CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage, **http,//chimie-biologie.ubm.ro/carpathian_journal/index.html**

IMPACT OF REFRIGERATED CURD ON KASHKAVAL QUALITY I. CHEMICAL CHARACTERISTICS

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https://doi.org/10.34302/crpjfst/2021.13.2.5

Article history:
Received,
17 January 2021
Accepted,
25 May 2021

Keywords:
Cagliata cheese;
Pasta filata cheese;
Proteolysis;
Oxidation;
Biogenic amines.

ABSTRACT
The present study examined the impact of refrigerated curd (1 °C, 2 months), so called "Cagliata" on Kashkaval cheese chemical characteristics. Changes of cheese components during ripening as well as proteolysis, biogenic amines formation, fatty acid profile, lipolysis and oxidation of milk fat were investigated. Kashkaval cheese samples were characterized by similar dry matters and protein contents but the results obtained for the components such as milk fat, minerals, salt and active acidity were significantly different ($P<0.05$). A more pronounced proteolysis was found in the cheese produced from refrigerated curd but no accumulation of biogenic amines in both analyzed samples was established. A greater lipolysis and oxidation of milk fat was observed in the cheese obtained from fresh milk. Cheese produced from refrigerated curd could be successfully used as a cheaper alternative of traditional Kashkaval or when a fresh curd is not available without compromising on its quality.

1. Introduction

In recent years, technologies are expanding, in order to meet the increased demands of the consumer for a safe, high-quality and affordable product. This can be done by value-added products as cheese supplemented with different compounds (Bukvicki *et al.*, 2018; Ullah *et al.*, 2018; El-Sayed *et al.*, 2020), cost-effective technologies (Simov and Ivanov, 2005; Marcuzzo *et al.*, 2012; Beykont and Kilic-Akyilmaz, 2013; Sharma *et al.*, 2018; Alinovi *et al.*, 2020a; 2020b) or innovative technologies (Johnson, 2017; Cabral *et al.*, 2019; Alinovi and Mucchetti, 2020a; 2020b).

In individual European countries, the production of raw cow milk varies widely (Bórawski *et al.*, 2020). There are producers on the market from the regions generating more quantities of cow milk at a cheaper price. They offer quality intermediate semi-finished product or so-called "Cagliata", which can be used as a raw material for the production of various type of cheese. This is an opportunity for manufacturers in countries, where milk is at high cost, to obtain a product with characteristics similar to the traditional product, but at a lower price.

The storage of raw materials in a refrigerated or frozen state is a common approach in food industry in order to extend their shelf life (Prakash, 2018). Different studies suggested the use of frozen milk or deep frozen curd as alternative to produce different kind of cheeses as Teleme cheese (Alichanidis *et al.*, 1981), soft caprine cheese (Van Hekken *et al.*, 2005), sheep cheese (Zhang *et al.*, 2006; Pazzola et al., 2013; Fava *et al.*, 2014), Mozzarella from buffalo milk (Manzo *et al.*, 2017), white brined goat cheese (Kljajevic *et al.*, 2017), Hispánico cheese from ewe milk (Alonso *et al.*, 2012), high-pressure treated raw goat milk curd (Picon *et al.*, 2012) etc. They reported that the production of cheese from frozen curd is possible with some minor changes in their composition and quality.

Kashkaval cheese is a typical hard cheese (Bulgarian National Standard (BNS) 14:2010). It is produced in the region of the Balkans and Eastern Europe. It is frequently associated with the "Pasta filata" cheese production technology because it includes cheddaring, cooking, and stretching of the fresh curd as well as molding and ripening of fresh cheese (Bylund, 2015).

The possibility to obtain cost effective production of Kashkaval by storing it in a frozen state before or after ripening is discussed by Simov and Ivanov (2005). They studied the proteolytic processes in frozen Kashkaval cheese and found enhanced proteolysis during ripening of thawed Kashkaval cheese. To the best of our knowledge any scientific studies about the possibility to obtain Kashkaval cheese from refrigerated curd were not available. Given that, we hypothesized that different raw material than fresh cow milk can represent a cost effective technology for Kashkaval production and similar cheeses, saving the chemical properties of the product.

