

Fatty acid profiles and health fatty acid indices of traditional cheeses of southeastern European countries

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Abstract

Different types of traditional cheeses have a central place in the markets of southeast European countries. They are usually produced as artisanal from cow's, sheep's and rarely goat's milk. In the last twenty years, these cheeses have attracted numerous researchers' attention. Most of these investigations have been focused on the specificity of their production and the proteolytic changes that influence the nutritional and sensory properties of specific cheese varieties. Less attention has been paid to their fatty acid profiles and their possible effects on human health. Since fatty acids content in cheeses are affected by numerous factors (type of milk and its characteristics, breeding, feeding, geographical area, and the production process), it can be assumed that their fatty acid composition (profiles) is significantly different. This paper provides an overview of the current knowledge of fatty acid profiles and health-related fat indices of traditional cheeses from several southeast European countries including Serbia, Bosnia and Herzegovina, Montenegro and North Macedonia.

Keywords: cheese; fatty acids profiles; health fatty acid indice

Introduction

Cheese has been a part of food culture since the dawn of history. It is produced using unique food processing methods, such as fermentation and ripening. Different types of cheese are produced around the world to meet different tastes, nutritional and health requirements. In recent years, cheese has been attributed a variety of health-promoting effects, often associated with the presence of various bioactive peptides and proteins, some vitamins and minerals (Barać and Sarić, 2023). Besides these compounds, cheese is also a good source of highly digestible lipids containing various spectra of fatty acids. Cheese contains saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Their ratio and content in cheese varies considerably depending on numerous factors. However, SFAs of different chain lengths (short, medium and long-chain fatty acids) dominate (López-Expósito et al., 2012).

Various traditional cheeses are a valuable part of the diet of people in the southeastern Europe. They are mostly produced as artisanal from cow's, sheep's and rarely from goat's milk and are named according to their production regions. For example, white cheese in brine, the most commonly consumed type of cheese in Serbia, is produced as Sjenica cheese, Zlatar cheese, Svrlijig cheese, Homolje cheese and Zlatibor cheese. These cheeses can be very similar, but they also differ in terms of the type of milk, production region, production protocols, composition and sensory properties, etc. (Radulović et al., 2011). Over the last twenty years, the traditional cheeses of southeastern Europe have attracted the attention of numerous researchers (Sarić, 2002; Sarić et al., 2002; Barać et al., 2006, 2013, 2016; Jovanović et al., 2005). However, most of these studies have been based on the proteolytic changes and their effects on the nutritional and sensory properties of certain cheeses, while less attention has been paid to fatty acid profiles and health fatty acid indices.

This article provides an overview of the current state of knowledge regarding the fatty acid profiles and health fatty acid indices of traditional cheeses from several southeastern European countries (Serbia, Bosnia and Herzegovina, Montenegro and North Macedonia).

Fatty acids in cheese

Cheese is an important source of easily digestible dietary fats containing a broad spectrum of fatty acids. The digestibility of cheese lipids is 88–94 % (Renner, 1987). Cheese lipids contain saturated, monounsaturated and polyunsaturated fatty acids. Their content and ratio in cheeses varies significantly and depends on numerous factors, including type and characteristics of milk and the method of cheese production (Sanz Sampelayo et al., 2007; Domagala et al., 2010; Markiewicz-Kęszczycka et al., 2013). However, SFAs are the predominant class of fatty acids in milk fat and cheeses. This group of fatty acids includes: short-chain fatty acids (SCFA), medium- and long-chain fatty acids as well as odd-chain fatty acids (OCFA) and

branched-chain fatty acids (BCFA, iso- and anteiso) (Jensen, 2000; Månsson, 2008). In general, cheese lipids contain about 66 % saturated fatty acids (SFA) (among them 57.4 % palmitic acid, 21.6 % myristic acid and 17.6 % stearic acid), 30 % monounsaturated fatty acids (MUFA) and 4 % polyunsaturated fatty acids (PUFA) (López-Expósito et al., 2012).

Most of the fatty acids in cheeses are in esterified form, while some of them exist as free fatty acids, some of which are the result of lipolysis triggered by lipases of various origins. Free forms of fatty acids contribute directly to cheese flavour, especially when balanced by proteolysis products and other reactions (Mallatou et al., 2004).

Due to the predominance of saturated fatty acids (60–70 %) and the presence of cholesterol, which are associated with cardiovascular disease, metabolic disorders and obesity, cheese is often characterized negatively from a nutritional point of view. Although many authors believe that SFAs contribute significantly to the development of heart disease, to date there is no study that directly and unequivocally demonstrates the link between the consumption of cheese lipids and its SFAs and the occurrence of heart disease. Cheese is also a rich source of short and some medium-chain fatty acids, which are associated with lowering cholesterol, anti-carcinogenic, anti-microbial and anti-diabetic effects. Additionally, cheese also contains certain trans-fatty acids that are now considered to be part of a healthy diet (Barać and Sarić, 2023).

