Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.63322

Protein and fatty acid profiles of Kajmak ripened at two different temperatures

Miroljub BARAĆ[1*], Tanja VUČIĆ[1], Bojana ŠPIROVIĆ-TRIFUNOVIĆ[1], Nevena BARAĆ[1], Milenko SMILJANIĆ[2]

Abstract

Kajmak is a specific dairy product with long term tradition of manufacture in Serbia and few South-East European countries. It is manufactured from the thin layer formed on the surface of hot milk during long cooling process. It may be consumed as a fresh, immediately after manufacture, or after a maturation period, as a ripened Kajmak. Kajmak is ripened usually up to 30 days at 15-18 ^{0}C or stored in refrigerator at 4 ^{0}C. In this work we investigated the effects of ripening of Kajmak in refrigerator at 4 ^{0}C and at 16 ^{0}C up to 28 days on protein profiles, fatty acid profiles and health fatty indices. Ripening temperature significantly affects protein and fatty acid profiles of Kajmak. Ripening at low temperature induces slow proteolysis, the change of fatty acid profile and health fatty acid indices. At low temperature the most susceptible to proteolysis was β-casein. Due to similar fatty acid profiles, these samples were characterized with high health fatty acid indices such as atherogenicity index (3.81-4.03) and the thrombogenicity index (4.62-4.95). Ripening at 16 ^{0}C induces complete hydrolysis of κ-CN, significant decrease of α_s- and β-CN content and improvement of health fatty indices.

Keywords: Kajmak; ripening; fatty acid profile; protein profile.

Practical Application: Effect of ripening conditions on Kajmak properties.

1 Introduction

Kajmak is a traditional dairy product of the several South-East European countries such as Serbia, Montenegro and Bosnia and Herzegovina. Usually it is produced as homemade or artisanal from the thin layer formed on the surface of hot milk during long cooling process. However, in recent decades, there has been a growing interest in the implementation of Kajmak production in the dairy industry. The process of traditional production of Kajmak is well described by several authors (Dozet et al., 2004; Pudja, 2006; Pudja et al., 2008; Radovanovic et al., 2020).

Kajmak is characterized with unique composition and sensory characteristics. It is mainly composed of milk fat aggregates and proteins; milk fat represents 40-55% of fresh and 60-70% of ripened Kajmak (Pudja et al., 2008). Kajmak, like other dairy products, is a significant source of highly digestible fat which contains different varieties of fatty acids. In general, digestibility of milk fat is 88-94% (Renner, 1987). However, milk fat is often related to the adverse nutritional image mainly due to the association with saturated fatty acids (which are the major fatty acids of dairy fat) and cholesterol with cardiovascular diseases. Besides the fact that many researchers have considered SFAs as one of the contributory factors in heart disease, until now there has been no real study to demonstrate conclusively a direct connection between cardiovascular disease and milk fat or to implicate dairy products in heart disease (Kanekanian, 2014). Furthermore, it is also known that individual SFAs have different effects on blood cholesterol levels (Legrand & Rioux, 2010). Total plasma cholesterol raising effects of SFAs are generally greater with medium- chain lengths acids such as lauric and myristic acid than with those with longer chain length (palmitic and stearic acid) (German & Dillard, 2006). Since stearic acid can be rapidly converted into C18:1 (oleic acid) fatty acid (Jakobsen et al., 2009), it is considered as healthier than other SFAs and is not related with cardiovascular risk. In addition, Kajmak like other dairy products may be a source of SFAs such as C15:0 and C17:0, which are, today, positively associated with improvement of insulin sensitivity (Pedersen et al., 2016) and the reduction of risk of developing diabetes type II (Forouhi & Wareham, 2014; Hernandez, 2016). Besides that, Kajmak is widely regarded as a source of monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) and is considered as much healthier than SFAs.

Milk proteins are the second most abundant component of Kajmak presented in concentration of 5-10% (fresh Kajmak) and 5-7% (ripened Kajmak) (Pudja et al., 2008). According to Radovanovic et al. (2020) proteins are mainly located in the upper part of Kajmak which is formed during the so called "hot phase" of Kajmak production. The same authors reported that about 7% of proteins are incorporated into upper, whereas about 2% of proteins are allocated in the bottom part of the final product. In general, protein fraction of Kajmak is composed of caseins, whey proteins, milk- fat globule membrane proteins (MFGM) and other minor proteins of milk. Their content and composition depend on numerous factors including type and quality of milk and processing conditions, especially regime of heat-treatment of milk. The production of Kajmak involves severe long-term heat treatment and long-term cooling process (up to 24 h). It is well documented that severe heat treatment and slow cooling process induced denaturation of milk proteins especially major serum proteins, their reorganization, interactions and

Received 28 May, 2022
Accepted 14 July, 2022
[1] *Faculty of Agriculture, University of Belgrade, Belgrade, Serbia*
[2] *Faculty of Technology, University of East Sarajevo, Zvornik, Bosnia and Herzegovina*
*Corresponding author: baracm@agrif.bg.ac.rs

formation of chemical complexes, so called WP-CN-complexes (Considine et al., 2007; Chandrapala et al., 2010; Chevalier & Kelly, 2010). These complexes as high molecular weight compounds may interact with milk fat and may be incorporated into Kajmak. Radovanovic et al. (2020) showed that the level of these complexes incorporated in Kajmak increased as the temperature during heat treatment of milk rised from 75 ^{0}C to 95 ^{0}C.

