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ALTERATIONS IN HEALTH-RELATED FATTY ACIDS IN BUFFALO MILK AFTER PROCESSING TO TRADITIONAL DAIRY PRODUCTS

– Research paper –

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Abstract: Milk provides some beneficial fatty acids which in dairy processing are subjected to pasteurization and fermentation. With the aim to assess such changes, aliquot parts of milk from 12 buffaloes were pooled and processed to germinated yoghurt and brined cheese, and to non-germinated curd – the respective samples of raw and dairy material subjected to lipid analysis. The results show that in cheese positive and negative changes are generally balanced, rumenic acid decreasing and other CLAs altered but not total CLA and PUFA; omega ratio and atherogenicity index worsened to little extent, due to adverse change in n-3, myristic and lauric acid. In yoghurt and curd CLA dramatically decreased, excluding rumenic acid; but vaccenic acid increased, though total *trans* isomers decreased; the worsened n-6/n-3 ratio and atherogenicity index is mostly because of the adverse effect on PUFAn-3 but also on myristic and lauric acid. In all products SFA and MUFA did not change, including palmitic, stearic, and oleic acid. It can be concluded that the decrease of CLA in yoghurt and curd is partially compensated by the increase in the vaccenic acid, while cheese making altered individual isomers but not groups of beneficial acids.

Keywords: Bulgarian yoghurt, brined cheese, acid curd, fatty acids, buffalo

INTRODUCTION

Buffalo milk is a delicacy product marked with higher content of lactose, protein, ash, and vitamins A and C, as well as with the presence of biliverdin, bioactive pentasaccharide and gangliosides, which are not present in bovine milk (Abd El-Salam and El-Shibiny, 2011). It provides high concentrations of amino acids – leucine, lysine, valine, tyrosine, glutamic and aspartic acid (Naydenova, 2005; Becskei et al., 2020). Buffalo milk is characterized by high density, associated not only with its higher dry matter but also with the higher proportion of high-melting triglycerides (Ramamurthy and Narayanan, 1971; Khan, et al., 2019), and with characteristic, appealing odor, attributed to the specific volatile organic compounds (Moio et al., 1993) and to the high concentrations of short-chained fatty acids (Naydenova, 2005; Güler et al., 2005). The fat percentage of buffalo milk is double higher but cholesterol is lower, and the lipid globules are larger, as compared to cow milk (Zicarelli, 2004; Islam et al., 2014).

It is rich in whey proteins, calcium and in particular colloidal calcium (Nguyen et al., 2014; Islam et al., 2014), which enhance the effect of probiotic bacteria during dairy processing (Chandan et al., 2006). The strains *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* inhibit the absorption of cholesterol, play anti-inflammatory and immunomodulatory effects, and improves intestinal microbial balance (Van de Water et al., 1999; Ebringer et al., 2008; Abesinghe et al., 2020).

Bulgarian yoghurt, with the presence of these lactic-acid bacteria, is a specific type of fermented dairy product that has gained recognition on the world market. Such probiotic microflora has synergistic effects that result in specific texture, composition and sensory properties of yoghurt (Ebringer et al., 2008). Buffalo yoghurt is characterized by microstructure that causes higher syneresis – interrupted by large fat globules, binding less protein and featuring more serum pores (Nguyen et al., 2014; Abesinghe et al., 2020), as well as by higher titratable acidity associated with firmer and smoother coagulum at cutting (Naydenova, 2005).

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Casein in buffalo milk is roughly 80% of total protein (20% whey protein) and almost all of it is in the form of larger, more numerous micels, as compared to bovine milk (Ahmad et al., 2013). It has outstanding rennet coagulation, gelling and firming properties for the production of curd – in particular high k-casein and superb proportions amongst protein, whey protein, types of casein and fats (Ariota et al., 2007; Abesinghe et al., 2020; Islam et al., 2014).

