 'Bulgarian White Dairy' breed (BWD) and its crossings with 'Toggenburg' (BWDxTG) and Anglo-Nubian (BWDxAN), to establish its physicochemical composition on the 3rd and 14th day of storage as well as to study its fatty acid profile in view of its healthy effect on the human body.

MATERIALS AND METHODS

Milk from experimental animals at the Research Institute of Mountain Stockbreeding and Agriculture - Troyan are used, that have been raised in one herd under the same production conditions consisting of three groups - 'Bulgarian White Dairy' and its crossings with 'Toggenburg' and 'Anglo-Nubian'. The rearing system is pasture-barn based, as in the period of April-November the animals were on a natural pasture of transitional type and in the barn, during the rest of the year. Several batches of kefir were prepared at the beginning (April), the middle (June) and the end (September) of the lactation period. For this purpose, the milk is pasteurized at a temperature of 85-90˚C, with a delay of 10-15 s., cooled to 29˚C and fermented with dry kefir leaven. It is poured into suitable containers and allow to ferment at 29°C for 16-18 hours, then cool and transfer to 0-4°C for refrigerated storage. Samples of the obtained kefir batches were examined to determine their physicochemical composition on the 3rd and 14th day of storage and are presented on arithmetic mean for the lactation period. The main groups of fatty acids in kefir on the 14th day of the storage process were determined and lipid indices were calculated.

The samples were tested in the technological laboratory for milk and dairy products at RIMSA - Troyan.

The following indicators were found on the Food Scan device (Lab, 78800): protein, fat, dry matter, water content, salts.

The titratable acidity was determined with 0.1 NaOH (BDS 1111-80), the active acidity with pH meter, the density by weight method, calcium by complexometry (according to Kodratenko et al., 1981), and the fatty acid composition with gas chromatograph "Shimadzu 2010" with flame ionization detector and column CP 7420 (100m x 0.25mm id, 0.2 μm film). The analysis was performed in the laboratory "Technology of milk, dairy products and fats" at the Institute of Cryobiology and Food Technology - Sofia.

Based on the obtained fatty acid composition, the following indices were calculated:

1) Atherogenic index (AI) - calculated on the basis of the content of medium-long fatty acids - C12: 0, C14: 0 and C16: 0 and the groups of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) (Chilliard et al., 2003):

$$AI = \frac{C12:0 + 4 \times C14:0 + C16:0}{MUFAs + PUFAs}$$

2) Thrombogenic index (TI) - according to Ulbricht and Southgate (1991):

$$TI=(C14:0+C16:0+C18:0)/0.5\times C18:1+0.5\times \Sigma MUFAs+0.5\times \Sigma PUFAn6+3\times PUFAn-3+(PUFAn3/PUFAn6)$$

3) Lipid preventive score (LPS) according to the equation of Richard and Charbonnier (1994):

$$LPS=FAT+2xSFA-MUFA-0.5PUFA$$

4)Ratio between hyper and hypocholesterolemic fatty acids (h/H);

$$h/H=(C18:1n\text{-}9+C18:1n\text{-}7+C18:2n\text{-}6+C18:3n\text{-}3+C18:3n\text{-}6+C20:3n\text{-}6+C20:4n\text{-}6+C20:5n\text{-}3+C22:4n\text{-}6+C22:5n\text{-}3+C22:6n\text{-}3)/(C14:0+C16:0).$$

The variational-statistical data processing is done through Statistica software package. The mean values of the groups were compared according to the tables of Student-Fisher t-test.

RESULTS AND DISCUSSIONS

The chemical composition of kefir depends on many factors such as the type of milk and technological conditions (Sady et al., 2007).

In the studied kefir samples on the 3rd day of the storage process (Table 1.) the content of protein, fat and dry matter is the highest in kefir from the milk of BWDxAN (5.42%, 4.77%, 15.84%), and the lowest in kefir of BWD breed (5.07%, 4.14%, 14.92%), as the results are statistically significant.