The fatty acids of cheese mainly originate from milk fat which is mostly (95–98 %) composed of triacylglycerides built from fatty acids with different chain lengths (4–24 carbon atoms), different degrees of saturation and different positional specificity, and alcohol glycerol. These triacylglycerides form the core of the fat globule, which is surrounded by a membrane consisting of phospholipids, cholesterol, and esters of these compounds (Lock et al., 2014). The presence of more than 400 different fatty acids makes milk fat the most complex natural source of these compounds (Parodi, 2004, 2009). However, only 15 of them are present in concentration of more than 1 % (Božanić et al., 2018). The origin of fatty acids in milk is the result of two processes, endogenous *de novo* synthesis in the mammary glands from the precursors in feed and the activity of microorganisms in the rumen (Hellgren and Nordby, 2017). The fatty acid content and composition of milk change under the influence of various factors such as animal species, feeding, breed, stage of lactation, individual characteristics, climatic conditions, health, age and others (Paszczyk et al., 2022). According to these authors, feeding is the most important of the above-mentioned factors. This study also showed that the chemical composition, trans-isomeric fatty acid content and lipid quality indices varied between summer and winter cheeses. Summer cheeses had more MUFAs and PUFAs compared to the winter cheeses. Summer cheeses were also characterized by lower content of SFA, higher content of n–3, lower n–6/n–3 ratio and higher desirable fatty acids ratio (DFA). Typical contents of the most important fatty acids in cow's, goat's and sheep's milk are shown in Table 1.

Bioactive fatty acids of cheese

As previously mentioned SFAs are dominant fatty acid of cheese. These fatty acids have poor image because there is some evidence of negative influence on blood lipids. However, despite the presence of considerable amounts of these fatty acids, there is no clear evidence relating cheese consumption to any disease (López-Expósito et al., 2012). It is also known that individual SFAs influence blood cholesterol level differently (Legrand and Rioux, 2010). Total plasma cholesterol raising effects of SFAs are generally greater with medium chain lengths acids (C12:0, C14:0) than for those with longer chain lengths (C16:0 and C18:0) (German and Dillard, 2006). Furthermore, C12:0 is more potent than C14:0 and C16:0 (Parodi, 2009). Also, Parodi (2009) suggests that other saturated fatty acids found in cheese neutralize their effect since they increase HDL- level. In addition, C18:0 as one of the most abundant SFAs can be rapidly converted to the oleic acid (C18:1) which is considered to be healthier and is not related with cardiovascular risk (Jakobsen et al., 2009).

Short-chain SFAs are highly digestible as they are preferentially hydrolyzed from the triglycerides and directly transferred from the intestine to the portal circulation without re- synthesis of triglycerides (Sanz Sampelayo et al., 2007). Thus, there is only low tendency of adipose formation. These acids contribute to reducing of cholesterol level in serum and liver as well as triglyceride levels (Sanz Sampelayo et al., 2007). It has also been shown *in vivo* that butyric acid (C4:0) is a stimulant of normal colonic cell proliferation. Also, it can inhibit the growth and the proliferation of colon cancer cell lines. Furthermore, butyric acid increases thermogenesis to increase energy expenditure, contributing to reduced body weight and other markers of metabolic syndrome (Aluko, 2012). On the other side, caprylic acid (C8:0) and capric acid (C10:0) expressed antiviral and antibacterial properties (Nair et al., 2005; Chen and Liu, 2020).

Milk, cheese and other dairy products are the major source of odd and branched-chain fatty acids in human

diet (Bainbridge et al., 2016). These groups of fatty acids are produced by rumen microbiota from food and their concentrations in milk vary depending on the lactation stage and diet. Several studies have shown that these fatty acids exhibit high bioactivity (Bainbridge et al., 2016), especially anteiso-series fatty acids, which have a cytotoxic effect on breast cancer cells (Wongtangintharn and Rutkowska, 2004), as well as those that have also been determined to stimulate the β -oxidation of fatty acids (Adamska and Rutkowska, 2014).

It is well known that the monounsaturated and polyunsaturated fatty acids (PUFA) are biologically beneficial to human health. Most of the unsaturated fatty acids of milk and dairy products are *cis*-fatty acids. These products also contain 2-4 % *trans*-fatty acids (TFA), mostly *trans*-C18:1 and to lesser extent *trans*-PUFAs. In fact, milk, cheese and other dairy products are the best source of natural *trans*-fatty acids, such as: vaccenic acid (C18:1 *trans*-11, VA) and conjugated linoleic acid (C18:2 *cis*-9 *trans*-11; CLA), which exhibit favorable properties compared to artificial *trans*-fatty acids in partially hydrogenated oils. Today VA is associated with anti-tumor and anti-atherosclerotic activities (Lim et al., 2014). According to several authors (Parodi, 2003; Park, 2009a,b; Barać and Sarić, 2023), CLA has a number of health-promoting properties, including anti-carcinogenic, anti-atherosclerotic, antioxidant and anti-inflammatory effects. Anti-cancer and anti-atherogenic activities are mainly attributed to the rumenic acid (C18:2 *cis*-9 *trans*-11) as the major isomer of CLA in cheese. This isomer represents 80-90 % of CLA in cheese. Also, anti-cancer and anti-atherosclerotic properties are attributed to C18:1 *cis*-9 acid and C18:3 *cis*-9 *cis*-12 *cis*-15 acid (Williams, 2000; Haug et al., 2007). In addition, it was shown that *trans*-C16:1n7 (palmitoleic acid) was associated with anti-diabetic activity. Also, n-3 PUFAs prevent heart diseases and improve immune response whereas the consumption of n-6 PUFAs has positive effects on human health (Arnould and Soyeyurt, 2009).