Therefore, the aim of the present research was to determine the effect of using refrigerated curd on the chemical composition of Kashkaval cheese.

2. Materials and methods

2.1. Materials

Fresh cow milk, meeting the national and European requirements, was obtained by the local farmers supplying milk to the company "Mlechni Producti Trakia" Ltd. - MP "Serdika Haskovo" was used in order to obtain a fresh curd with a studied composition (Table 1). A refrigerated (at 1 °C for 2 months) "Cagliata" curd was used as a raw material supplied by a foreign company with a composition presented in Table 1.

Table 1. Chemical composition of fresh ad refrigerated curd

Component	Refrigerated curd	Fresh curd
Dry matter, %	62.90 ± 1.95^{a}	57.21 ± 1.35^{b}
Milk fat, %	27.5 ± 0.5^{a}	25.5 ± 0.5^{b}
Total protein, %	24.69 ± 1.24^{a}	24.50 ± 1.23^{a}
Ash, %	3.11 ± 0.08^{a}	2.01 ± 0.01^{b}
Milk fat in dry matter, %	43.75 ± 1.22^{a}	44.60 ± 1.04^{a}
pH	5.57 ± 0.01^{a}	5.23 ± 0.05^{b}

[a, b] Means with different letters within a row are significantly different ($p<0.05$)

Starter culture containing *Str. thermophilus*, *Lb. delbrueckii* ssp. *bulgaricus* and *Lb. helveticus* was supplied by LB Bulgaricum Ltd. Calcium dichloride solution (50%) was purchased from Biokom Trendafilov Ltd. Rennet enzyme CHY-MAX® M was delivered by Chr. Hansen. The chemicals and reagents used for analysis were analytical grade and were used without further purification.

2.2. Cheese preparation

2.2.1. Kashkaval cheese produced from fresh curd

Kashkaval cheese was prepared from two batches of cow milk. It was produced in the company "Mlechni Producti Trakia" Ltd. - MP "Serdika Haskovo". A classic production process included the following technological operations (Ivanova *et al.*, 2020): cow milk qualification; clarification and standardisation of milk fat (at 35-45 °C) in order to achieve casein to fat content ratio 0.70; thermisation (at 63 ± 2 °C for 15-20 s); cooling (at 33 ± 1 °C); addition of starter culture in amount of 1%, calcium dichloride solution (50%) in amount of 30 cm^3 per 100 dm^3 of milk (previously diluted in water in 1:10 ratio) and rennet (previously diluted in water in 1:10 ratio) in such amount that the coagulation started 10-12 min after enzyme addition and a set coagulum was formed after 45 min; cutting the obtained coagulum at two stages - into 3-4 cm grains and after 5-10 min into 6-8 mm grains; stirring and stabilizing the grains for 5-15 min; heating the formed grains (at 40-42 °C for 40-60 min); draining (separated whey titratable acidity 16-24 °T); collecting of curd for pressing (6 kg weight for 1 kg curd for 15-20 min, when pH reached 5.8-5.9); cutting the curd in 50-60 cm parallelepiped slices; cheddaring (1-1.5 h, pH 5.2-5.3); milling of the curd (slices with length of 20-30 cm and width 8-10 mm) and salting in a hot water solution (at 72 °C and 14% salt content); forming in 0.5 kg mould; stabilizing the forms by 3-4 turnings of the moulds; drying the fresh cheese (at 8-10 °C for 15 h); unmoulding and drying the cheese (at 6-8 °C for 2 days); packing and ripening (at 8-10 °C and relative humidity *80-85% for 60 days).*

2.2.2. Kashkaval cheese produced from refrigerated curd

Kashkaval cheese was prepared from two batches of refrigerated “Cagliata” curd. It was produced in the company "Mlechni Producti Trakia" Ltd. - MP "Serdika Haskovo". The production process included the following technological operations: refrigerated curd qualification; cutting the curd in slices; milling of the curd and salting in a hot water solution (at 72 °C and 14% salt content); forming in 0.5 kg mould; stabilizing the forms by 3-4 turning of the moulds; drying fresh cheese (at 8-10 °C for 15 h); unmoulding and drying (at 6-8 °C for 2 days); packing and ripening (at 8-10 °C and relative humidity 80-85% for 60 days).