Much lower results indicated Nacheva et al. (2017) for kefir from goat's milk on the 1st day of storage, respectively (3.41%; 3.47%; 12.20%), as well as Akgul et al., (2018) for dry matter and protein (10.01%; 3.23%) for kefir of 5th day of cow's milk, which was probably due to differences in technological process as well as differences in the composition of milk from different breeds of animals.

Table 1. Composition of kefir on the 3rd day of storage, % (n = 9)

	Breed groups		
	BWD	**BWDxTG**	**BWDxAN**
INDICATORS	**x±Sx**	**x±Sx**	**x±Sx**
Protein,%	5.07±0.144	5.34±0.139	5.42±0.078
Fat,%	4.14±0.050	4.73±0.081b***	4.77±0.209a*
Water content,%	85.25±0.385	84.39±0.269	84.14±0.081a*
Salts,%	0.86±0.055	0.82±0.044	0.81±0.067
Dry matter,%	14.92±0.302	15.75±0.229b*	15.84±0.105a*
Titrable acidity,T°	80.33±0.837	82.78±1.160	83.11±1.976
Active acidity, pH	4.46±0.018	4.50±0.029	4.52±0.035
Density,%	1.051±0.003	1.065±0.010	1.071±0.012
Ca, mg %	0.160±0.003	0.161±0.003	0.155±0.005

Note: a: BWD/BWDxAN; b: BWD/BWDxTG *P≤0.05;***P≤0.001.

The water content varied from 84.14% for BWDxAN to 85.25% for kefir from BWD, and the salts were in the range of 0.81% for BWDxAN to 0.86% for kefir from BWD.

The titratable and active acidity were the highest in BWDxAN - 83.11 T° and 4.52, which was close to the results indicated by Nacheva (2019) pH-4.50 and titratable acidity - 98°T in kefir from goat's milk fermented with the addition of lactulose.

Chen et al. (2009) also found, as in the present study, a decrease in active acidity and an increase in titratable acidity in kefir produced with starter cultures and kefir grains with different fermentation cycles.

The density and calcium content varied within relatively narrow limits between the different batches of kefir (1.051 – 1.071%; 0.154 - 0.160%). The lowest density was found in kefir from BWD with 1.051, which was because of the lower content of dry matter and milk fat.

The composition of kefir on the 14th day of storage is presented in Table 2.

Table 2. Composition of kefir on the 14th day of storage, % (n = 9)

	Breed groups		
	BWD	**BWDxTG**	BWDxAN
INDICATORS	**x±Sx**	**x±Sx**	**x±Sx**
Proteins,%	4.99±0.145	5.27±0.136	5.34±0.044
Fat,%	4.05±0.066	4.55±0.038b***	4.66±0.192a*
Water content,%	85.78±0.325	84.86±0.153b*	83.68±0.140a**c***
Salts,%	0.85±0.037	0.78±0.050	0.80±0.025
Dry matter,%	14.15±0.290	15.14±0.160a*	15.74±0.069a*c*
Titrable acidity,T°	84.33±1.020	87±1.345	88±2.696
Active acidity, pH	4.40±0.003	4.43±0.020	4.44±0.012
Density,%	1.062±0.009	1.060±0.004	1.064±0.005
Ca, mg %	0.155±0.004	0.161±0.003	0.157±0.003

Note: a: BWD/BWDxAN; b: BWD/BWDxTG *P≤0.05;***P≤0.001.

The values for protein, fat and dry matter were the highest as well as on the 3rd day of kefir storage from BWDxAN milk (5.34%, 4.66%, 15.74%), and the lowest for kefir from BWDxAN (4.99%, 4.05%, 14.15), as there was statistical reliability for fat and dry matter,