Table 1. Typical content (%) of the major fatty acids of cow's, goat's and sheep's milk (Božanić et al., 2018)

Fatty acid		Goat's milk	Sheep's milk	Cow's milk
		%		
C _{4:0}	butiric	2.99	3.51	3.30
C _{6:0}	caproic	6.25	2.90	1.60
C _{8:0}	caprilic	2.52	2.64	1.30
C _{10:0}	capric	10.41	7.82	3.00
C _{12:0}	lauric	5.64	4.38	3.10
C _{14:0}	miristic	12.81	10.40	9.50
C _{16:0}	palmitic	34.80	25.90	28.80
C _{18:0}	stearic	6.84	9.57	14.60
C _{18:1}	henicosanoic	13.26	21.10	29.80
C _{18:2}	behenic	3.60	3.21	2.50
C _{18:3}	linoleaidic	0.88	0.80	-

Health-related fatty acid indices of cheese

In current literature different parameters are used to characterize health effects of lipids from milk and dairy products. All of these indices are based on the determination of fatty acid content and composition. Besides Σ SFA, Σ MUFA, Σ PUFA, Σ n-6PUFA, Σ n-3 PUFA and n-6 PUFA/n-3 PUFA in recent years several other parameters including PUFA/SFA, index of atherogenicity (AI), index of thrombogenicity (TI), hypocholesterolemic/hypercholesterolemic ratio (HH), health-promoting index (HPI), unsaturation index (UI), the sum of eicosapentaenoic acid and docosahexaenoic acid (EPA + DHA) are in use (Table 2).

The index of atherogenicity (AI) reflects the atherogenic potential of FA (Ubricht and Southgate, 1991). The AI indicates the relationship between the sum of SFAs and the sum of unsaturated fatty acids (UFAs). The main classes of SFAs, which include C12:0, C14:0, and C16:0, with the exception of C18:0, are considered pro-atherogenic (they favor the adhesion of lipids to cells of the circulatory and immunological systems). UFAs are considered to be anti-atherogenic as they inhibit the accumulation of plaque and reduce the levels of phospholipids, cholesterol, and esterified fatty acids. Thus, Yurchenko et al. (2018) suggested that the consumption of foods or products with lower AI can reduce the levels of total cholesterol and LDL-C in human blood plasma. The TI characterizes the thrombogenic potential of FAs, indicating the tendency to form clots in blood vessels and provides the contribution of different FAs, which denotes the relationship between the pro-thrombogenic FAs (C12:0, C14:0, and C16:0) and the anti-thrombogenic FAs (MUFAs and the n-3 and n-6 families) (Prandini et al., 2011). Therefore, as in the case of AI, the consumption of foods or products with a lower TI is beneficial for human health. The level of AI and TI of milk and cheese reported in literature varies within wide range (1.42-5.02 and 1.00-5.04, respectively, Chen and Liu, 2020). The HH ratio reflects the effect of fatty acids on cardiovascular diseases and in milk and dairy products this ratio varies between 0.32 and 1.29 (Chen and Liu, 2020).

The health-promoting index (HPI) is the inverse of AI. Thus, dairy products with high HPI value are assumed to

be more beneficial to human health. It is mainly used in research on dairy products such as milk (Bonanno et al., 2016; Chen et al., 2004) and cheese (Giorgio et al., 2019). According to these investigations the HPI is in the range of 0.16-0.68.

Fatty acid profiles and health fatty indices of Serbian white brined cheese

More than 2/3 of annual production and consumption of cheese in Serbia is related to the white brined cheese. White brined cheese usually named as "kriska cheese" is a traditional type of cheese which is ripened and kept in brine. It is manufactured from cow's, sheep's, goat's milk or from any mixture of these types of milk. Due to different geographical areas of production, type of milk, animal breed and different autochthonous technological processes even though they belong to the same group of cheese, they have different physicochemical, nutritive and sensory characteristics.

The production processes of different types of Serbian white cheese, as well as their nutritional and sensory characteristics, have been well characterized by a number of authors (Dozet et al., 1996; Maćej et al., 2005, 2006; Jovanović et al., 2005; Barać et al., 2006, 2013; 2016a,b, 2019; Vesković Moravčanin et al., 2012a,b; Miočinović et al., 2016; Smiljanić et al., 2014). However, most of the investigations presented in current literature are related to their properties and specificities of their production and, especially proteolytic changes during ripening and storage. However, to the best of our knowledge, less attention has been paid to their fatty acid profiles and health fatty acid qualities. Barać et al. (2018) investigated fatty acid profiles and health lipid indices of four artisanal Serbian traditional white brined cheeses: Zlatar cheese (cow's milk cheese), Sjenica cheeses (sheep's and cow's milk cheeses), Svrlijig cheese (sheep's milk cheese) and Homolje cheeses (sheep's and cow's milk cheeses). These cheeses were two months old and collected from specific geographical area in the period from April to September. Fatty acid profiles of investigated cheeses are presented in Table 3.

Table 2. Parameters usually used as health fatty acid indices

Index	Full name	Calculation formula
PUFA/SFA	polyunsaturated/saturated fatty acid ratio	Σ PUFA/ Σ SFA
AI	Index of atherogenicity	$[C12:0 + (4 \times C14:0) + C16:0]/\Sigma$ UFA
TI	Index of thrombogenicity	$(C14:0 + C16:0 + C18:0)/[(0.5 \times \Sigma$ MUFA) + (0.5 $\times \Sigma$ n-6 PUFA) + (3 $\times \Sigma$ n-3 PUFA) + (n-3/n-6)]
HH	Hypocholesterolemic/hypercholesterolemic ratio	$(cis-C18:1 + \Sigma$ PUFA)/(C12:0 + C14:0 + C16:0)
HPI	Health-promoting index	Σ UFA/[C12:0+(4 \times C14:0) + C16:0]
UI	Unsaturation index	1 \times (% monoenoics) + 2 \times (% dienoics) + 3 \times (% trienoics) + 4 \times (% tetraenoics) + 5 \times (% pentaenoics) + 6 \times (% hexaenoics)
LA/ALA	Linoleic acid/ α -linolenic acid ratio	C18:2 n-6/C18:3 n-3
TFA	Trans fatty acids	Σ TFA

In these cheeses, the authors detected up to twenty one fatty acids. Twelve of them were saturated whereas up to nine were unsaturated, monounsaturated (MUFA) and polyunsaturated (PUFA) acids. They showed that the fatty acid profiles of traditional white cheeses were similar qualitatively, especially regarding to SFAs. More pronounced qualitative differences they observed in the case of unsaturated fatty acids (UFA). For example, cis-10-pentadecanoic acid (C15:1) was found only in Zlatar cheese whereas linoleic acid (C18:2n6t) was detected only in sheep's milk cheese from Svrljig.