Manufacture and consumption of Kajmak has a long tradition in Serbia. It may be consumed as a fresh Kajmak (ripened up to 7 days), or after a maturation period (usually up to 30 days) as a ripened Kajmak (Pudja et al., 2008). Namely, after production, fresh Kajmak is stored in a refrigerator at 4 ^{0}C or is ripened usually up to 30 days at 15-18 ^{0}C. It can be assumed that storing at these temperatures affects its nutritional characteristics differently. According to the authors knowledge, there is a lack of data related to the influence of the storage of Kajmak at low temperatures on fatty acid and protein profiles. Thus, the goal of this work was to characterize the change of fatty acid profiles and health fatty acid indices as well as the change of protein composition during the storage of Kajmak at low temperature (4 ^{0}C; in further text it was called ripening at low temperature) and to compare with those induced at 16 ^{0}C.

2 Material and methods

2.1 Sampling of Kajmak

Kajmak prepared according to traditional procedure was obtained from the mountain village near Mionica. Kajmak was sampled from the same producer three times during the period of May-September 2021. Each time eight 250 g-portions of fresh Kajmak were sampled. The four of them were storage at 4 ^{0}C in refrigerator during different period (7, 14, 21 and 28 days) and frozen until analysis. The other four were stored at 16 ^{0}C during the same periods and frozen.

2.2 Physico-chemical analyses

The composition of Kajmak was determined by following methods: dry matter by standard drying method at 102 ± 2 ^{0}C (International Dairy Federation, 1982); fat content according to the Van-Gulik method (International Dairy Federation, 1986), also expressed as fat in dry matter (FDM); protein content by Kjeldahl method (Association of Official Analytical Chemists, 1998) and expressed as total protein in dry matter (TP/DM). NaCl content was determined according to the Volhard method (International Dairy Federation, 1988). The pH was measured using digital pH-meter (Consort, Turnhout, Belgium) (Ardö & Polychroniadou, 1999).

2.3 Assessment of proteolysis

The change of protein profiles during storage of Kajmak at different temperatures was followed using SDS-PAGE under reducing conditions and desytometric analysis of obtained gels. Proteins of Kajmak was extracted according to the following procedure: A portion of 2 g of defrosted Kajmak was extracted in 10 mL of 0.05M Tris-HCl sample buffer pH 6.8 (containing 2% SDS, 4% urea, 5% β-mercaptoethanol (V/V), 7% glycerol) for 1 hour at 50 ^{0}C. After that, the extract was immediately cooled in ice-water bath and centrifuged at 4.000 x g for 30 min and the lipid layer was carefully removed and additionally filtered through Whatman No1. Clear supernatant was diluted with the same sample buffer in which bromphenol blue (0.0025%) is added.

The SDS-PAGE method of Fling & Gregerson (1986) under dissociating and reducing conditions was used. Electrophoresis were performed on 5% (wt/vol.) stacking and 12.5% (wt/vol.) resolving gel (Gel electrophoresis apparatus, LKB-2001- 100, LKB, Uppsala, Sweden) as described by Šertović et al. (2022). The Low molecular mass calibration kit (Sigma-Aldrich, St.Louis, USA) was used to estimate molecular masses of the identified polypeptides and proteins. Also, caseins were identified using the mixture of α_s- and β-CN. The scanned gels were analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafalel, CA). Caseins and polypeptides were quantitatively determined by integration of peak volumes. Intensity of detected bands was quantified from the gel on which 25µl of the samples were applied.

2.4 Fatty acid profiles

The fatty acid content of Kamak was determined as suggested by Barać et al. (2018). A quantity of 0.5 g of Kajmak was extracted in 10 mL of heptane in an ultrasonic water bath for 1 h and then for 24 h at room temperature. The extract was filtered through Whatman No. 1 filter paper and evaporated under a stream of nitrogen. Fatty acids were dissolved in 1 ml of hexane and transformed into methyl esters (FAME`s) using 1 mL of 14% BF3/MeOH. The mixture was heated at 100 ^{0}C for 1 h, cooled to room temperature and the metal esters were separated in the hexane phase after the addition of 1 mL of deionized water.

FAME's were separated using capillary gas chromatography with flame ionization detector (GC/FID) The GC/FID (Agilent Technologies 6890, Santa Clara, CA, USA) equipped with split/splitless injector and SP-2560 (length 100 m, i.d. 0.25 mm, film thickness 0.20 µm, Supelco, Bellefonte, USA). The obtained chromatographic peaks were identified using Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA). Fatty acid content was calculated in mg/g of lipids and expressed in relative quantities as the mass percent of total fatty acids.