2.3. Analysis

2.3.1. Chemical analysis

Determination of cheese dry matter – Bulgarian National Standard (BNS) EN ISO 5534:2005; Determination of cheese fat content ISO 3433:2008; Determination of fat in dry matter – empirical calculation; Determination of cheese total protein content - BNS EN ISO 8968-1:2014; Determination of water soluble nitrogen (WSN), noncasein nitrogen (NCN), nonprotein nitrogen (NPN) and total nitrogen (TN) content - Vakaleris and Price (1959) with some slight modifications by Ivanova et al. (2021); Determination of biogenic amines – Ivanova et al. (2021); Determination of cheese ash content - BNS 6154:1974; Determination of cheese salt (NaCl) content – BNS 8274:1982; Determination of salt in moisture – empirically calculated; Determination of cheese fat fatty acids – milk fat extraction - ISO 1735/IDF 5:2004, preparation of methyl esters of fatty acids - ISO 5509:2001, chromatographic analysis of methyl esters of the fatty acids - ISO 5508:2000; Determination of fat indices - milk fat extraction – Bligh and Dyer (1959), peroxide value determination - ISO EN 3960:2008, acid value determination - ISO EN 660:2009; Potentiometric determination of active acidity (pH).

2.3.2. Statistical analysis

Statistical processing of obtained results was performed by Microsoft Excel 2010 according to one-way ANOVA method for analysis of variance. Multiple comparisons were made with the LSD (Least Significant Difference) procedure. Results were presented as the mean ± SD (standard deviation) of four replicates (n=4) and were considered significantly different when $P<0.05$.

3. Results and discussions

3.1. Chemical composition

As shown in Table 2 Kashkaval produced from refrigerated curd differed from the same produced from a fresh curd by some chemical parameters.

Despite the difference with the dry matter of the raw materials, no statistically significant difference ($P>0.05$) was observed of this component in the analyzed samples during the repining period. This can be explained by the higher hydrophilicity of the proteins in the refrigerated curd and their ability to absorb more water than the proteins in the fresh curd during the salting process in the cooker-stretcher production unit. Kljajevic *et al*. (2017) suggested that heating the curds in whey was an attempt to rehydrate proteins after thawing frozen curd. Our study showed that when cheese was produced from refrigerated curd, milk casein kept its ability to retain water – an effect clearly demonstrated after heating the curd in the salting solution. This property of the milk protein was lost when the cheese was produced from frozen curd and significant water losses were reported (Alichanidis *et al*., 1981). Greater fat losses were obtained in the sample with refrigerated curd compared to the control ($P<0.05$). It was likely that the protein-fat matrix in the curd obtained from chilled curd was less dense and had a reduced ability to retain milk fat. Similar explanation was given by Kljajevic *et al.* (2017) who suggested that proteins, damaged during frozen (refrigerated) storage, released more fat into whey (salting solution) if the curd was not pressed before freezing (refrigeration), because a significant whey separation occurred after thawing (tempering the curd in the production unit). No statistical difference in fat content during ripening period was observed in the both samples ($P>0.05$). The salt content in the experimental sample produced from chilled curd was higher than the control one. This phenomenon could be explained by the higher initial pH of this curd as well as its lower fat content (Kozhev, 2006). This tendency continued until the end of the ripening period of the cheese. According to Kozhev (2006) the salt dissolved in the water phase was continuously redistributed in the colloidal bound water during ripening which explained the decrease in the salt in moisture content. The active acidity of the control sample was found to be lower than the experimental one which was due to the lower initial pH of the fresh curd. Increased active acidity in the beginning of maturation was explained by the partial separation of significant part of the lactic acid present in the curd after cheddaring. This tendency persisted during the ripening process despite the lactose fermentation process, because protein proteolysis took place and its products increased cheese buffer capacity. Similar results were obtained for cheese produced from frozen curd (Alichanidis *et al*., 1981; Kljajevic *et al*., 2017).