As expected, SFA dominated in fatty acid profiles of all Serbian traditional white brined cheeses. Their content varied significantly ($p < 0.05$) depending on cheese variety and ranged from 65.97 % to 76.61 % (cow's milk cheeses) and 69.68 % to 74.52 % (sheep's milk cheese, Table 4). The lowest content of SFA was observed in Sjenica cow's milk cheese whereas the highest ratio of these fatty acids was in Homolje cow's milk cheese. Based on this investigation the ratio of UFAs of traditional cheeses which are composed of MUFAs and PUFAs was quite different and it was found to be in the range of 23.39 % - 34.03 % (Table 3). The largest part of UFAs was accounted for MUFAs and among them C18:1 acid is characterized by the highest content (17.71 % - 26.43 %) which is in a good

agreement with data reported for milk Markiewicz-Kęszycka et al. (2013) and other types of cheeses such as Ementaler, Gouda and Camembert (Domagała et al., 2010).

PUFAs are significant part of identified fatty acids of investigated cheeses. Depending on the type of cheese, their proportions were 11.03 % to 1.66 % (Table 4). The highest content of these fatty acids was detected in Homolje sheep's milk cheese due to the presence of linoleic acid (C18:2n6t; 4.55 %) and C20:1n9 cis-11-eicosenoic acids (2.56 %). These PUFAs were not detected in other cheeses and the major polyunsaturated fatty acid in all other cheeses was linoleic (C18:2n6c). Under conditions used in this study, due to absence of standards, CLAs were not detected and quantified.

It is interesting to note that traditional white cheeses contained relatively high content (more than 5 %) of odd fatty acids such as C15:0 and C17:0, which are being associated nowadays with several human health benefits (Barać and Sarić, 2023).

As a result of different fatty acid compositions, white brined cheeses had different health lipid indices including index of atherogenicity (IA), index of thrombogenicity (IT), desirably fatty acids ratio (DFA) and UFA/SFA ratio (Table 4). Due to the lowest content of UFAs (23.39 %) Homolje cow's milk cheese is characterized with the most unfavorable DFA, AI-,

Table 3. Fatty acid content (%) of traditional Serbian white brined cheeses (Barać et al., 2018)

Saturated fatty acid (%)	White cheese in brine					
	Sheep's milk cheeses			Cow's milk cheeses		
	Svrljig	Homolje	Sjenica	Homolje	Sjenica	Zlatar
C4:0 butyric	2.51±0.21 ^a	2.26±0.15 ^{ab}	2.12±0.05 ^b	1.54±0.09 ^c	2.17±0.10 ^b	1.51±0.06 ^c
C6:0 caproic	2.63±0.03 ^a	2.44±0.09 ^b	2.39±0.05 ^b	1.81±0.10 ^{c,d}	2.04±0.03 ^c	1.62±0.10 ^d
C8:0 caprylic	2.12±0.11 ^b	2.38±0.22 ^b	2.40±0.12 ^b	1.38±0.10 ^c	1.28±0.17 ^c	1.13±0.08 ^c
C10:0 capric	5.02±0.14 ^b	7.12±0.21 ^a	7.36±0.32 ^a	3.44±0.17 ^c	2.69±0.11 ^d	2.59±0.20 ^d
C12:0 lauric	3.81±0.05 ^a	4.02±0.10 ^a	4.06±0.11 ^a	3.33±0.01 ^c	2.93±0.08 ^b	3.06±0.02 ^b
C14:0 miristic	11.78±0.25 ^b	11.95±0.27 ^b	12.38±0.09 ^a	11.95±0.13 ^b	10.78±0.10 ^c	11.88±0.22 ^c
C15:0 pentadecanoic	2.78±0.04 ^d	2.94±0.07 ^c	2.84±0.10 ^c	5.34±0.23 ^a	3.05±0.06 ^c	4.0±0.16 ^b
C16:0 palmitic	21.11±0.15 ^b	18.06±0.24 ^c	18.08±0.09 ^c	20.56±0.10 ^b	18.36±0.26 ^c	22.48±0.19 ^a
C17:0 heptadecanoic	2.36±0.08 ^c	2.46±0.15 ^c	2.68±0.10 ^c	4.29±0.21 ^a	2.73±0.26 ^c	3.35±0.15 ^b
C18:0 stearic	9.45±0.21 ^f	13.22±0.24 ^a	10.05±0.10 ^d	11.06±0.16 ^c	12.31±0.22 ^b	9.91±0.31 ^{d,f}
C21:0 heneicosanoic	n.d. ^b	n.d. ^b	1.66±0.09 ^a	n.d. ^b	1.56±0.04 ^a	n.d. ^b
C22:0 behenic	6.11±0.14 ^c	7.67±0.11 ^a	3.9±0.12 ^d	1.54±0.09 ^c	6.07±0.15 ^c	6.91±0.24 ^b
Unsaturated fatty acids (%)	Svrljig	Homolje	Sjenica	Homolje	Sjenica	Zlatar
C14:1 miristoleic	n.d. ^e	1.41±0.05 ^d	1.64±0.008 ^c	2.46±0.07 ^a	1.84±0.02 ^b	2.40±0.11 ^a
C15:1 cis-10-pentadecanoic	n.d. ^b	n.d. ^b	n.d. b	n.d. ^b	n.d. ^b	1.18±0.09 ^a
C16:1 palmitoleic	n.d. ^f	n.d. ^f	1.16±0.10 ^d	1.56±0.05 ^c	2.04±0.07 ^b	2.59±0.10 ^a
C18:1n9c (oleic)	11.05±0.24 ^e	10.26±0.15 ^f	14.34±0.31 ^d	6.63±0.31 ^a	16.62±0.24 ^a	15.55±0.32 ^c
C18:1n9t (elaidic)	7.4±0.23 ^c	11.29±0.15 ^a	9.38±0.11 ^b	11.08±0.12 ^a	9.81±0.21 ^b	6.73±0.18 ^d
C18:2n6c linoleic	3.83±0.12 ^a	2.52±0.17 ^d	3.13±0.10 ^c	1.20±0.08 ^e	3.38±0.09 ^b	3.11±0.10 ^c
C18:2n6t linoleic acid	4.55±0.21 ^a	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b
C18:3n6 γ-linoleic	n.d. ^c	n.d. ^c	0.43±0.03 ^a	0.46±0.02 ^a	0.34±0.01 ^b	n.d. ^c
C20:1n9 cis-11-eicosenoic	2.65±0.01 ^a	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b