The higher fat content of buffalo milk is very important as it leads to higher ratio with protein and hence to superior elastic property of the curd (Ariota et al., 2007), and these are the major components influencing the quality of the manufactured cheese (Guinee et al., 2000; Barron et al., 2001). In association with the higher fat content and dry matter, during ripening buffalo cheese is characterized by lower intensity of physical and chemical changes and lower lipolytic activity, as Ivanov et al. (2016) conclude.

The essential nutritional values of milk and dairy products are indisputable, but still there is criticism against their mass consumption because of the saturated nature of the lactic lipids (Givens and Shingfield, 2006). But, as ruminant products, they are a dominant provider of the beneficial conjugated linoleic acid (CLA), vaccenic and other monounsaturated, and even some saturated fatty acids like butyric and stearic (German, 1999; Lawson et al., 2001; Vargas-Bello-Pérez and Garnsworthy, 2013). In addition, fatty acids are strongly responsible for the quality of the different types of cheese, forming the flavor and being precursors for other volatile aromatic compounds (Khalid and Marth, 1996). Therefore, an important feature of a dairy product is what alterations its lipids undergo in the respective technology and to what extent these essential fatty acids are preserved during milk processing.

The objective of the present study was to analyze the changes in the fatty acids of buffalo milk as processed to the traditional dairy products Bulgarian yoghurt, white-brined cheese and curd, with a focus on the beneficial and health-related acids and isomers.

MATERIAL AND METHODS

For the aim of the study, 12 lactating buffalo cows of the *Bulgarian Murrah* breed from the farm of Agricultural Institute – Shumen were assigned. The housing system is tie stalls with exercise yard.

The daily diet from July to October involves 18 kg green herbage, 4 kg wheat straw, and 4 kg compound feed per capita. The concentrate feed provides 1629 kcal energy and 96 g digestible protein and has the following composition: wheat – 15%, barley – 12%, corn – 56%, wheat bran – 10%, sunflower oilcake – 5%, dicalcium phosphate 0.6%, salt – 0.4%, and chalk – 1%.

The buffaloes were selected to be at second-plus parity and to have calved within a close range – from April 6th to April 25th, also to have minimal daily milk yield of 8 kg, and to have intermediate body condition score of 3.0 to 4.0. A bulk milk sample of 30 kg was pooled after taking an individual sample of 2.5 kg from the morning milking of each animal on May 7th, 2020.

The bulk milk was technologically processed to Bulgarian yoghurt (further referred to as just "yoghurt"), curd and white brined cheese in laboratory conditions. The preparation of the yoghurt included the following operations: the raw buffalo milk was pasteurized at 85 ºC for 15 min, cooling to 43-46 ºC and germination of the milk by 20 mL/L commercial yeast (Apolon-69 EOOD) including *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus (2:1*), fermentation/ coagulation for 2.5-3.5 hours at 41-44 ºC, cooling for 1-2 hours, at 20 ºC and cooling to 2-6ºC by acidity 75-80 ºT.

The curd was produced from whole milk, not as a byproduct from the production of cheese. The preparation of the curd included pasteurization of raw milk at 85 ºC for 15 min, heating to 100 ºC, coagulation of the milk by citric acid (1%), cooling to 20-25 ºC and percolation for 6 hours at 4 ºC. In this way it is in fact an acid-curd cheese (herein refered to as just "curd"), which, unlike the other two products, is a non-probiotic foodstuff.

The preparation of the white brined cheese (further referred to as just "cheese") included the following operations: the raw buffalo milk was pasteurized at 70-75 ºC for 20 min, cooling to 43-46 ºC and germination of the milk by 20 mL/10L commercial yeast (Apolon-69 EOOD) including *Lactobacillus delbrueckii ssp. Bulgaricus* and *Streptococcus thermophiles (2:1*) and 2 mL/10L commercial rennet (Apolon-69 EOOD), fermentation/ coagulation for 1-2 hours at 30-34 ºC, the rennet coagulum was cut into prisms of size 2cm x 2cm x 2cm, pressing the cheese for 12-16 hours, dry salting for 24 hours, washing and maturation in 10% NaCl solution at 10-12 ºC for 60 days.