Table 2. Chemical composition of Kashkaval from refrigerated and fresh curd

Component	Kashkaval from refrigerated curd		Kashkaval from fresh curd	
	Beginning of ripening	End of ripening (60 days)	Beginning of ripening	End of ripening (60 days)
Dry matter, %	57.57 ± 2.67^a	57.33 ± 3.50^a	59.89 ± 2.08^a	58.21 ± 1.99^a
Milk fat, %	25.0 ± 0.5^a	24.0 ± 0.5^a	27.0 ± 0.3^b	27.5 ± 0.3^b
Total protein, %	24.37 ± 1.22^a	24.75 ± 1.24^a	24.56 ± 1.23^a	24.63 ± 1.23^a
Ash, %	4.64 ± 0.03^a	4.40 ± 0.01^b	3.93 ± 0.02^c	3.83 ± 0.01^d
Salt, %	2.8 ± 0.1^a	2.6 ± 0.1^a	2.3 ± 0.1^b	2.2 ± 0.1^b
Salt in moisture, %	6.73 ± 0.26^c	2.6 ± 0.1^a	5.93 ± 0.36^c	2.2 ± 0.1^a
pH	5.80 ± 0.00^a	5.90 ± 0.11^a	5.87 ± 0.05^b	5.86 ± 0.00^b

$^{a\text{-}d}$ Means with different letters within a row are significantly different ($P<0.05$)

3.2. Proteolytic processes

The proteolytic processes in the analyzed samples are represented in Table 3.

Table 3. Proteolytic processes of Kashkaval from refrigerated and fresh curd

Component	Kashkaval from refrigerated curd		Kashkaval from fresh curd	
	Beginning of ripening	End of ripening (60 days)	Beginning of ripening	End of ripening (60 days)
†TN, mgN/g	3.82 ± 0.19[a]	3.88 ± 0.20[a]	3.85 ± 0.19[a]	3.86 ± 0.19[a]
WSN, mgN/g	0.40 ± 0.02[a]	0.48 ± 0.03[b]	0.32 ± 0.02[c]	0.36 ± 0.02[a]
NCN, mgN/g	0.38 ± 0.02[a]	0.46 ± 0.02[b]	0.29 ± 0.02[c]	0.33 ± 0.02[d]
NPN, mgN/g	0.28 ± 0.02[a]	0.34 ± 0.02[b]	0.10 ± 0.01[c]	0.18 ± 0.01[d]
WSN/TN, %	10.47 ± 0.53[a]	12.37 ± 0.62[b]	8.31 ± 0.42[c]	9.33 ± 0.47[a]
NCN/TN, %	9.82 ± 0.49[a]	11.92 ± 0.60[b]	7.47 ± 0.37[c]	8.42 ± 0.42[d]
NPN/TN, %	7.20 ± 0.36[a]	8.76 ± 0.44[c]	2.60 ± 0.13[c]	4.66 ± 0.23[d]
Putrescine, mg/kg	0	0	0	0.73 ± 0.01[NS]
Cadaverine, mg/kg	0	0	0	1.35 ± 0.02[NS]
Histamine, mg/kg	0	0	0	0
Tyramine, mg/kg	0	3.70 ± 0.01[NS]	0	2.13 ± 0.01[NS]

[a-d] Means with different letters within a row are significantly different ($P<0.05$)

[NS]Non Significant (values below 10 mg/kg are below the limit of quantification (LOQ), ie. they are beyond the accuracy of the standard curve); †TN – total nitrogen; WSN/TN - water-soluble nitrogen in total nitrogen; NCN/TN - noncasein nitrogen in total nitrogen; NPN/TN - nonprotein nitrogen in total nitrogen.