* values represent means ± SD; values with the different letter within the same row are significantly different at $p < 0.05$;

n.d. - not detected

TI- values and USFA/SFA ratio. In opposite to this, the best health lipid indices had cow's milk cheese from Sjenica (AI 1.89; TI 1.37 and DFA 46.34 %, Table 4).

The fatty acid profiles and nutritive quality of lipid fractions of white brined cheeses was also investigated by Paskaš et al. (2024). They analyzed commercial cow's milk cheeses and cheeses prepared from goat's and sheep's milk according to traditional procedure as suggested by Paskaš et al. (2023). In opposite to the investigation of Barać et al. (2018), this investigation highlighted the health-promoting fatty acid profile of sheep's and goat's milk white brined cheeses, in particular the more favorable values of the nutritional indices: atherogenic, thrombogenic, and health-promoting index, as well as hypocholesterolemic/hypercholesterolemic and n-6/n-3 ratio compared to commercial cow's milk brined cheeses. Such disagreement can be associated with numerous factors including those related to cheese production procedures, initial milk characteristics, area of cheese production etc.

In another work, Barać et al. (2021) followed the influence of ripening environment (brine or vacuum plastic bags without brine) on the fatty acid profile of industrially produced reduced-fat white cheese. They showed that both the removal of part of milk fat during Kajmak production and ripening environment greatly affected fatty acid composition of reduced-fat white cheese. According to the results of this investigation much more intensive lipolysis occurred in cheeses ripened in brine than in vacuum. Consequently, already after 20 days of ripening reduced-fat white brined cheese had only three fatty acids, myristic (C14:0), palmitic (C16:0) and linoleaidic (C18:2n6t) acid.

Fatty acid profiles and health fatty indices of Bosnia and Herzegovina cheeses

Different types of traditional cheeses are present in the Bosnian market. The most famous are Livno, Travnik/Vlašić and Kupres cheeses. Their quality, nutritive and sensory characteristics as well as production processes including proteolytic changes during ripening are characterized by several authors (Dozet et al., 1983; 1996; Dizdarević, 2009; Sarić, 2002; Sarić et al., 2002; Bijeljac and Sarić, 2005; Kalit et al., 2016). However, the fatty acid composition and health characteristics of the traditional cheeses of this region have not yet sufficiently attracted the attention of researchers. Namely, in the available literature, only a few works related to the lipid fraction of these products are presented. The attention has been paid only to the fatty acid quality of Livno and Travnik cheese (Hrković-Porobija, 2016; Hrković-Porobija et al., 2018) and recently Kupres cheese (Sarić et al., 2022).

Traditional Livno cheese belongs to the group of full-fat hard cheeses produced from raw sheep's milk or from different mixtures of sheep's and cow's milk without the addition of a starter culture. This cheese has been produced in the Livno area for more than 130 years. Production of this cheese is seasonal (from May to October), when sheep are kept in the mountains surrounding the town of Livno, in the southwestern part of Bosnia and Herzegovina (Dizdarević et al., 2023). It has moderately salty, spicy flavor typical for sheep cheeses. Livno cheese is specific mainly due to the presence of a specific plant cover in mountain area, climatic conditions and milk of autochthonous sheep (Hrković-Porobija et al., 2018).