2.5 Indexes of lipid quality

Based on the fatty acid profile analysis the unsaturated/saturated fatty acids (SFA/UFA) ratios, desirable fatty acids (DFA, hypocholesterolemic fatty acids), the ratios of hypocholesterolemic and hypercholesterolemic fatty acids (DFA/OFA), the atherogenicity index (AI) and the thrombogenicity (TI) indices of Kajmak were calculated. DFA was calculated according to following equation (Equation 1):

$$DFA = \Sigma MUFA + \Sigma PUFA + C18:0 \quad (1)$$

OFA (hypercholesterolemic fatty acids) was calculated according to following equation (Equation 2):

$$OFA = C12:0 + C14:0 + C16:0 \quad (2)$$

The AI and TI indices were calculated, as proposed by Brandielli et al. (2020) through the equations (Equations 3-4):

$$AI = [(4 \times C14:0) + C16:0] / \Sigma MUFA + \Sigma PUFA \quad (3)$$

$$TI = \left(C14:0 + C16:0 + C18:0\right) / \left(0.5MUFA + 0.5PUFA - n6 + 3PUFAn3 + PUFA - n3 / PUFA - n6\right) \quad (4)$$

AI indicates the relationship between the sum of the main saturated FAs (considered as proatherogenic) and the main classes of unsaturated FAs (considered as anti atherogenic). TI reflects the tendency to form clots in the blood vessels and represents the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (Liu et al., 2020).

2.6 Statistical analysis

All measurements were done in triplicate. Analysis of variance (ANOVA) was performed on all data with IBM-SPSS v20 software (IBM Corp., New York, NY, USA). Comparisons of mean values were followed by t-test with significance level set on $p < 0.05$.

3 Results and discussion

3.1 Physico-chemical analyses of Kajmak

Due to its specific composition (mainly milk fat and proteins) Kajmak may be classified between cheese and butter (Pudja et al., 2008). Therefore, similar as in cheese, during ripening numerous factors including ripening temperature regulate and control the biochemical processes that affect the formation of specific flavor, aroma and texture of Kajmak (McSweeney, 2004; Vučić et al., 2008). The physico-chemical characteristics of Kajmak during ripening period are shown in Table 1.

Traditionally, Kajmak is consumed as fresh, immediately after production or ripened up to 7 days. Values of DM, fat, FDM and proteins for both Kajmak samples (after 7 days of ripening) were in the range of those reported for fresh Kajmak (Mijačević et al., 1990). In Kajmak production, high heat treatment is used, and no starter culture is added, so initial acidity is the result of residual indigenous bacteria, and/or microbial population, probably derived from external contamination during formation of Kajmak. Since higher ripening temperature is more suitable for the growth of microorganisms, acidification was significantly slower ($p < 0.05$) in Kajmak ripened at 4 ^{0}C. Therefore, the pH of fresh Kajmak (7-days ripened) was 6.08 and 4.88, respectively. During ripening decrease of pH was observed in both variants of Kajmak. In Kajmak ripened at 4 ^{0}C, pH decreased significantly ($p < 0.05$) throughout whole ripening period, and reached 5.05 after 28 days. On the other hand, in Kajmak ripened at 16 ^{0}C, pH decreased during 21 days, and then remained constant. Besides pH, significant effect of ripening temperature was also observed in DM, fat and TP/DM content of Kajmak ($p < 0.05$). Ripening at 16 ^{0}C had the highest effect on fat and consequently dry matter of Kajmak. After 28 days of ripening significantly lower ($p < 0.05$) fat content of Kajmak ripened at 16 ^{0}C can be attributed to intensive lipolysis and resultant release of fat. The average content of fat was 65.28% and 50.08%, whereas DM content was 75.75% and 56.51%, respectively. Since fat is the most abundant component of DM of Kajmak, after 28 days of ripening its reduction did not affect FDM which was 86.14% and 88.63%, respectively. Furthermore, with the exception of values obtained for 14-days old Kajmak, FDM was not influenced by ripening temperature. These FDM values agree with the results reported for similar products from Serbia and Montenegro (Vučić et al., 2008; Mirecki et al., 2017). In Kajmak ripened at low temperature there was no change in TP/DM content during 21 days of ripening. After this period, slow proteolysis induced significant decrease of TP/DM to 11.85% ($p < 0.05$). Since higher ripening temperature induces more intensive proteolysis, during 21 days in Kajmak samples ripened at 16 ^{0}C significant decrease ($p < 0.05$) of TP/DM content was observed. The average content of TP/DM content was 9.99% (7-days ripened Kajmak) and 7.74% (21-days ripened Kajmak), respectively. However, after 28 days of ripening, reduction of dry matter due to loss of fat caused by lipolysis led to significant ($p < 0.05$) increase of TP/DM to 9.71%. Nevertheless, results of

Table 1. Composition of Kajmak ripened at 4 ^{0}C and 16 ^{0}C during 28 days.