It was known that the process of proteolysis started during curd cheddaring (Kozhev, 2006; Kalit *et al*., 2016). Significant and more pronounced processes of proteolysis in Kashkaval produced from refrigerated curd were observed. This was despite the equal dry matter (water content respectively) of both cheeses and the higher salt in moisture content in the cheese produced from refrigerated curd. Some of the potential explanations could be changes in casein structure during refrigerated storage which made it more easily attacked by proteolytic enzymes. The higher pH of the refrigerated curd which represented a more favorable medium for the activity of these enzymes could be another possible justification. Alichanidis *et al*. (1981) and Picon et al. (2012) reported the same tendency of faster proteolysis for cheese produced from frozen curd. Although the accelerated rate of proteolysis it was found no significant differences in the quantity of biogenic amines accumulated in both cheeses.

3.3. Lipolytic processes

The fatty acid composition of obtained cheeses is presented in Table 4.

The fatty acids in Kashkaval cheese were formed not only by the hydrolysis of milk fat, but also by the fermentation of lactose and the desamination of amino acids (Kozhev, 2006).

Manzo *et al*. (2017) found no effect on the cheese fatty acid concentrations after the freezing storage of the curd. Our results confirmed this statement and suggested that the refrigeration of the curd did not affect Kashkaval traditional fatty acid profile described by Ivanova *et al*. (2020). Some individual differences between fatty acids were observed but not for the total amount of saturated and unsaturated fatty acids. This was probably due to the different raw materials for the preparation of two type of cheese.

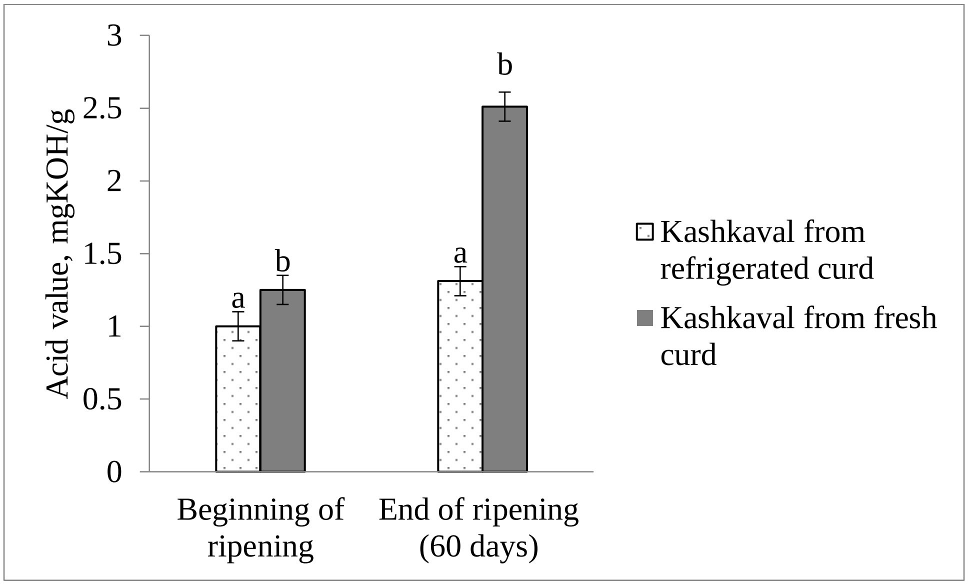
The lipolysis and oxidation of milk fat are presented in Figure 1 (A) and (B).

