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# Effect of High Temperature Application on Mineral Matter Levels, Fatty Acids and Protein Fractions of White Cheese

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#### Abstract

The aim of this study was to increase the yield of white cheese by applying high temperature to milk. The average yield was determined as 17.58% in high-temperature white cheese samples and 16.40% in the control white cheese sample. During ripening periods, the average degree of ripening in cheese samples treated with a high temperature and control cheese samples was determined as 17.3% and 18.3%, respectively. It was determined that the average butyric acid amount (1.743%) white cheese samples treated with a high temperature was higher than that of the control samples (1.589%). The reason for the low rate of free fatty acids in this study may be due to the inactivation of the natural lipase enzyme of milk as a result of the high temperature applied to milk. It was determined that as the ripening times increased, the amount of both  $\alpha$ -casein and  $\beta$ -casein decreased, whereas the protein degradation products increased.

Keywords: White cheese, High temperature, Starter culture, Casein fractions, Free fatty acids, Minerals

# Yüksek Sıcaklık Uygulamasının Beyaz Peynirin Mineral Madde, Yağ Asitleri ve Protein Fraksiyonlarına Etkisi

#### Öz

Bu çalışmanın amacı süte yüksek sıcaklık uygulaması yaparak yapılan beyaz peynirin randımanını artırmaktır. Ortalama randıman yüksek sıcaklık uygulanan beyaz peynirlerde % 17.58, kontrol beyaz peynirde ise %16.40 olarak saptanmıştır. Olgunlaşma süresince yüksek sıcaklık uygulaması ve kontrol peynir örneklerinde ortalama olgunlaşma derecesi sırasıyla %17,3 ve %18,3 olarak belirlenmiştir. Yüksek sıcaklık uygulanmış beyaz peynir örneklerindeki ortalama butirik asit miktarının (%1,743) kontrol örneklerinden (%1,589) daha yüksek olduğu belirlenmiştir. Bu çalışmada serbest yağ asitleri oranının düşük olmasının nedeni süte yüksek ısıl işlem uygulanması sonucu sütün doğal lipaz enziminin inaktif olmasından kaynaklanabilir. Olgunlaşma süresi arttıkça  $\alpha$ -kazein ve  $\beta$ -kazein miktarının azalmasına karşılık parçalanma ürünlerinin arttığı belirlenmiştir.

Anahtar Kelimeler: Beyaz peynir, Yüksek sıcaklık, Starter kültür, Kazein fraksiyonları, Serbest yağ asitleri, Mineraller

# 1. Introduction

High temperature application causes protein denaturation and interactions occur between denatured proteins and casein micelles, minerals and fat globules. These interactions have both positive and negative effects on cheese production. While there is an increase in cheese yield according to denatured proteins, the interactions between them and casein micelles, this prevents coagulation and creates a weak clot structure with a long coagulation time (Singh & Waungana, 2001). In a research 5 different pasteurization norms were used and it was observed that norms other than the 10 min norm at 68°C caused an increase in the clotting time of cow's milk. It is estimated that this increase is due to the interaction of  $\beta$ -lactoglobulin and  $\kappa$ -casein, which are denatured by heat, making enzyme-substrate complex formation more difficult and decreasing the concentration of calcium ions (Koçak & Devrim, 1989). Many studies have been made on the use of culture in white cheese production (Karakuş et al., 1992; Tunail, 1999).

The fat loss in whey in cheeses made from pasteurized milk is less than in cheeses made from raw milk (Cankara & Karacaoğlu, 1983). It has been determined that the amount of dry matter passing into the whey is high when raw milk is used in making white cheese, and that the amount of dry matter in the whey is the lowest in cheese pasteurized at 85°C for 15 s and produced by adding 0.03% CaCl<sub>2</sub> (Şakiroğlu & Üçüncü, 1986). the effects of different pasteurization temperatures on the chemical, microbiological and sensory qualities of cheese were examined in a study by Nizamlıoğlu et al. (1998). Researchers tested the cheese samples made from pasteurized milk at 65°C for 15 minutes and at 75°C for 10 minutes and at 85°C for 5 minutes.