Fatty acid profiles of ripened Livno cheese (90 days) were characterized by Hrković-Porobija et al. (2018). However, according to our knowledge in current literature there are no data related to lipolysis and the change of fatty acid compositions of Livno cheese during ripening. Hrković-Porobija et al. (2018) showed an obvious variation in the

Table 4. Health fatty acid indices of traditional Serbian white brined cheeses (Barać et al., 2018)*

Parameters	White cheese in brine					
	Sheep's milk cheeses			Cow's milk cheeses		
	Svrljig	Homolje	Sjenica	Homolje	Sjenica	Zlatar
SFA (%)	69.68 ^c	74.52 ^b	69.92 ^c	76.61 ^a	65.97	68.44 ^d
SCSFA C4:0-C6:0 (%)	5.14 ^a	4.70 ^b	4.51 ^c	3.35 ^e	4.21 ^d	3.13 ^f
MCFA C8:0-C15:0 (%)	25.51 ^c	28.41 ^b	29.04 ^a	25.44 ^c	20.73 ^e	22.66 ^d
LCSFAC 16:0-C24:0 (%)	39.03 ^e	41.41 ^b	36.37 ^a	47.82 ^a	41.03 ^f	42.65 ^c
MUFA (%)	18.45 ^e	22.96 ^d	26.52 ^c	21.73 ^d	30.31 ^a	28.45 ^b
PUFA (%)	11.03 ^a	2.52 ^e	3.56 ^c	1.66 ^f	3.72 ^b	3.11 ^d
DFA	38.93 ^d	38.7 ^d	40.13 ^c	34.45 ^e	46.34 ^a	41.47 ^b
AI	2.44 ^c	2.74 ^b	2.38 ^e	3.06 ^a	1.89 ^e	2.31 ^d
TI	1.87 ^b	1.84 ^b	1.51 ^d	2.12 ^a	1.37 ^e	1.64 ^c
USFA/SFA	0.42 ^c	0.34 ^d	0.43 ^c	0.30 ^d	0.51 ^a	0.46 ^b

*data were presented as average values; values with the same letter within the same row are not statistically significant at $p > 0.05$; AI - atherogenicity index; TI - thrombogenicity index

content of individual fatty acids in the analyzed Livno cheese samples by sampling periods. As a result, quality parameters such as SFA/MUFA, SFA/UFA, etc. also varied (Table 5).

As Table 5 shows, the content of SFAs and UFAs was 53.60-56.48 % and 32.62-34.51 %, respectively. In addition, PUFAs made up 4.28-4.41 % of total fatty acids.

Kupres cheese is a full-fat hard cheese prepared from cow's, sheep's, goat's milk and their mixture. It is produced in the Kupres area at an altitude of 1200 meters, with unique conditions for livestock breeding and the production of milk and dairy products. Its production process and technological characteristics were reported by Rizvanović et al. (2018, 2019), but until the work of Sarić et al. (2022) fatty acid composition of this type of cheese were not characterized. These authors followed the change of fatty acid profiles and healthy fatty indices of Kupres cheese during 90 days of ripening (Table 6). This study clearly showed that ripening greatly improved fatty acid composition and health fatty indices of this type of traditional cheeses. In fact, this investigation showed that ripening for at least 30 days greatly improved health quality of Kupres cheese.

As in the case of other cheese varieties, SFAs were dominant in all analyzed Kupres cheeses, and they represented 57.99 % (one-day-old) and 56.63 % (90-day-old cheese) of all identified fatty acids. The most abundant saturated fatty acids in all of the samples were myristic (C14:0), palmitic (C16:0) and stearic (C18:0), which represented 8.10-6.70 %, 13.07-12.02 % and 26.76-28.30 % of identified fatty acids, respectively. Furthermore, it is interesting to note that analyzed cheeses did not contain short-chain fatty acids (C4:0 - C6:0, SCFA). Also, lauric acid (C12:0) and capric acid (C10:0) were not detected, while myristic acid (C14:0) was the most abundant medium-chain fatty acid. In addition, Kupres cheese also contained C15:0 and C17:0; their content depending on time of ripening was in the range of 5.01-4.92 % and 1.0-1.20 %. These fatty acids originated from milk and are the result of microbial fermentation in rumen. For a long time, they were considered to have no biological function. Nowadays, they

are positively associated with the improvement of insulin sensitivity and the reduction of risk of developing diabetes type II (Forouhi et al., 2014). High content of these fatty acids is related to organic production of milk (Kusche et al., 2015). Thus, they are generally accepted as biomarkers of milk fat intake (Yakoob et al., 2014). Kupres cheese contained higher level of UFAs compared to Livno cheese and especially of PUFAs (11.60 %- fresh cheese and 15.80 %, 90-days-old cheese). In fact, Kupres cheese had more than two times higher content of PUFAs than Livno cheese (Tables 5 and 6). The high content of unsaturated fatty acids in Kupres cheese could be attributed to grazing cows on mountain pastures whereas the observed difference between Kupres and Livno cheeses can be the specificity of grazing composition. It was established that cow's milk fat composition and grazing on mountain pastures were related (De Noni and Battelli, 2008). It was also shown that cows grazing on mountains increased the level of unsaturated acids, especially PUFAs (Romanzin et al. 2013). High content of PUFAs in Kupres cheese can be also a result of strong initial activity of bacterial fatty acid desaturases. These enzymes catalyze the biosynthesis of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) by conversion of single bonds (C-C) into double bonds (C=C) in the acyl chain. Conversion of saturated FAs into MUFAs is catalyzed mainly by stearyl-CoA desaturase-1 which primarily uses stearic acid as a substrate but can also convert other SFAs (Czumaj and Śledziński, 2020). PUFAs are result of the reactions catalyzed by another group of desaturases, unsaturated fatty acid desaturases. Strong activity of both types of desaturases during ripening of Kajmak was also reported by Barać et al. (2022). Thanks to the high UFA and especially PUFA content and composition, Kupres cheese has more favorable AI and TI values than other hard cheeses investigated by Paszczyk et al. (2020) and Hirigoyen et al. (2018).