Table 4. Fatty acid profiles of Kashkaval from refrigerated and fresh curd

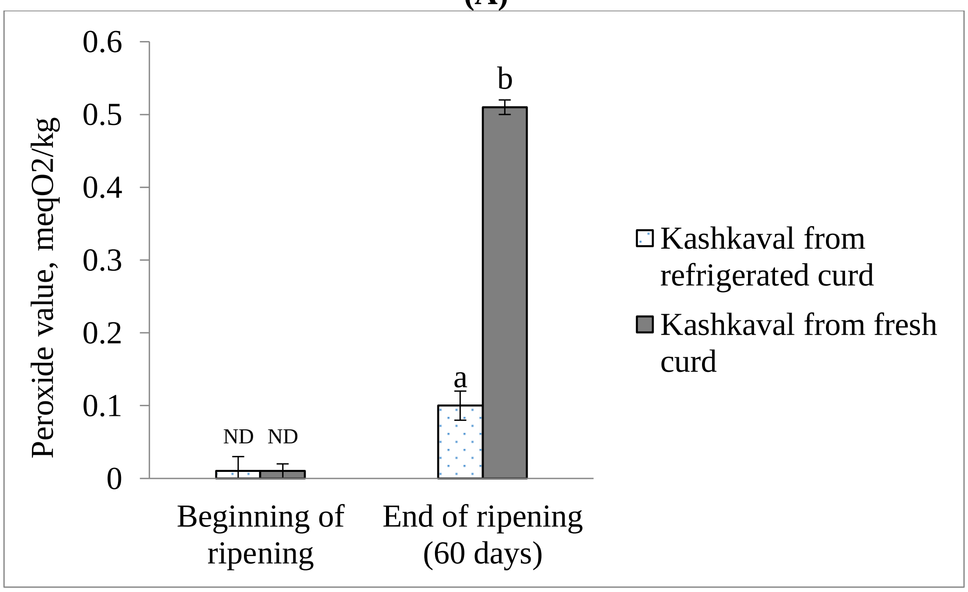
Fatty acid, g/100 g extracted fat	Kashkaval from refrigerated curd		Kashkaval from fresh curd	
	Beginning of ripening	End of ripening (60 days)	Beginning of ripening	End of ripening (60 days)
C4:0	1.22 ± 0.05^{a}	0.87 ± 0.02^{b}	0.91 ± 0.01^{b}	1.04 ± 0.02^{c}
C6:0	1.32 ± 0.0^{4a}	0.99 ± 0.03^{b}	0.98 ± 0.02^{b}	1.00 ± 0.04^{b}
C8:0	3.60 ± 0.09^{a}	2.89 ± 0.09^{a}	2.61 ± 0.07^{b}	2.66 ± 0.08^{b}
C10:0	4.80 ± 0.08^{a}	4.22 ± 0.08^{b}	3.30 ± 0.06^{c}	3.28 ± 0.04^{c}
C11:0	0.19 ± 0.02^{a}	0.20 ± 0.01^{a}	0.09 ± 0.00^{b}	0.13 ± 0.05^{c}
C12:0	13.49 ± 0.93^{a}	12.86 ± 1.10^{a}	11.98 ± 0.90^{a}	11.68 ± 0.99^{a}
C13:0	0.56 ± 0.08^{a}	0.47 ± 0.07^{a}	0.71 ± 0.05^{b}	0.73 ± 0.06^{b}
C14:0	2.22 ± 0.15^{a}	2.35 ± 0.08^{a}	2.24 ± 0.07^{a}	2.28 ± 0.09^{a}
C14:1	0.21 ± 0.01^{a}	0.19 ± 0.05^{a}	0.44 ± 0.09^{b}	0.34 ± 0.06^{b}
C16:0	36.57 ± 1.00^{a}	34.16 ± 1.10^{b}	32.45 ± 1.11^{c}	30.20 ± 1.17^{d}
C16:1	2.20 ± 0.07^{a}	2.13 ± 0.08^{a}	2.05 ± 0.06^{a}	1.60 ± 0.10^{b}
C17:0	0.73 ± 0.02^{a}	0.57 ± 0.02^{b}	0.92 ± 0.03^{c}	0.89 ± 0.02^{c}
C17:1	0.34 ± 0.01^{a}	0.13 ± 0.01^{b}	0.26 ± 0.02^{c}	0.23 ± 0.03^{c}
C18:0	10.58 ± 1.00^{a}	10.96 ± 0.09^{a}	13.74 ± 1.12^{b}	13.01 ± 0.90^{b}
C18:1	19.4 ± 1.10^{a}	20.94 ± 1.30^{a}	23.33 ± 1.20^{b}	21.75 ± 1.13^{a}
C18:2	1.69 ± 0.04^{a}	2.79 ± 0.04^{b}	2.35 ± 0.05^{c}	5.16 ± 0.07^{d}
C18:3	ND	1.68 ± 0.04^{a}	ND	1.82 ± 0.05^{b}
C20:0	0.53 ± 0.01^{a}	0.45 ± 0.02^{b}	0.79 ± 0.02^{c}	0.76 ± 0.03^{c}
C20:1	0.35 ± 0.01^{a}	1.17 ± 0.01^{b}	0.85 ± 0.04^{c}	1.45 ± 0.05^{d}
Saturated fatty acids	75.81 ± 3.57^{a}	70.99 ± 3.14^{a}	70.72 ± 3.90^{a}	67.66 ± 3.48^{a}
Unsaturated fatty acids	24.19 ± 2.11^{a}	29.03 ± 2.80^{a}	29.28 ± 2.91^{a}	32.35 ± 2.48^{a}