The aim of this research was to determine the effect of different pasteurization applications on cheese quality in the production of brined white cheese.

# 2. Material and Method

## 2.1. Establishment of the experiment

In order to produce white cheese, milk standardized to 3% fat content was heated at 85°C for 5 min. and 15 min. After being pasteurized, it was cooled to 37°C. Thermophilic and mesophilic cultures were added to the pasteurized and cooled milk and incubated for 10 minutes. Subsequently 0.03-0.04% CaCl<sub>2</sub> was added and left for 20 minutes (pH 6.0-6.1). Liquid rennet was added and left for 90 minutes. Coagulation was achieved over a period of time. The clot that was ripe for cutting was broken and pressure was applied for 2 hours. After removing the whey, the cheeses (pH 5.8-5.9) were cut and kept in pasteurized brine with 14% bome degree for 4 hours. Cheeses whose pH levels dropped to 5.1-5.2 were placed in 2 kg boxes and pasteurized brine and adding 12.5% bome degree and ripened at +4 °C for 2 months. Traditional white cheese was used as a control application, the milk was pasteurized at 68 °C for 30 minutes, mesophilic culture and 0.015% CaCl<sub>2</sub> were added. Some physical and chemical analyzes were performed on the 2nd, 15th, 30th and 60th days of storage and fatty acids, casein fractions and mineral matter analyzes were performed on the 2nd (fresh) and 60th days. White cheese production was repeated twice. The treatments applied in white cheese production and sample numbers are given in Table 1. The production steps are given in Figure 1 and Figure 2.

Pasteurization	Culture	$CaCl_2$	Sample No
norm	Туре	(%)	INO
	Thermophilic	0.03	1
85°C / 5 min.	Thermophine	0.04	2
65°C / 5 mm.	Macaphilia	0.03	3
	Mesophilic	0.04	4
	Thermophilic	0.03	5
85°C / 15 min.	Thermophine	0.04	6
65 C / 15 mm.	Mesophilic	0.03	7
	Mesophine	0.04	8
68°C /30 min. (cont.)	Mesophilic	0.015	9

Table 1. Treatments and sample numbers applied in cheese production



Figure 1. Experimental white cheese production steps



Figure 2. Control white cheese production steps

### 2.2. Methods

Nitrogen and water-soluble nitrogen content in white cheese samples was determined with the micro-Kjeldahl method. The degree of ripening was obtained by dividing the amount of water-soluble nitrogen in white cheese samples by the total amount of nitrogen (Kurt et al.,1996). Mineral contents of white cheeses were determined with the method specified by Naumann et al. (1983). The mini alkaline urea gel electrophoresis technique, specified by Creamer (1991), was used to determine the casein fractions of white cheese samples on the 2nd and 60th days of the ripening period. The analysis of free fatty acid, which plays an important role on the taste and aroma of cheese, was carried out by gas chromatography on the 2nd and 60th days of the ripening period (Akalın et al., 1998).

The research was carried out according to the "completely randomized block trial plan" in a factorial arrangement (2 pasteurization norms x 2 culture combinations x 2 CaCl<sub>2</sub> amount x 2 replications) (Yıldız & Bircan, 1994). Statistical analyzes were subjected to variance analysis with a package program and significant averages were compared with the Duncan multiple comparison test.