Traditional Travnik/Vlašić cheese is a very popular cheese in Bosnia and Herzegovina which belongs to the group of white-brined cheeses. Production of traditional Travnik/Vlašić

Table 5. Some quality parameters of fatty acids of traditional Livno cheese (Hrković-Porobija et al., 2018)

Parameter	I	II	III
Σn-3	1.66	1.24	1.41
Σn-6	2.23	2.39	2.38
ΣSFA	55.44	53.6	56.48
ΣMUFA	28.14	30.30	28.44
ΣPUFA	4.48	4.21	4.39
ΣUFA	32.62	34.10	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	12.80
MUFA/PUFA	6.28	7.19	6.47
SFA/UFA	1.69	1.55	1.72
UFA/MUFA	1,16	1,13	1.16
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

Table 6. Changes in health lipid indices of Kupres cheese during 90 days of ripening (Sarić et al., 2022)*

	1 day	30 days	90 days
Sum SFA	57.99 ^a	58.70 ^a	56.63 ^c
SCSFA C4:0-C6:0	n.d. ^a	n.d. ^a	n.d. ^a
MCSFA C8:0-C15:0	16.69 ^a	16.22 ^a	14.89 ^b
LCSFA C16:0-C24:0	41.30 ^b	42.48 ^a	41.84 ^b
UFA	42.01 ^b	41.30 ^c	43.37 ^a
MUFA	30.39 ^a	27.96 ^b	27.47 ^c
PUFA	11.62 ^c	13.34 ^b	15.80 ^a
DFA	68.77 ^b	68.73 ^b	71.67 ^a
SFA/UFA	1.38 ^b	1.42 ^a	1.31 ^c
AI	1.72 ^a	1.76 ^a	1.55 ^b

*data were presented as average values; values with the different letter within the same row are significantly different at $p < 0.05$; SFA - saturated fatty acids; SCSFA - short chain saturated fatty acids, MCSFA - medium chain saturated fatty acids, LCSFA - long chain saturated fatty acids, UFA - unsaturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, DFA - desirable fatty acids, AI - atherogenicity index; TI - thrombogenicity index.

cheese is located on the mountain Vlačić and surrounding mountains (near the town Travnik), situated in the central part of the country. It is produced from raw sheep's milk (autochthonous sheep Pramenka) on small farms. Production of traditional Travnik/Vlačić cheese is seasonal, usually starts in mid-May and lasts until early October. During this period, sheep are fed only on the pastures (Sakić-Dizdarević et al., 2009). Traditional production of this cheese is still not standardized. Besides other factors, this can induce significant variation in content and composition of the major cheese compounds. Fatty acid composition of Travnik/Vlačić sheep's milk and cheese was the object of investigation of Hrković-Porobija (2016) and Hrković-Porobija et al. (2018). These studies demonstrated that the fatty acid content and composition of sheep's milk in this area varied within and between sampling areas. It was characterized by a relatively high content of saturated fatty acid (SFA) during the harvest period. Comparing characteristics of sheep's milk used for Travnik and Livno cheese production, Hrković-Porobija (2016) reported that fatty acid composition of milk varied significantly. Milk from Travnik region had more C4:0, CLA, DHA (C22:4 n6), EPA (C20:5 n3), ARA (C20:4 n6) than milk from Livno. Consequently, Travnik cheese had more PUFAs (especially CLA and vacenic acid) than Livno cheese.

Fatty acid profiles of Montenegro and North Macedonia cheeses

For centuries, milk production and processing have been the main activities of the large part of the agricultural population of Montenegro and North Macedonia. Consequently, on the market of these countries there is a wide range of traditional cheeses. According to Mirecki and Sevgi (2020) the most famous, the highest quality and, according to the quantity of production and consumption, the most common cheeses of Montenegro are Pljevaljski, Njeguški, Lisnati, and Prljo. They are all characterized by high nutritional value, piquant, unique and specific aromas. Their traditional production, physico-chemical and sensory characteristics are well documented

by numerous authors (Dozet et al., 1996; Bojanić-Rašević et al., 2010; Mirecki and Adžić, 2006; Mirecki and Kirdary, 2020; Mirecki et al., 2012, 2015; Mirecki and Konatar, 2014). But according to our best knowledge, the current literature still lacks data related to the fatty acid composition and the health fatty acid parameters of these cheeses. Recently, Barać et al. (2024) characterized quality and health aspects of Lider cheese at different stages of ripening. Lider cheese is a commercial full-fat semi-hard cheese produced according to traditional procedure from pasteurized goat's milk in mountain area of Nikšić. In this work it was characterized the change of protein and fatty acid profiles, mineral content and antioxidant potential during the period of one month of its ripening. It was shown that ripening during only 30 days significantly improved fatty acid profiles and consequently health indices of Lider cheese (Table 7). The 30-days-ripened cheeses are characterized with lower values of SFA/UFA ratio, AI and TI compared to one-day-old samples. This was attributed to the strong bacterial desaturases activity. The observed values of AI and TI parameters of 30-days-old Lider cheese (2.31, 2.13) were lower than those of semi-hard goat's milk cheeses reported by Bodnár et al. (2021). These authors showed that AI and TI of goat's cheeses prepared from milk obtained from extensively grazed goats were 2.47 and 2.68, respectively. This can be attributed to the higher content of PUFAs, especially linoleic acid.