The lipid analysis of samples of the raw milk, the yoghurt, the cheese and the curd was carried out at the Laboratory of the Department of Food Technology, Institute of Cryobiology and Food

Technology, Sofia. The extraction of total lipids was carried out by the Rose-Gottlieb method, using diethyl ether and petroleum ether and subsequent methylation with sodium methylate (CH_3ONa, Merck, Darmstadt) and drying with $NaHSO_4.H_2O$. Fatty acid methyl esters (FAME) were analyzed using a Shimadzu-2010 gas chromatograph (Kioto, Japan) equipped with a flame ionization detector and an automatic injection system (AOC-2010i). The analysis was performed on a CP 7420 capillary column (100m x 0.25mm i.d., 0.2μm film, Varian Inc., Palo Alto, CA). Hydrogen was used as the carrier gas, and as a make-up gas – nitrogen. Four-step furnace mode was programmed – the column's initial temperature is 80 ° C / min, maintained for 15 minutes, then increased by 12ºC/min to 170ºC and maintained for 20 minutes, followed by a further increase of 4ºC/min 186ºC for 19 minutes and up to 220ºC with 4ºC/min until the process is complete.

The overall spectrum of the lipid analyses includes 22 saturated fatty acids (SFA), 24 monounsaturated fatty acids (MUFA), 20 polyunsaturated fatty acids (PUFA), and 8 isomers. The content of individual FAs and groups of FAs was expressed as a percentage of total FAs in milk using the "%" symbol, while the relative differences between a dairy product and the milk are commented as "percent".

The atherogenicity (IA) index was calculated using the equation developed by Ulbricht and Southgate (1991), as follows:

$$IA = (C12:0 + 4*C14:0 + C16:0) / (MUFA + PUFA) \quad (1)$$

RESULTS AND DISCUSSION

The fatty acids (FA) in the buffalo milk and in the derived dairy products are presented in Table 1. It is seen that the curd has preserved the saturated characteristics of the milk, expressed in practically unchanged total SFA and individual SFAs, the biggest difference being the increase in lauric acid by nearly 5 percent. No great changes are also observed in total SFA in the other two dairy products. In the yoghurt lipids there are some increased values of the atherogenic myristic and lauric acid, as well as of the capric acid – relatively by 5.3 to 7.1 percent. Similarly, in the cheese making where of these fatty acids greatest is the difference in capric acid (13.5 percent). What is noteworthy for the white brined cheese processing is the increased short-chained FAs, to similar degree as C10:0. Of them, most important is the higher concentration of butyric acid – by 10 percent as compared to its level in the milk. In none of the dairy foodstuffs the dominant stearic acid changed.

Small changes have taken place also in the fraction of MUFAs, as shown also in the table. This applies also to the most highly presented oleic acid and to the total of *cis* isomers in all studied dairy foodstuffs. The vaccenic C18:1*trans-11* has little higher value in the curd – by 8.7 percent as compared to its concentration of 0.50% in the raw milk. In the production of the yoghurt, although *trans*-vaccenic acid has also increased (by 10.6 percent), the total content of *trans* MUFAs has decreased by 23.3 percent. There is only little decrease by 4.4 percent of MUFA in the production of the cheese.

A trend of increase in the cheese and decrease in the yoghurt and curd is observed in most of the PUFAn-6, especially in the long-chained C20:2n-6 and C20:4n-6. This applies also to the most presented and valuable n-6, the γ-linolenic acid C18:3 (GLA), where the relative increase in the cheese is by 54 percent and the decrease in the curd and yoghurt – by 36 and 12 percent respectively. The dihomo-GLA (C20:3n-6) has behaved differently in the production of the yoghurt and curd, increasing respectively by 35 and 113 percent. The n-6 fatty acids that decreased in the cheese are the dominating C18:2*cis-9 cis-12* and especially the long-chained C22:2n-6. As a result of these changes, the only alteration of the total omega-6 FAs is the little decrease in the yoghurt – by 7 percent.