respectively ($p<0.05$), ($p<0.001$) for BWDxAN and BWDxTG.
The reduction in dry matter, fat, and protein found in the present study from the 3rd day to the 14th day of storage coincided with data by Irigouen et al. (2005) and Nacheva et al. (2017). Irigouen et al. (2005) indicated a value for fat and dry matter - 3.59-3.54% and 11.5-11.4% on the 14th day of kefir storage with 1% and 5% kefir grains.
Changes in the amount of fat are mainly due to the action of lipases obtained from kefir grains during fermentation (Vujicic et al., 1992). However, the most significant changes in fat values occurred after the 14th day of storage and were associated with the development of molds, which are the main lipolytic agents in fermented milk (Tamime & Deeth, 1980).
The active acidity ranged from 4.40 in BWD to 4.44 in BWDxAN, and the titratable with 84.33-88 Tº, as in all three batches of kefir there was a decrease on the 14th day of active acidity, and there was an increase in titratable acidity.
Sung-Ho Yoo et al. (2013), as well as in the present study, found in the production of kefir by two-stage fermentation a decrease of pH from zero to 24 days of storage, respectively, from 3.99 - 3.94 and 4.52 - 4.45 and increase in the titratable acidity from 0.60 to 0.71 and 0.83% - 0.90%.
According to Kang et al. (2013), the pH decreases due to the increase in acidity in the early stage of storage caused by the continuing metabolic activity of lactic acid bacteria.
Paseephol et al. (2008) did not find significant differences in pH values (4.2-5.5) and titratable acidity (0.81.0%) among samples of yogurt on the 1st day and after the 28th day of storage at 4℃. The acidity of all samples increased slightly (1.0-1.2%) and the pH decreased slightly (4.1-4.3). This is a proof for the continuous metabolic and enzymatic activity of the mixed cultures during low temperature storage.
The results for the main groups of fatty acids in kefir on the 14th day of storage are presented in Table 3.
Modern aspects concerning the composition of milk fat in ruminants relate mainly to the content of conjugated linoleic acid (CLA), which can reduce the risk of many diseases such as obesity, atherosclerosis, cancer etc. (Lawson et al., 2001). CLA-containing products contribute to the reduction of body fat by inhibiting lipogenesis and stimulating lipolysis (Raff et al., 2009).
The amount of conjugated linoleic acid in ruminants changes during the season due to changes in diet (Chilliard et al., 2005).
The differences between the three batches of kefir were insignificant and ranged from 0.59 g/100 g fat in BWD to 0.61 g/100 g fat in BWDxTG.
The amount of trans-isomers during the analyzed period was high in kefir from BWDxAN with 3.89 g/100 g fat, and of cis-isomers in kefir from BWD with 26.45 g/100 g fat, as the differences were statistically insignificant.

Table 3. Main groups of fatty acids in kefir on the 14th day of storage, g/100 g fat

Groups of fatty acids	Breed groups		
	BWD	BWDxTG	BWDxAN
	x±Sx	x±Sx	x±Sx
ΣCLA	0.59±0.123	0.61±0.091	0.60±0.0116
Σ C-18:1 trans forms	3.28±0.554	3.40±0.628	3.89±0.834
Σ C-18:1 cis forms	26.45±2.030	25.48±1.799	25.08±1.655
ΣSFAs	71.89±2.354	73.16±2.449	73.88±2.232
ΣMUFAs	25.38±1.817a*	24.37±1.701a*	24.71±1.521
ΣPUFAs	5.75±0.908	5.32±1.015	5.12±0.855
Σ n-3	0.64±0.092	0.65±0.092	0.59±0.087
Σ n-6	5.04±0.778	4.83±0.920	4.33±0.759
Σn-6/Σn-3	7.90±0.423	7.43±0.354	7.33±0.523
ΣBranched chain fatty acids	1.30±0.138	1.29±0.205	1.24±0.231
CLA	0.51±0.091	0.55±0.063	0.53±0.091

Note: a: BWD/BWDxTG; *P≤ 0.05

The total amount of SFAs, which are the main components of milk fat and are considered harmful to human health because they increase cholesterol (USDA, 2002), was relatively high in the studied samples, respectively 71÷73 g/ 00 g fat ($p < 0.05$ in BWD/BWDxTG).

The amounts of MUFAs and PUFAs predominated in kefir from the milk of BWD goats with 25.38 g/100 g fat and 5.75 g/100 g fat, while the lowest values were found in BWDxTG with 24.37 g/100 g fat and 5.32 g/100 g fat.