Traditional cheeses are specific products of the Republic of North Macedonia, mainly produced in small-scale farms or farm houses located in high mountains and rural areas. Some of the best-known cheese types are produced in almost all regions in North Macedonia: kashkaval, white brined cheese and beaten cheese ("bieno sirenje"). Kashkaval is a type of hard yellow cheese with a natural rind and it belongs to the Pasta filata cheese group. Traditionally, it is made from sheep's milk. White brined cheese is the most commonly consumed type of cheese that can be produced from sheep's, cow's and goat's milk. Beaten cheese is a type of yellow hard cheese and the scalding procedure is a crucial step in its production. The great diversity in the manufacturing procedures results in variations in the physical, chemical and microbiological composition, as well as with variations in the proteolysis,

Table 7. Changes in health indices of Lider cheese during 30 days of ripening (Barać et al., 2024)*

Health indice	Ripening time (days)		
	1	15	30
Sum SFA	66.81 ^a	63.81 ^b	60.40 ^c
SCSFA C4:0-C6:0	n.d. ^a	n.d. ^a	n.d. ^a
MCSFA C8:0-C15:0	24.20 ^b	26.89 ^a	24.35 ^b
LCSFA C16:0-C24:0	42.61 ^a	36.90 ^b	36.05 ^c
UFA	33.19 ^c	36.19 ^b	39.60 ^a
MUFA	20.46 ^c	25.48 ^b	26.35 ^a
PUFA	12.73 ^b	10.71 ^c	13.25 ^a
DFA	59.19 ^b	58.79 ^b	61.38 ^a
SFA/UFA	2.01 ^a	1.76 ^b	1.52 ^c
AI	3.04 ^a	2.77 ^b	2.31 ^c
TI	3.29 ^a	2.81 ^b	2.13 ^c
OFA	28.56 ^a	28.45 ^a	26.19 ^b
DFA/OFA	2.07 ^b	2.07 ^b	2.34 ^a

*data were presented as average values; values with the different letter within the same row are significantly different at $p < 0.05$; SFA - saturated fatty acids; SCSFA - short chain saturated fatty acids, MCSFA - medium chain saturated fatty acids, LCSFA - long chain saturated fatty acids, UFA - unsaturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, DFA - desirable fatty acids, AI - atherogenicity index; TI - thrombogenicity index; OFA - hypercholesterolemic fatty acids.

the texture and volatiles. As in the case of Montenegrin cheeses, the manufacturing and the characteristics (chemical composition and physical properties) of the major traditional cheeses from North Macedonia are well documented by numerous authors including Mojsova et al. (2013), Sulejmani et al. (2014a,b), Santa and Srbinovska (2014); Sulejmani and Hayaloglu (2018), Mateva-Dubrova et al. (2008), Mateva et al., (2019), Makarijoski and Dimitrovska (2021). However, less attention has been paid to fatty acid composition of these cheeses. In fact, only two works related to free fatty acid composition of traditional South Macedonian cheeses can be found. Srbinovska and Santa (2018) investigated only free fatty acid profiles and sensory characteristics of Galichki sheep kashkaval cheese from mountain named Bistra. They showed that three fatty acids i.e. palmitic, oleic and miristic acid represented the major part of free fatty acids of this type of cheese and their contents were 30.57 ± 4.17 g/100 g, 24.89 ± 1.05 g/100 g and 11.01 ± 0.69 g/100 g, respectively. In another study, Makarijoski and Dimitrovska (2021) reported that four fatty acids (oleic, palmytic, stearic and miristic acid) dominated in free fatty acid profiles of North Macedonian white brined cheese.

Conclusions

Based on the data presented in this paper, it is obvious that despite the dominant presence of saturated fatty acids, cheese is a very important source of bioactive fatty acids that can play a significant role in the healthy diet of modern people. Furthermore, the data present in the literature clearly indicate that the ratio of certain fatty acids, including saturated ones, is of key importance for considering the health aspect of cheese consuming. Given the fact that the traditional cheeses have central place on the market

of Serbia, Bosnia and Herzegovina, Montenegro and North Macedonia there is a need for closer characterization of fatty acid profiles and parameters that indicate the health effect of their consumption. However, it seems that there is still a lack of data that would more closely indicate the effect of numerous factors on their fatty acid profiles and consequently on health aspect of their consumption.

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Masnokiselinski profili i zdravstveni indikatori masnih kiselina tradicionalnih sireva zemalja jugoistočne Europe

Sažetak

Različite vrste tradicionalnih sireva imaju središnje mjesto na tržištima zemalja jugoistočne Europe. Tradicionalno se proizvode od kravljeg, ovčjeg i rijetko kozjeg mlijeka. U posljednjih dvadesetak godina ovi su sirevi privukli pažnju brojnih istraživača. Većina ovih studija usmjerena je na specifičnosti njihove proizvodnje i proteolitičke promjene koje utječu na nutritivna i senzorska svojstva pojedinih vrsta sireva. Manje se pažnje posvećuje njihovim profilima masnih kiselina te mogućim učincima na ljudsko zdravlje. Kako na sadržaj masnih kiselina u sirevima utječu brojni čimbenici (vrsta mlijeka i njegove karakteristike, uzgoj i hranidba životinja, zemljopisno područje i proces proizvodnje), može se pretpostaviti da se i sastav (profil) njihovih masnih kiselina razlikuje. Ovaj rad daje pregled trenutnog znanja o profilima masnih kiselina i zdravstvenim indeksima masti tradicionalnih sireva iz nekoliko zemalja jugoistočne Europe uključujući Srbiju, Bosnu i Hercegovinu, Crnu Goru i Sjevernu Makedoniju.

Ključne riječi: sir; masno-kiselinski profili; zdravstveni indikatori masnih kiselina

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