# **3.Results and Discussion**

### 3.1 Physical and Chemical Analysis Results

### 3.1.1. Cheese yield

The highest yield was obtained from the cheese samples that were pasteurized at 85°C for 5 minutes and adding 0.03% CaCl<sub>2</sub> and thermophilic culture. The lowest yield (16.60%) was obtained from the white cheese sample pasteurized for 15 minutes at the same temperature and adding 0.04% CaCl<sub>2</sub> and mesophilic culture (Table 2). The average yield was determined as 17.58% in high-temperature treated white cheese samples and 16.40% in the control white cheese sample (Table 2). The yields obtained from white cheese samples treated with high temperatures were found to be higher than the control sample. This is due to the serum proteins being denatured and remaining in the cheese curd as a result of the high temperature application. Kurt & Çakmakçı (1991) reported that cheese yield is linked to milk composition, acidity, temperature application and the entire production stage. Dağdemir et al. (2003) and Pappa et al. (2006) reported that the use of different cultures had no effect on cheese yield.

Rynee et al. (2004) produced semi-fat Cheddar cheese from milk pasteurized at 72°C, 77°C, 82°C and 87°C for 26 s and analyzed after ripening. They reported that the denaturation rate of total serum proteins was 2.8%, 8.4%;20.2% and 34.1% respectively.

Hazir (1995) reported that applying heat to cow's milk at 80°C for 5 minutes and adding 0.04% CaCl<sub>2</sub> reduced the milk components lost with whey and therefore increased cheese yield. While Öksüz (1989) obtained the highest cheese yield of 20.033% from cheeses produced by adding 0.03% CaCl<sub>2</sub> to milk pasteurized at 77°C for 1 minute, Şakiroğlu (1986) reported that the highest cheese yield was obtained from cheese produced from milk pasteurized at 85°C for 15 s and addeing 0.03% CaCl<sub>2</sub> which parallels our results.

Wolfschoon-Pombo (1997) reported that the cheese yield was 38kg/100kg when 0.01% calcium chloride was added to cheese milk,. He also stated that seasonal changes were observed and significant differences in yield occurred in the spring and summer months. Other studies have reported that high temperature application caused protein denaturation and that cheese yield increased according to denatured proteins (Guinee et al., 2004; Singh & Waungana, 2001). In their study, Celik et al. (2005) obtained the highest yield of cheese from milk that was subjected to high heat treatment which is also commensurate with the results of this study.

#### 3.1.2.Nitrogen ratio

Nitrogen ratio is a parameter used to determine the amount of protein in cheese and calculate the degree of ripening along with water-soluble protein. Protein has the largest share in cheese solids after fat. The nitrogen content of the cheese samples varied between 1.39-2.58% (Table 2). As a result of variance analysis, the difference between CaCl<sub>2</sub> additions (p<0.05), different culture usage and maturation times (p<0.01) was found to be significant. The nitrogen rate in white cheeses was determined as  $1.970\pm0.023\%$  in applications with thermophilic culture and  $2.116\pm0.023\%$  in applications with mesophilic culture. The nitrogen content (2.58%) in the present study was determined in fresh cheese samples pasteurized at 85°C for15 min and supplemented with mesophilic culture and 0.03% CaCl<sub>2</sub>.

In a study carried out by Gürsel et al. (1987) in which thermophilic culture and 0-0.02-0.03-0.04-0.05% CaCl<sub>2</sub> was added to the milk pasteurized at  $72\pm1$ °C for  $2\pm0.3$  min., the highest nitrogen content was achieved by adding 0.03% calcium chloride. In the control white cheese sample, the highest average nitrogen rate was obtained from fresh cheese as 2.60%, and this rate was found to be higher than that of the value determined in the trial cheese samples (Table 2). This may be due to the fact that the dry matter content of the control white cheese samples was higher than the trial white cheese samples. Lau et al. (1989) investigated the effect of pasteurization temperature on the amount of fat and protein and reported that as the pasteurization temperature increased, the amount of nitrogen remaining in the cheese increased as well. This finding did not parallel the results of this research.

#### **3.1.3.** Proportion of water-soluble nitrogen

The water-soluble nitrogen ratio uses to express ripening in cheese; They are fractions consist of small molecular weight nitrogenous components such as protein, short-chain peptide, ammonia, free amino acids and urea. The water-soluble nitrogen value varies depending on the protein and water content of the cheeses. The lowest and highest water-soluble nitrogen content in high-temperature white cheese samples was determined as 0.17% and 0.59%. In the control white cheese sample, the lowest amount of water-soluble nitrogen was determined as 0.29% and the highest was 0.51% (Table 2).