As in the group of FAn-6, the most highly presented FAn-3 – the α-linolenic C18:3 (ALA) – is marked with a drop in the three dairy products, best expressed in the cheese – by 16 percent as compared to the milk. The processing to yoghurt has led to even greater drop in all other FAn-3 (by 32 to 88 percent) and in total omega-3 content (34 percent). The same occurred to all FAn-3 in the curd, except for the increase in C20:3n-3, the relative differences ranging from 53 to 83 percent and that in the total FAn-3 being 20 percent. As for the second most highly presented omega-3 (C20:3n-3), it is one of the two fatty acids that are marked with higher level in the cheese. Even greater increase is observed in C20:5n-3 in the cheese – by 150 percent. Hence, the decrease in total omega-3 in the cheese is much lesser – by 5.5 percent.

Table 1. Fatty-acid composition of the buffalo milk and of the dairy foodstuffs produced thereof – presented as % of total fatty acids

Fatty acids	Milk	Yoghurt	Cheese	Curd
C4:0	8.15	8.38	8.98	8.26
C8:0	1.119	1.157	1.261	1.120
C10:0	1.761	1.886	1.998	1.818
C12:0	1.857	1.984	1.984	1.949
C14:0	9.22	9.72	9.66	9.47
C16:0	31.0	31.6	31.1	31.0
C18.0	12.45	12.54	12.17	12.49
C18:1 [a]	25.77	26.02	24.54	26.20
C18:1*trans*-11	0.501	0.563	0.500	0.553
∑C18:1*trans*	1.731	1.328	1.812	1.599
∑C18:1*cis*	26.04	26.21	24.84	26.39
C18:2*cis*-9,12n6	1.574	1.620	1.427	1.679
CLAc9*trans*-11	0.1455	0.1557	0.1251	0.1471
CLA*cis*-9.11	0.0417	0.0110	0.0481	0.0085
CLA*trans*-9.11	0.0174	0.0062	0.0069	0.0097
C18:3n3	0.2309	0.2023	0.1935	0.2156
C18:3n6	0.1822	0.1609	0.2805	0.1171
C18:4n3	0.0241	0.0030	0.0168	0.0042
C20:2n6	0.0333	0.0048	0.0494	0.0134
C20:3n6	0.0394	0.0530	0.0594	0.0841
C20:3n3	0.1121	0.0764	0.1338	0.1242
C20:4n6	0.0441	0.0179	0.0676	0.0237
C20:5n3	0.0252	0.0089	0.0632	0.0055
C22:2n6	0.0692	0.0520	0.0397	0.0718
C22:5n3	0.0658	0.0229	0.0371	0.0310
C22:6n3	0.0235	0.0052	0.0105	0.0069
∑SFA	69.6	71.2	71.6	70.2
∑MUFA	30.50	29.60	29.17	30.18
∑PUFA	2.716	2.450	2.687	2.602
∑CLA	0.2262	0.1749	0.2247	0.1688
∑n3	0.482	0.319	0.455	0.388
∑n6	2.125	1.978	2.115	2.072
n6/n3	4.41	6.21	4.65	5.35
IA	2.17	2.28	2.27	2.19

[a] Sum of C18:1*cis-9*, C18:1*trans-12* and C18:1*trans-13*

Based on these observations, as the table shows, the relatively good omega ratio of the milk of 4.41 is worsened to a great extent in the yoghurt processing, the relative difference being 41 percent. In the curd the ratio is by 21 percent higher, while in the cheese the increase of the n-6/n-3 ratio is as little as 5.4 percent.

The alterations in the isomers of the CLAs are also shown in Table 1. In comparison to its level in the milk (0.146%), the dominating rumenic acid (CLA *cis*-9, *trans*-11) has increased in the yoghurt (by 8 percent), decreased in the brined cheese (by 13 percent), and remained unchanged in the curd. The other three CLA isomers follow the same general trend of alterations in the yoghurt and curd as in omega-3 FAs. The substantial decrease in the yoghurt and curd is 45 to 92 percent, as a result the total CLA in both products dropping by over 20 percent as related to the raw milk. In the cheese the least presented CLA*trans-9,trans-11* has also decreased (by 60 percent), while the *cis-9,cis-11* positional isomer increased. Hence, the total CLA in the cheese has practically preserved its value of 0.226% from the milk.