Parameter	Ripening temperature	Days of ripening			
		7	14	21	28
Dry matter (%)	4 ^{0}C	63.74 ± 0.22Cb	63.23 ± 0.13Cb	73.71 ± 0.11Ba	75.75 ± 0.11Aa
	16 ^{0}C	65.74 ± 0.21Ba	71.83 ± 0.04Aa	72.52 ± 0.24Ab	56.51 ± 0.25Cb
Fat (%)	4 ^{0}C	53.33 ± 1.03Bb	52.00 ± 1.58Bb	62.08 ± 1.50Aa	65.25 ± 0.52Aa
	16 ^{0}C	58.33 ± 0.52Ba	64.25 ± 0.25Aa	61.08 ± 0.66ABa	50.08 ± 0.80Cb
FDM (%)	4 ^{0}C	83.67 ± 1.39Aa	82.25 ± 2.88Ab	84.23 ± 1.93Aa	86.14 ± 0.79Aa
	16 ^{0}C	88.73 ± 0.56ABa	89.44 ± 1.70Aa	84.23 ± 1.16Ba	88.63 ± 1.15ABa
TP/DM	4 ^{0}C	12.79 ± 0.08Aa	12.28 ± 0.08Aa	13.06 ± 0.23Aa	11.85 ± 0.16Ba
	16 ^{0}C	9.99 ± 0.39Ab	8.78 ± 0.10Bb	7.74 ± 0.18Cb	9.71 ± 0.24Ab
NaCl (%)	4 ^{0}C	0.55 ± 0.03ABa	0.47 ± 0.02Ba	0.59 ± 0.04Aa	0.57 ± 0.02Aa
	16 ^{0}C	0.52 ± 0.02Aa	0.50 ± 0.02Aa	0.53 ± 0.02Aa	0.50 ± 0.01Aa
pH	4 ^{0}C	6.08 ± 0.04Aa	5.47 ± 0.03Ba	5.18 ± 0.03Ca	5.05 ± 0.04Da
	16 ^{0}C	4.88 ± 0.04Ab	4.66 ± 0.04Bb	4.51 ± 0.03Cb	4.53 ± 0.02Cb

A, B, C, D: means in the same row followed by different letters were significantly different ($p < 0.05$); a, b: means in the same column followed by different letters significantly different ($p < 0.05$); FDM: fat in dry matter; TP/DM: total protein in dry matter.

compositional analysis obtained for all Kajmak samples during ripening were in agreement with requirements defined by Serbian regulations (Serbia, 2014).

3.2 Protein profiles of Kajmak

Polypeptide profiles of Kajmak obtained by the SDS-PAGE under reducing conditions are shown in Figure 1. The change of relative concentrations of detected fractions is presented in Table 2.

The applied electrophoretic method separated proteins of Kajmak into multiple components with molecular weights ranged from 180 kDa-6.5 kDa which can be divided into three fractions, the fraction of high-molecular weight (HMWF > 50 kDa) peptides, the fraction of the major caseins and the fraction of low- molecular weight (LMWF < 19 kDa) peptides. As expected, the fraction of caseins dominated. In this fraction of the 7-days-old Kajmak samples three major bands, α_s-, β- and κ-CN with molecular weight consistent with those reported in literature (Pesic et al., 2012) were observed. These caseins represented 67.68% and 62.24% of identified components of the SDS-profiles of the 7-days-old Kajmak ripened at 4 ^{0}C and 16 ^{0}C, respectively (Table 2). The HMWF-fraction of these samples consists of several diffused bands which represented 8.16% (7-days-old sample ripened at 4 ^{0}C) and 13.69% (7-days-old sample ripened at 16 ^{0}C). According to the data reported by Spitsberg (2005), these bands mainly correspond to the major MFGM-proteins (proteins of milk-fat globule membrane) such as Mucin I, Xantine oxidase, CD36 and Butyrophilin.

In general, from Figure 1 and Table 2 it is obvious that relatively slow proteolysis occurred during ripening of Kajmak. However, the choice of ripening temperature affected the distribution of the major proteins and polypeptides. Furthermore, it is obvious that ripening at 16 ^{0}C induced more intensive degradation of the major casein fraction than the ripening at 4 ^{0}C. Ripening of Kajmak at 16 °C reduced the relative content of all of three identified caseins but to the different extent. The most susceptible was κ-CN; this casein was completely hydrolyzed between 7th and 14th day of ripening (Figure 1). Also, β-CN was more prone to hydrolysis than α_s-CN. Between 7th and 28th day of ripening the ratio of β-CN was reduced from 24.83% to 20.57% whereas the relative content of α_s-CN was reduced from 30.86% to 28.20% (Table 1). As a result of their degradation, the relative content of LMWP significantly ($p < 0.05$) increased up to 35.32%.