As it is known, C-18:2 and C-18:3 perform a number of important functions as C-18:2 is characterized by very high biological activity, as in the body in the presence of vitamin B_6 it passes into the essential arachidonic acid (C-20:4) (Gladky & Fedyakina, 2006).

Vieira et al. (2015) found in kefir from cow's milk on the 14th day of storage values close to the present study for SFAs, MUFAs and PUFAs, respectively 67.2 g/100 g fat, 28.2 g/100 g fat, 4.54 g/100 g fat.

Vital for metabolism fatty acids from the group omega-3, omega-6 are in the range of 0.59-0.64 g/100 g fat and 4.33-5.04 g/100 g fat, which is close to the results obtained by Nacheva et al., (2018) omega-3 - 0.52 g/100 g fat, omega-6 - 4.13 g/100 g fat in kefir from goat's milk.

The qualitative assessment of milk fat was made on the basis of the following indicators: lipid preventive score, atherogenic and thrombogenic index and the ratio between hyper- and hypocholesterolemic fatty acids (Table 4).

Lipid preventive score (LPS) calculated by equation, is used to assess the preventive effect of a fat on the risk of cardiovascular disease. In the studied samples with the highest LPS is kefir from BWDxTG with 9.89 g/100 g fat, and the lowest in BWD breed with 8.12 g/100 g fat, and the results are statistically significant, which means that the product has a well-balanced fatty acid composition.

The atherogenic index giving the relationship between the sum of basic saturated and unsaturated fatty acids and the thrombogenic index expressing the ratio between saturated fatty acids and monounsaturated and polyunsaturated omega-3 and omega-6 fatty acids (Ghaeni et al., 2013) are the highest in kefir from the milk of BWDxAN - 4.52; 3.27, and the hypocholesterolemic index in kefir from BWD - 0.56.

Vieira et al. (2015) indicate for kefir from cow's milk on the 14th day of storage values for AI, TI and h/H of 1.94; 2.98; 0.82.

Table 4. Kefir indices on the 14th day of storage, g/100 g product

Indices	BWD	BWDxTG	BWDxAN
	x±Sx	x±Sx	x±Sx
LPS	8.12±1.588	9.89±0.643a*	9.20±0.632
AI	3.11±0.244	4.30±0.840	4.52±0.891b*
TI	2.16±0.363	2.14±0.550	3.27±1.163
h/H	0.56±1.476	0.42±1.886	0.43±1.746
Trans fatty acids	0.12±0.593	0.14±0.810	0.17±0.847
SFAs+TFAs	3.0±0.502	3.30±.555	3.59±0.482

Note: a: BWD/BWDxTG; b:BWD/BWDxAN $^{*}P \leq 0.05$

Trans fatty acids (TFAs), naturally obtained and important in human nutrition, varied in individual kefir from 0.12 g/100 g fat in BWD to 0.17 g/100 g fat in BWDxAN, which gives us reason to refer to products with a low TFAs content according to Regulation (EC) No 1924/2006.

CONCLUSIONS

The highest content of protein, fat and dry matter in kefir on the 3rd and 14th day of storage was found in milk from BWDxAN breed (5.42%, 5.34%; 4.77%, 4.66% and 15.84%, 15.74%), and the lowest in kefir from the milk of BWD breed (5.07%, 4.99%; 4.14%, 4.05%; 14.92%, 14.15%).

Water content and active acidity decreased, and titratable acidity increased from the 3rd to the 14th day of storage.

The results for protein, fat and dry matter decreased in all three batches of kefir on the 14th day of storage compared to the 3rd day.

Kefir from the milk of BWDxAN had the highest content of saturated fatty acids, while MUFAs and PUFAs predominated in kefir from purebred goats.

The content of saturated fatty acids in the studied kefir from goat's milk was from 3.0 g/100 g fat to 3.59 g/100 g fat. Therefore, it is defined as products with high content of saturated fatty acids (over 1.5 g/100 g product) and low content of trans fatty acids (0.12-0.17

g/100 g product) according to Regulation (EC) No 1924/2006.

LPS values show a well-balanced fatty acid composition of kefir from the milk of the three breed groups, which defines it as a product with pronounced health effects.

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