The table also shows that in the cheese making the concentration of 2.72% of total PUFA from the

milk was also preserved, and the PUFA/SFA ratio changed from 0.39 to 0.38. In the curd PUFA dropped by only 4.2 percent, and similarly the PUFA/SFA ratio. While in these two dairy products only the individual PUFAs are decreased, in the yoghurt the total PUFA content is by nearly 10 percent lower, and the PUFA/SFA ratio – by 12 percent. The data also indicate that total CLA constitutes 8.3% of total PUFA, which was practically preserved during the cheese processing, while in the yoghurt and curd it is reduced to 7.1 and 6.5% respectively.

The atherogenicity index in the yoghurt has deteriorated (Table 1), which is on the basis both on the increase of lauric, myristic and palmitic and on the decrease of total PUFA. This index is higher in the cheese as well, mostly due to the increase of the atherogenic SFA but also to the decrease of MUFA.

In fact, the yoghurt is the only dairy foodstuff where overall fatty-acid profile (SFA/MUFA/PUFA) is altered, as in the three products no substantial changes have occurred in total SFA and total MUFA and in their major representatives – the palmitic, stearic, and oleic acid.

Discussion of the results

The processing of milk to dairy products in this study represents in laboratory conditions the respective technologies applied in the dairy industry. In all three dairy products subjected to lipid analysis in our study the content of myristic acid has changed to little extent its level from the milk, which is actually lower than the value established in our previous study on the same herd (Ilieva et al., 2020). As compared to milk, in the yoghurt from Bulgarian Murrah buffaloes in two studies (Naydenova, 2005; Naydenova et al., 2013) were observed similar changes in the 4- to 14-carbon FAs (especially the increase of capric, lauric and miristic) while the higher content of the palmitic acid and the lower concentration of the valuable stearic and oleic acid are in disagreement with our unchangable values. In agreement, the authors found total dienes decreasing in yoghurt, while they established an increase in the trienes and decrease in total MUFA in contradiction to our findings. In the same time, using technological methods to elongation of shelf life in yoghurt from buffalo milk, Ivanova et al. (2011) have found unchangeable SFA, and similarly Gerchev and Mihaylova (2012) in two breeds of sheep.

As in our study, such alterations of the SFAs have been reported for yogurts with the same microflora in camels (Gassem et al., 2016), in goats (Sumarmono et al., 2015), and in bovine cows (Santos-Junior et al., 2012). Only the latter report is in agreement with our results about the constant levels of total MUFA and in particular the oleic acid, while the former two have found definite increase in C18:1.

Known to provide for better protection against coronary heart diseases (Schwingshackl and Hoffmann, 2012), the MUFA proportion in the studied buffalo milk is high, as compared to other previous studies (Naydenova, 2005; Ilieva et al., 2020), and this beneficial level is preserved also in the other two studied dairy products, as established for different ripening stages of bubaline (Van Nieuwenhove et al., 2007) and bovine (Bonanno et al., 2013) cheese making.

The preserved values of the *trans*-vaccenic acid, the increase of the *trans* isomers and the decrease of *cis* in the cheese are commensurate with the results of Corazzin et al. (2019) in cows, while in a cow yellow cheese with *L. d. bulgaricus* and *S. thermophilus* Bonanno et al. (2013) found increased vaccenic acid together with total MUFA.

As for the saturation of the cheese, the lauric acid that showed some increase in the cheese, except for its adverse atherogenic effect, has also some benefits in association with antivirus and antibacterial effects (Sun et al., 2002; Thormar and Hilmarsson, 2007). The higher levels of caprylic and capric acids are also associated with such benefits (Thormar and Hilmarsson, 2007) and together with butyric acid form the specific flavor of cheese (Naydenova, 2005; Güler et al., 2005).