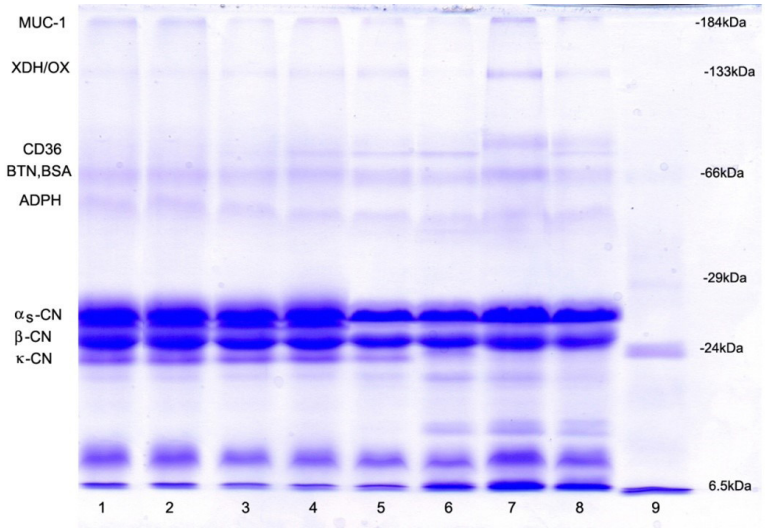

Figure 1. The change of SDS-polypeptide profiles of Kajmak ripened at 4 ^{0}C and 16 ^{0}C during 28 days; 1, 2, 3, 4: samples ripened for 7, 14, 21 and 28 days at 4 ^{0}C; 5, 6, 7, 8: samples ripened for 7, 14, 21 and 28 days at 16 ^{0}C; 9: Molecular weight standard; XDH/OX: Xanthine dehydrogenase/oxidase; CD36: Cluster of differentiation 36; BTN: Butyrophilin; ADPH: Adipophilin; BSA: Bovine serum albumin.

Table 2. The change of polypeptide composition of Kajmak during ripening at different temperature.

	Ripening temperature							
	4 °C				16 °C			
	Days of ripening				Days of ripening			
	7	14	21	28	7	14	21	28
SUM HMWF	8.16^f	12.25^e	12.56^e	15.00^b	13.69^d	12.15^e	15.56^a	14.33^c
α_s-CN	36.12^a	35.68^a	36.77^a	36.70^a	30.86^b	29.01^c	27.29^e	28.20^d
β-CN	25.81^a	23.68^c	22.42^d	22.87^d	24.83^b	21.80^e	19.53^g	20.57^f
κ-CN	5.75^b	5.38^b	6.33^a	5.02^c	6.55^a	n.d.^{d}	n.d.^{d}	n.d.^{d}
SUM LMWP	24.15^c	23.06^d	20.60^e	19.40^f	24.06^c	32.82^b	35.65^a	35.32^a

Means in the same row followed by different letters were significantly different ($p < 0.05$); HMWF: high molecular weight fraction; LMWP: low molecular weight polypeptides; n.d.-not detected.

In opposite to the ripening at 16 ^{0}C, ripening of Kajmak at 4 ^{0}C caused much slower proteolysis of the main caseins. In fact, no significant ($p < 0.05$) changes between relative contents of α-CN of 7-days-old and 28-days-old samples ripened at 4 ^{0}C were detected. In contrast to α_s-CN, the ratio of β-CN after 28 days decreased to 22.87% (Table 2). Furthermore, κ-CN was much more resistant to proteolysis at low temperature than at 16 ^{0}C. This casein was detected in all samples ripened at low temperature; its concentration was in the range of 5.75% (7-day-old sample) to 5.02% (28-days-old sample).

Due to several specifics of Kajmak production including the use of severe heat treatment, the absence of rennet enzymes and the absence of starter-culture addition, slow proteolysis during ripening could be expected. Namely, milk is usually boiled for one hour and slowly cooling during 24 h. Such long-heat and slow-cooling processes significantly reduce non-starter lactic bacteria (NSLAB), favor formation of WPC-CN complexes and induce the release of heat- stable plasmin. The WPC-CN complexes which are partly incorporated in Kajmak (Radovanovic et al., 2020) are more resistant to proteolysis than individual ones (Barac et al., 2019). Furthermore, it is known that native and denatured whey proteins are resistant to proteolysis (Huppertz et al., 2006).

According to the results of this study it seems that proteolysis at 16 ^{0}C occurred due to the activity of residual indigenous milk NSLAB, NSLAB which originate from environment and probably the low activity of plasmin. However, due to unfavorable low pH of these samples (4.88-4.51, Table 1), according to Larsson et al. (2006) any activity of plasmin can hardly be expected. In opposite, at low temperature (4 ^{0}C) the slow proteolysis of Kajmak is mainly based on the activity of plasmin which preferably hydrolyzed β-CN (Ardö et al., 2017). This is supported by the absence of significant changes of the α_s-CN content, more favorable pH values (Table 1) of these samples and the fact that at 4 ^{0}C about 20% of plasmin activity retained (Maćej et al., 2007). In addition, the activity of plasmin and the absence of rennet enzyme could be the main reasons of different resistance of α_s-CN and β-CN during ripening of Kajmak compared to ripening of cheeses.

As a result of hydrolysis of caseins, during ripening the relative content of HMWF increased up to about 15%. As previously mentioned this fraction is mostly composed of MFGM proteins. In recent years, these proteins are increasingly considered as a potential nutraceuticals due to their antiviral, antibacterial, anticancer and other activities (Kosmerl et al., 2021). According to the result of this study, these proteins appear to be resistant to the action of proteolytic enzymes probably due to their incorporation into milk fat globule which make them less available to enzymes.

3.3 Fatty acid profiles and health fatty indices of Kajmak

The change of fatty acid profiles and healthy fatty acid indices during ripening of Kajmak is shown in Tables 3-4.

Under experimental conditions used in this study, depending on the ripening conditions in Kajmak samples, up to twenty seven fatty acids were detected; 14 were saturated and 13 belong to unsaturated fatty acids (MUFAs and PUFAs).