The lowest water-soluble nitrogen rate (0.17%) was found at  $85^{\circ}C/5$  min. pasteurization norm. The highest water-soluble nitrogen rate (0.59%) was determined at  $85^{\circ}C/15$  min norm. As seen in Figure 3, at the beginning of the ripening, the water-soluble nitrogen rate in white cheeses produced with both cultures gave approximate values, and as the ripening period extended, a significant increase in the water-soluble nitrogen rate was observed. The increase in the amount of the water-soluble nitrogen was greater in cheeses produced with mesophilic culture. This can be sourced from high proteolysis. Guizani et al. (2006), Dağdemir and Özdemir (2008), Göncü and Alpkent (2005) and Özdemir (1990) reported in their studies that the rate of water-soluble nitrogen increases as ripening periods increase.



Figure 3. Different culture x ripening time interaction of water-soluble nitrogen in the cheese samples

#### 3.1.4. Degree of ripening

The lowest and highest degree of ripening in samples were determined as 7.01% and 29.01%, respectively (Table 2). Likewise, the lowest (8.8%) and highest (29.0%) ripening degrees in control samples were determined in fresh and 2-month-ripened cheeses, respectively (Table 2). The ripening degrees of white cheese samples showed a significant increase in parallel with the increase in the amount of water-soluble nitrogen during ripening (Göncü & Alpkent, 2005). The average ripening degree of high temperature applied cheese samples and control cheese samples was determined as 17.3% and 18.3%, respectively (Table 2). This is probably due to the higher water content of white cheese samples treated at high temperatures. Çelik et al. (2005) reported that the ripening index of cheeses produced from milk treated with high temperatures was higher. It was different from our result.

#### 3.2. Mineral Substance Analysis Results

Mineral matter (potassium, sodium, phosphorus, calcium and magnesium) analysis results of high temperature applied white cheese samples are given in Table 3.

#### 3.2.1. Amount of calcium

The calcium content of white cheese samples treated with high temperature was between 455.51-1182.88 mg/100 g, the average was detected as 768.64 mg/100 g, and the calcium content in white cheese samples decreased as the ripening time increased. While the average amount of calcium in fresh cheeses (day 2) was  $949.61\pm33.63 \text{ mg}/100\text{ g}$ , this amount decreased to  $575.04\pm33.63 \text{ mg}/100\text{ g}$  on the 60th day of ripening. Other studies have reported that the amount of Ca in cheese decreases during ripening which parallels our results (Cichoscki et al., 2002; Sağun et al., 2005). In the production of high-temperature white cheese, the amount of calcium obtained in applications when 0.03% CaCl<sub>2</sub> was added was  $686.34\pm33.63 \text{ mg}/100\text{ g}$  while the awerage amount of Ca was  $838.31\pm33.63 \text{ mg}/100\text{ g}$ , and in samples reinforced with 0.04% CaCl<sub>2</sub>.



Figure 4. Pasteurization time x culture addition interaction regarding Ca content of cheese samples