$^{a-d}$ Means with different letters within a row are significantly different ($p<0.05$)

ND Not Detected



(A)



(B)

[a-b] Means with different letters within columns are significantly different ($p<0.05$)

[ND] Not Detected

Figure 1. Acid (A) and peroxide (B) values of Kashkaval milk fat extracted from refrigerated and fresh curd

Lipolytic processes were significantly less pronounced than proteolytic processes. This was due to the starter culture which was known to produce more thermoresistant enzymes with proteolytic activity than lipolytic during cheddaring of the curd and not during ripening (Kozhev, 2006). At Figure 1 (A) it can be seen that the lipolysis was more pronounced in the Kashkaval obtained from fresh curd which could be explained by the lower salt content in this product and the more active lactic acid process which took place in the fresh curd. This was inconsistent with the results obtained from Alonso *et al*. (2012) who found that cheese containing frozen curd from pasteurized ewe milk had concentrations of free fatty acids similar to those of the control sample. Probably these differences were due to the type of raw material used (sheep's milk), as well as some technological factors - used starter culture, processed curd, etc. The oxidation processes presented in Figure 1 (B) were not very marked in both cheeses. They were more pronounced in the cheese from fresh curd which correlated to the higher lipolysis established in this sample. Similar results were reported by Picon *et al*. (2012) who established lower levels of free fatty acids in the cheeses produced from frozen goat curd.

4. Conclusions

The obtained results showed that refrigerated curd, so called "Cagliata" cheese, could be successfully used for the production of Kashkaval cheese or similar Pasta filata-type cheeses. However when making cheese from refrigerated curd, it must be taken into account the fact that the cheese produced from this raw material reached earlier the stage of full breakdown of macropeptides to peptides of low molecular weight and aminoacids which was not the case of the control sample where protein was partially hydrolyzed for the same ripening period. Its casein water-bounding ability was more pronounced compared to the control sample. The effects of refrigerated curd application in the production of Kashkaval cheese on the microbiological and sensory profile as well as on its rheological properties during ripening is yet to be evaluated.

Our results demonstrated that the production of cheese from refrigerated curd was possible with some minor changes in their composition but retaining quality characteristics. The use of chilled curd for the production of Kashkaval or similar cheese is an opportunity to achieve significantly lower cost compared to the production of cheese obtained from raw cow's milk in countries where the unit price is higher.

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Acknowledgment

We acknowledge Mariya Dushkova, Liliya Kuneva, Mariana Topchieva and (Lutsian Krastev) for their technical support during this research as well as Mr. Todor Todorov, director of the company "Mlechni Producti Trakia" Ltd. - MP "Serdika Haskovo" for the provided production area for this study.