In general, investigated parameters (temperature and duration of ripening) affected fatty acid profiles of Kajmak. Qualitative and quantitative differences could be observed even after 7 days of ripening. Qualitatively, these differences are reflected through the presence or the absence of some of SFAs and especially those which belong to unsaturated fatty acids. More precisely, in 7-day-old sample ripened at 4 ^{0}C less common SFAs were not detected, including C11:0, C13:0, C21:0 and C24:0 as well as several MFAs (C14:1- C17:1) and PUFAs such as α-linolenic, C20:3n6cis-8,11,14-eicosatrienoic and C20:3n3cis-11,14,17-eicosatrienoic (Table 3). As in the most other dairy products, 7-day-old Kajmak samples were dominated by SFAs. These fatty acids represented 72.92% and 69.99% of 7-day-old samples ripened at 4 ˚C and 16 ˚C, respectively. Three of them, C14:0, C16:0 and C18:0 were the most abundant in sample ripened at 4 ^{0}C whereas the 7-days-old Kajmak ripened at 16 ^{0}C had no stearic acid (C18:0).

Despite the slightly higher content, the seven-day-old Kajmak ripened at 4 ^{0}C had a more favorable SFA composition compared to the counterpart matured at a higher temperature. This sample contained more short-chain (5.07%) and long-chain fatty acids (44.35%) and less undesirable MCSFAs such as C12:0 and C14:0 than 7-days-old sample ripened at 16 ^{0}C (Table 3). It is known that short-chain fatty acids such as C4:0 and C6:0 as well as some of the medium-chain fatty acids (C8:0, C10:0) are easily digestible, easily hydrolyzed from triglycerides and reach the body directly and circulate without re-synthesis of triglycerides (Sampelayo et al., 2007). Therefore, these fatty acids contribute to the reduction of triglycerides and cholesterol in serum and liver. In addition, butyric acid has antimicrobial properties (Huang et al., 2011), while *in vitro* studies have shown that it stimulates the proliferation of normal colon cells, and can inhibit the growth and proliferation of some cancer cell lines (Blank-Porat et al., 2007; Aluko, 2012). Also, research by Nair et al. (2005) showed that caprylic and capric acid have antiviral and antimicrobial activity. On the other hand, the lower content of short and medium chain fatty acids in the sample matured at higher temperature suggests more intensive lipid metabolism and higher degree of lipolysis induced by residual indigenous bacteria. These acids are known to be more accessible to bacterial enzymes and are precursors of cheese-like flavor compounds (Fox et al., 2017; Tekin & Güler, 2019). Thus, it can be assumed that at higher ripening temperature induces faster formation of Kajmak aroma compounds.

In opposite to SFAs, 7-days-old sample ripened at 16 ^{0}C had more USFAs (30%) and more favorable composition of these fatty acids compared to 7-days-old sample ripened at 4 ^{0}C (25.71%, Table 4). It is evident from Table 4 that sample ripened at higher temperature contained more MUFAs and almost twice higher content of PUFAs mainly due to the relatively high content of linoleic acid (2.92%) which is considered to be a precursor of several metabolites (eicosanoids, thromboxanes, prostacyclins, prostaglandins and leukotrienes) which are associated with immune and inflammatory responses (Viana et al., 2020). In addition, the most common MUFA in both samples was C18:1, but while in sample ripened at low temperature dominated *cis*- form (oleic acid) the ratio of *cis*- and *trans*- isomers was 13.24% and 9.06%, respectively (Table 3). Altered content of

Table 3. The change of fatty acid composition during ripening of Kajmak at two different temperatures.