Past. Norm	Starter culture	CaCl <sub>2</sub> %	Ripening times (day)	Nitrogen (%)	Water soluble nitro. (%)	Ripening degree (%)	Efficiency (%)		
	0	0.02	2. (fresh) 15.	2.06 2.04	0.26	12.67 12.36	17.05		
	Thermophilic	0.03	30.	1.83	0.36	21.52	17.85		
	lqo		60.	1.75	0.39				
	LT .		2. (fresh)	2.23	0.24	10.51			
_:	he	0.04	15.	1.99	0.28	11.66	17.15		
nin	Τ	0.04	30.	1.98	0.37	19.51	17.15		
85°C / 5 min.			60.	1.88	0.41	21.38			
$\overline{\mathbf{O}}$			2. (fresh)	2.20	0.24	10.89			
5°(		0.03	15.	2.11	0.25	11.89	16.95		
$\infty$	lic	0.03	30.	1.90	0.26	14.10	10.95		
	phi		60.	1.71	0.40	28.39			
	los		2. (fresh)	2.38	0.17	7.01			
	Mesophilic	0.04	15.			22.16	16.60		
			30.	2.14	0.56	23.42	16.60		
			60.	1.90	0.59	29.01			
	Thermophilic	0.03	2. (fresh)	2.17	0.23	12.92			
			15.	2.12	0.34	17.18	10.50		
			30.	1.97	0.37	17.63	18.50		
			60.	1.72	0.43	20.49	l		
		0.04	2. (fresh)	2.39	0.27	10.67			
85°C/ 15 min.			15.	2.09	0.32	14.30	10.00		
			30.	1.92	0.37	20.46	18.20		
			60.	1.39	0.44	25.92			
		0.03	2. (fresh)	2.58	0.24	9.09			
	lic		15.	2.09	0.30	14.81	10 15		
			30.	1.92	0.44	21.43	18.45		
	phi		60.	1.86	0.49	23.65			
	los	0.04	2. (fresh)	2.36	0.25	8.48			
	Mesophilic		15.	2.24	0.27	13.03	16.05		
			30.	2.20	0.39	17.86	16.95		
			60.	2.09	0.48	25.25			
کر in. ol)		0.015	2. (fresh)	2.60	0.29	8.81			
	ph.		15.	2.13	0.30	13.16			
65° C/ 30 min. (control)	Mesoph.		30.	1.97	0.36	20.07	16.40		
30 (co	Me		60.	1.69	0.51	29.41			

Table 2. Physical and chemical analysis results of trial white cheese samples

As seen in Figure 4, the Ca content in samples treated at higher temperatures for a longer time and using thermophilic culture was higher than those that were treated with mesophilic culture. In the control white cheese sample, the calcium content was determined as 1008.21 mg/100g on the 2nd day of ripening (fresh) and 509.01 mg/100g on the 60th day, and the average was determined as 758.61 mg/100g, It was determined that the average calcium content of white cheese samples treated with high temperature was higher than that of the control samples.

# **3.2.2. Amount of potassium**

The average potassium content of white cheese samples treated with high temperature was determined as 114.67 mg/100g (Table 3). The potassium content of white cheese samples decreased as the ripening time increased. The average amount of potassium in the trial white cheese samples decreased during storage, and in fresh white cheeses  $(2^{nd} day)$ , this amount was determined as  $138.94\pm4.83$  mg/100g. The average potassium content was determined as  $106.92\pm4.83$  mg/100g and  $121.26\pm4.83$  mg/100g in the samples added with 0.03% and 0.04% CaCl<sub>2</sub>, respectively. It was determined that the potassium content in the white cheese samples produced by adding a high amount of CaCl<sub>2</sub> (0.04%) was higher than the content of the other application (0.03%). The potassium content of control white cheese samples ranged between 87.16-159.21 mg/100g and the average was determined as 123.19 mg/100g (Table 3). The average potassium content (114.67 mg/100g) of white cheese samples subjected to the high temperature application was lower than that of the control samples. Demirci (1989) reported the potassium amount of white cheese samples as  $114.6\pm13.41$  mg/100g, and Özdemir (1990) reported it as 110-170 mg/100g.

#### 3.2.3. Amount of magnesium

The magnesium content of white cheese samples to which high temperature was applied varied between 39.21 mg/100 g and 58.94 mg/100 g ((Table 3). The magnesium content of the samples decreased in parallel with the prolongation of the ripening period. In the trial fresh white cheeses (2nd day), the average Mg was  $56.58 \pm 1.23 \text{ mg}$ . /100g, while in ripened white cheeses (60th day) it was determined as  $41.92 \pm 1.23 \text{ mg}/100 \text{g}$ . Sağun et al. (2005) and Prieto et al. (2002) reported that there was a significant decrease in the amount of Mg during ripening. The Mg amounts detected in this research were higher than the values determined by Sağun et al. (2005) and Özdemir (1990).