More special is the increase of C4:0 as it is found only in fats of ruminant origin and which, together with the fat soluble vitamins (A, D, E) and CLA, has protective function against various diseases (German, 1999; Parodi, 2004). Corazzin et al. (2019) have found relatively constant butyric acid in cheese from grazing bovine cows, as well as smaller increase in myristic, caprylic and capric acids, and practically unchangeable stearic acid, as in the present study. In buffalo unripened cheese, Van Nieuwenhove et al. (2007) found similar little increase in myristic but in disagreement also increase in rumenic, total CLA, stearic acid, and ∑C18:1*cis*.

Actually, as Laskaridis et al. (2013) found in feta (sheep white brined) cheese, CLA concentration increased during ripening but decreased during storage up to 48 days to reach, in similarity to our study, lower level than milk accompanied with a drop in PUFA, but also in MUFA unlike our study. Similar was the destiny of total PUFA and total CLA, in particular rumenic acid, in the 60-day

cheese of Bonanno et al. (2013), while in white brined cheese Ivanova and Angelov (2017a) found no significant alterations of fatty-acid profile of milk, regardless of the technology of farming.

Santos-Junior et al. (2012) also found a decrease of ALA in yoghurt but in disagreement they established that the studied omega-6 were increasing and rumenic acid – decreasing. And what is remarkable about the latter CLA isomer is that it was found to undergo dramatic drop due to pasteurization and to partially regain its content during fermentation. The authors' conclusion that the drastic decrease in CLA obliterates the efforts to increase it via diet supplementation explains our findings but is not commensurate with the following review study.

Gutiérrez (2016) finds the literature on the effect of fermentation during dairy processing rather controversial and concludes that the content of CLA in dairy foodstuffs depends more on feeding and management than on the specifications of the milk processing. According to Dave et al. (2002), the concentrations of CLA, vaccenic acid and omega-3 FAs remain unchanged in yoghurt produced via germination with *L. d. bulgaricus* and *S. thermophilus*, although the diet manipulation of fatty-acid profile resulted in substantial increase of beneficial FAs in milk. But still, there are evidences that these two probiotic strains efficiently biosynthesize CLA from linoleic acid (Lin et al., 1999; Yang et al., 2014).

As Gutiérrez (2016) also points out, the effects of thermal processing have shown to be also inconsistent across literature sources. Martínez-Monteagudo and Saldaña (2014) established that both CLA and vaccenic acid are oxidized to a great extent when the temperature increased from 90 to 120°C. On the other hand, according to Destaillats and Angers (2005), high temperature and pressure alter the isomers but not the total composition of CLA. In our study the thermal regime of the cheese making is way below that lower limit, for which our practical observations in laboratory conditions have shown that it does not affect fatty acids, so the alterations here are to be asociated with the further processes – to lesser extents with fermentation and to greater with ripening and aging, based on the latter two cited studies and on that of Laskaridis et al. (2013). In the yoghurt, where the temperature is higher but still below 90°C, no such transformations have occurred, as separate isomers together with total CLA were found to decrease. Hence, the different behavior of CLA can be explained with the difference in pasteurization specifications.

As for the *trans*-vaccenic acid in the present study, if such processes of oxidation have occurred, a further increase must have also occurred presumably due to biosynthesis. Though total CLA is seriously reduced, vaccenic acid is reported to be the only known precursor of rumenic acid and a definite portion of it from the human diet is converted into it (Turpeinen et al., 2002; Field et al., 2009). In this context, its higher level is one of the few assets of the Bulgarian yoghurt produced within our study as certain health-related functional properties, like anti-cardiovascular, anti-carcinogenic and other effects are attributed both to vaccenic acid and the *per se* conjugated linoleic acid (Belury, 2002; Field et al., 2009; Dilzer and Park, 2012).