Fatty acid	Fatty acid content (%)							
	Temperature of ripening							
	4 °C				16 °C			
	Ripening time (Days)							
	7	14	21	28	7	14	21	28
C4:0 (butiric)	2.48^{a}	2.58^{a}	2.20^{b}	2.10^{b}	0.96^{c}	0.95^{c}	0.75^{d}	0.92^{c}
C6:0 (caproic)	2.59^{a}	2.61^{a}	2.38^{b}	2.3^{b}	1.01^{c}	1.03^{c}	0.95^{c}	1.13^{c}
C8:0 (caprilic)	1.39^{a}	1.39^{a}	1.25^{a}	1.23^{a}	0.91^{b}	0.95^{b}	0.82^{b}	0.97^{b}
C10:0 (capric)	3.04^{a}	3.04^{a}	2.68^{b}	2.64^{b}	2.80^{b}	2.95^{a}	2.48^{c}	2.87^{b}
C11:0 (undecanoic)	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.27^{b}	0.35^{b}	0.30^{b}	0.42^{a}
C12:0 (lauric)	3.36^{c}	3.33^{c}	2.95^{d}	2.93^{d}	5.37^{a}	5.65^{a}	4.73^{b}	5.49^{a}
C13:0 (tridecanoic)	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.13^{a}	0.13^{a}	0.13^{a}	0.14^{a}
C14:0 (miristic)	15.05^{e}	15.54^{d}	15.14^{e}	15.18^{e}	17.85^{b}	18.54^{a}	15.78^{c}	18.25^{a}
C15:0 (pentadecanoic)	0.66^{c}	0.71^{c}	0.89^{c}	0.88^{c}	1.12^{b}	1.24^{b}	1.09^{b}	2.23^{a}
C16:0 (palmitic)	34.49^{d}	34.59^{d}	36.29^{c}	36.42^{c}	38.01^{b}	38.51^{a}	32.89^{e}	n.d.^{f}
C17:0 heptadecanoic	0.43^{e}	0.42^{e}	0.45^{e}	0.45^{e}	0.74^{c}	0.91^{b}	0.59^{d}	2.93^{a}
C18:0 (stearic)	9.86^{a}	9.95^{a}	9.16^{b}	9.18^{b}	n.d.^{d}	n.d.^{d}	n.d.^{d}	0.54^{c}
C21:0 (henicosanoic)	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.28^{b}	0.24^{b}	0.24^{b}	0.80^{a}
C24:0 (lignoceric)	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.54^{b}	n.d.^{c}	n.d.^{c}	0.71^{a}
C14:1(miristoleic)	n.d.^{b}	n.d. ^{b}	n.d.^{b}	n.d.^{b}	0.65^{a}	0.72^{a}	0.72^{a}	n.d.
C15:1 (cis-10-pentadecanoic)	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.70^{b}	0.72^{b}	0.61^{b}	0.94^{a}
C16:1 (palmitoleic)	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	1.61^{b}	1.76^{b}	1.50^{b}	20.03^{a}
C17:1 (cis-10-heptadecanoic)	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.21^{b}	n.d.^{c}	0.15^{b}	0.44^{a}
C18:1n9c (oleic)	22.07^{a}	21.32^{a}	22.05^{a}	21.99^{a}	13.24^{b}	13.37^{b}	4.17^{c}	4.41^{d}
C18:1n9t (elaidic)	1.62^{c}	1.60^{c}	1.81^{c}	1.82^{c}	9.06^{a}	7.78^{b}	n.d.^{d}	n.d.^{d}
C18:2n6c (linoleic)	1.79^{d}	1.67^{d}	1.52^{d}	1.59^{d}	2.92^{c}	2.82^{c}	28.06^{b}	31.49^{a}
C18:2n6t (linoleaidic)	0.11^{c}	0.11^{c}	0.11^{c}	0.16^{c}	n.d.^{d}	n.d.^{d}	2.66^{b}	3.11^{a}
C18:3n3 (α-linoleic)	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.20^{a}	0.15^{a}	0.29^{a}	n.d.
C20:1n9cis-11-eicosenoic	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.58^{a}	0.54^{a}	0.28^{b}	0.30^{b}
C20:2cis-11,14-eicosadienoic	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.65^{ca}	0.39^{b}	0.40^{b}	0.56^{a}
C20:3n6cis-8,11,14-eicosatrienoic	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.19^{a}
C20:3n3cis-11,14,17-eicosatrienoic	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.19^{a}	n.d.^{b}	n.d.^{b}	n.d.^{b}
C20:4n6arachidonic	0.12^{c}	0.14^{c}	n.d.^{d}	n.d.^{d}	n.d.^{d}	0.31^{b}	0.40^{b}	0.59^{a}
C20:5n3cis-5,8,11,14,17-eicosapentaenoic	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.07^{a}
C22:2cis-13,16-docosadienoic	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.45^{a}

Means in the same row followed by different letters were significantly different ($p < 0.05$); n.d.: not detected.

Table 4. The change of health fatty acid indices during ripening of Kajmak.

	Ripening temperature							
	4 °C				16 °C			
	Days of ripening				Days of ripening			
	7	14	21	28	7	14	21	28
SUM SCSFA C4:0-C6:0	5.07^{a}	5.19^{a}	4.58^{b}	4.41^{b}	1.97^{c}	1.98^{c}	1.70^{d}	2.05^{c}
SUM MCSFA C8:0-C15:0	23.50^{f}	24.01^{e}	22.91^{g}	22.86^{g}	28.45^{c}	29.81^{b}	25.33^{d}	30.37^{a}
LCSFA C16:0-C24:0	44.35^{b}	44.54^{b}	45.45^{a}	45.6^{a}	39.57^{c}	39.66^{c}	33.72^{d}	4.98^{e}
SUM SFA	72.92^{b}	73.74^{a}	72.94^{b}	72.87^{b}	69.99^{d}	71.45^{c}	60.75^{e}	37.40^{f}
SUM MUFA	23.69^{c}	22.92^{d}	23.86^{c}	23.81^{c}	26.04^{a}	24.89^{b}	7.43	26.12^{a}
SUM PUFA	2.02^{d}	1.92^{d}	1.63^{e}	1.75^{e}	3.96^{c}	3.67^{c}	31.81^{b}	36.46^{a}
SUM UFA	25.71^{e}	24.84	25.49^{e}	25.56^{e}	30.00^{c}	28.56^{d}	39.24^{b}	62.58^{a}
DFA	35.57^{c}	34.79^{d}	34.65^{d}	34.74^{d}	30.00^{e}	28.56^{f}	39.24^{b}	63.12^{a}
OFA	52.90^{e}	53.46^{d}	54.38^{c}	54.53^{c}	61.23^{b}	62.7^{a}	53.40^{d}	23.74^{f}
DFA/OFA	0.67^{c}	0.65^{c}	0.65^{c}	0.64^{c}	0.49^{d}	0.45^{d}	0.73^{b}	2.66^{a}
AI	3.81^{d}	4.03^{b}	3.91^{c}	3.91^{c}	3.83^{d}	4.14^{a}	2.57^{e}	1.25^{f}
TI	4.62^{c}	4.92^{a}	4.75^{b}	4.75^{b}	3.52^{e}	3.90^{d}	2.41^{f}	0.61^{g}

Means in the same row followed by different letters were significantly different ($p < 0.05$); AI: atherogenicity index; TI: thrombogenicity index; DFA: desirable fatty acids; OFA: hypercholesterolemic fatty acids; SCSFA- short chain saturated fatty acids; MCSFA- medium chain saturated fatty acids; LCSFA- long chain saturated fatty acids; SFA- saturated fatty acids; UFA- unsaturated fatty acids; PUFA- polyunsaturated fatty acids; MUFA- monounsaturated fatty acids.

cis- and *trans*- form of C18:1, the presence of a number of less represented monounsaturated (C15:1- C17:1) and several PUFAa as well as the absence of stearic acid in sample ripened at 16 ^{0}C suggest a 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 stearoyl-CoA desaturase-1 which primarily uses stearic acid as a substrate but can also convert other SFAs (Czumaj & Śledziński, 2020). PUFAs are result of the reactions catalyzed by another group of desaturases, unsaturated fatty acid desaturases. At 16 ^{0}C processes of desaturation of both saturated and monounsaturated fatty acids take place over 28 days. The most intensive transformations are registered during last 14 days of ripening. As a result, the content of SFAs after this period decreased up to 30.7% whereas USFAs due to the high content of PUFAs dominated (36.46%, Table 4). Similar effect of LAB from kefir grains on fatty acid profiles of milk was reported by Vieira et al. (2015). The most abundant PUFA in 21- and 28-days-old Kajmak was linoleic acid; their content was 28.06% and 31.49%, respectively (Table 3). In addition, 28-days-old Kajmak contained high percent of palmitoleic acid (20.03%; Table 3). Palmitoleic acid can be result of the desaturation of palmitic acid induced by Δ9-stearoyl-ACP desaturase (Hernández et al., 2016). It has been reported that palmitoleic acid has beneficial effects on insulin sensitivity, cholesterol metabolism, and hemostasis. Also, it was suggested that palmitoleic acid may prevent beta-cell apoptosis induced by glucose or saturated fatty acids (Morgan & Dhayal, 2010). Since unsaturated fatty acids are considered as much healthier than SFAs and that PUFAs are related do several health benefits (Mollica et al., 2021) it is obvious that longer ripening period (longer than 14 days) greatly improved health fatty acid indices of Kajmak. Indeed, 28-days-old Kajmak had more favorable values of AI, TI and DFA/OFA ratio compared to those of less-time ripened samples as well as those of samples ripened at 4 °C. Furthermore, AI and TI value of this sample (1.25 and 0.61) were better or in the range of those for numerous different dairy products summarized by Chen & Liu (2020).

In contrast to maturation at 16 ^{0}C, maturation at low temperature for 28 days did not cause significant changes in terms of fatty acid content and composition. Thus, the observed fatty acid profiles and the ratio of specific fatty acids were quite similar. The absence of significant metabolitic transformation of fatty acid at low temperature was in good agreement with slow proteolysis observed in these samples (Figure 1, Table 2). Similar trends were observed by Frau et al. (2021) during refrigerated storage of artisanal goat cheese. As a result of slow metabolitic transformation of fatty acids, samples ripened at low temperature are characterized with similar health fatty acid indices. For example, AI and TI indexes were in the range of 3.81-4.03 and 4.62-4.92 (Table 4).

Based on the results obtained in this study regarding composition, fatty acid and protein profiles it is obvious that the temperature of ripening significantly affects nutritive characteristics of Kajmak. From the nutritional point of view ripening at higher temperature (16 ^{0}C) is more favorable compared to ripening at 4 ^{0}C. Due to more intensive lipolytic and proteolytic changes Kajmak samples ripened for 28 days at 16 ^{0}C had lower fat content (50.08%, Table 1), higher ratio of UFAs (62.58, Table 4) and higer level of easily digestible LMW peptides (35.32%, Table 2) and MFGM proteins.

4 Conclusion

The results of this study clearly showed that the ripening conditions (temperature and ripening time) differently affected fatty acid and protein profiles of Kajmak. Due to unfavorable conditions for the activity of indogenic LAB, ripening at low temperature (4 ^{0}C) induces slow proteolysis and fatty acid metabolism compared to the ripening at 16 ^{0}C. Fatty acid profiles of samples ripened during 28 days in refrigerator were similar whereas decrease of only β-CN was detected. In opposite, strong desaturation activity of indigenous bacteria increased unsaturated fatty acid contents and improved health fatty acid indices.

Acknowledgements

The authors wish to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support of the research presented in this article (Grant no. 451-03-68/2022-14/200116).

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