Similar beneficial properties have been established also for the EPA and its ratio with oleic and omega-6 acids (Haug et al., 2007), as well as for DHA with added priority effect against obesity (Buckley and Howe, 2010). Except the drastic increase of the EPA in the cheese, these two n-3-FAs were found in drastically diminished concentrations in the studied dairy foodstuffs. The result for EPA in the yoghurt is commensurate with a study in goats with the same microflora plus *L. achidophylus* (Sumarmono et al., 2015), but not that for DHA, ALA, GLA, and arachidonic acid.

As for the controversial C20:4n-6, it has undergone remarkable alterations similar to those in EPA. As summarized by Calder (2007), arachidonic acid is a precursor of CLA, and has important role as a cell-signaling molecule with positive effect on neurological system, growth and immune system. The author also points out that it has also adverse effects in high diet concentrations (excess intake), which is mitigated by PUFAn-3 via conversion into oxidised derivatives.

The very little improvement of n-6/n-3 ratio in the cheese (compared to the milk) is practically commensurate with the constant value found by Bonanno et al. (2013) in bovine yellow cheese. Omega-3 FAs are established to derive from the main FAn-3, the ALA, and have beneficial effects on consumers' health similar to CLA (Leaf, 2008; Barceló-Coblijn and Murphy, 2009; Buckley and Howe, 2010), Nevertheless, while CLA in the human diet can be obtained predominantly from milk and meat of ruminant origin (Lawson et al., 2001; Vargas-Bello-Pérez and Garnsworthy, 2013), FAn-3 have low concentrations in livestock products and the desirable n-6/n-3 ratio should be achieved via consumption of other, n3-richer foods.

The only study on this type of traditionally produced curd – which is actually an acid-curd,

non-fermented cheese from whole milk – is the study of Ivanova and Angelov (2017b). It was found that regardless of the system of farming of the cows, the produced curd preserved the fatty-acid profile of the milk subjected to dairy processing, which is not confirmed in our results about the curd produced from intensively bred buffaloes. In a similarly produced but germinated ricotta cheese, Bergamaschi and Bittante (2017) reported also an increase in medium-chain SFA and a decrease in many polyunsaturated FAs during 6-month ripening. It should be noted that, according to Sommella et al. (2018), oxidation of fatty acids depends mostly on temperature rather than on pH and titratable acidity. And also, what concerns the yoghurt and cheese, the dominating lactic-acid strain used as a starter (*Lactobacillus delbrueckii ssp. bulgaricus*) is not a typical (strong) lypolitic culture (Thierry et al., 2017) in combination with the relatively low lipolytic activity of buffalo milk (Ivanov et al., 2016).

CONCLUSIONS

It can be summarized that our results about the yoghurt contribute to the controversy on the topic of the transformations of the health-related fatty acids from milk in the literature. The resemblance of the changes in the yoghurt and curd presumptively denies the existence of a notable effect of fermentation, as the technology of the curd excludes any germination, relying instead on acidification to generate coagulation. And as cited above, acidity is not such an extreme oxidative condition as high temperature, nor are the lipolytic activity of the lactic-acid culture and that of the *per se* buffalo milk. Hence, the alterations observed in the yoghurt are to be explained chiefly with the pasteurization, but it is not so with cheese where the technology is different.

The technological parameters of cheese making are different also from those in most studies, but still our results the sooner confirm the general conception that such technological processes alter the individual isomers but not the groups of beneficial fatty acids (CLA) as a whole. The small overall changes are to be attributed to the generally sole effect of ripening processes, but not to the rennet, which has no lypolitic activity and not to the pasteurization because of the low temperature involved. The focus is chiefly on CLA as it is obtained predominantly from milk and other foodstuffs of ruminant origin, which can be compensated with the presence of its most important precursor in the human diet, the *trans*-vaccenic acid, that was found increased in the dairy products with decreased CLA. Such generalizations might apply also to the other valuable PUFAs, the omega-3 fatty acids, but in human nutrition they are obtained mainly from sources alternative to livestock products.

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