The magnesium content of control white cheese samples varied between 43.77-60.38 mg/100g and the average was determined as 52.08 mg/100g. The average magnesium content of high temperature treated white cheese samples (49.23 mg/100g) was found to be lower than that of the control samples (52.08mg/100g). (Table 3). Demirci (1989) determined the magnesium content of white cheeses as  $39.6\pm3.48$  mg/100g which is lower than the results of this research.

#### 3.2.4. Amount of phosphorus

The phosphorus amount of the samples treated at high temperatures varied between 589.20-1378.73 mg/100g, and the average was determined as 892.55 mg/100g (Table 3.3). The graph of the interaction is given in Figure 3.3. The phosphorus content ( $793.95\pm55.58 \text{ mg}/100g$ ) of white cheeses produced from pasteurized milk was higher. The phosphorus content of white cheeses produced by adding 0.03% calcium chloride was determined as  $758.89\pm55.58 \text{ mg}/100g$ , and the phosphorus content of white cheeses produced by adding 0.04% CaCl<sub>2</sub> was  $1003.35\pm55.58 \text{ mg}/100g$ . As the CaCl<sub>2</sub> ratio used in the production of white cheese increased, the phosphorus content also increased. The phosphorus content of fresh ( $2^{nd}$  day) white cheeses ( $1009.91\pm55.58 \text{ mg}/100g$ ) was found to be higher than the phosphorus content of ripened ( $60^{th}$  day) white cheeses ( $752.33\pm55.58 \text{ mg}/100g$ ). Other researchers have also reported a decreasing in the amount of P during ripening of cheese which parallels our findings (Cichoscki et al. 2002; Prieto et al. 2002). The P amounts obtained in the study calculated by Özdemir (1990) and Sağun et al. (2005) were higher than the values determined.





As seen in Figure 5, the highest phosphorus content was determined in fresh white cheese samples (day 2) to which 0.04% CaCl<sub>2</sub> was added. At the end of storage, there was a highly increase in the amount of phosphorus in both CaCl<sub>2</sub> ratios and a decrease in the P amount. The phosphorus content of control white cheese samples ranged between 855.10-1025.68 mg/100g and the average was determined as 940.39 mg/100g. The average phosphorus content (892.55 mg/100g).

#### 3.2.5. Amount of sodium

The sodium amount of white cheese samples treated with high temperature varied between 1335.02 mg/100g and 3606.61 mg/100g, and the average was determined as 2378.64 mg/100g (Table 3). The Na content of white cheeses produced by adding mesophilic and thermophilic cultures was 2560.57±99.46 mg/100g, respectively. The average was determined as 2173.67±99.46 mg/100g. As the ripening time of the cheese samples increased, the sodium content also increased. Na content was determined as 1674.70±99.46 mg/100g in fresh white cheeses and 3059.54±99.46 mg/100g in ripened white cheeses. This was due to the increase in the amount of Na as a result of salt intake as the ripening time of the cheese increased. Sağun et al. (2005) reported that the Na content of cheeses increased to 3459.50±47.61 mg/100g on the 30th day and decreased slightly on the 90th day to 3379.50±54.40. The findings of Sağun et al. (2005) parallel our results. Özdemir (1990) reported an average content of 1627 mg/100g Na in cheeses ripened for three months. As seen in Figure 6, while the Na content was at the same level at the beginning of ripening in both culture uses, the Na content increased as the ripening period increased and it was determined that the Na content was at the highest level in the applications using mesophilic culture.



Figure 6. Different starter culture x ripening time interaction regarding Na content of cheese samples

The sodium content of control white cheese samples ranged between 1422.80-2968.70 mg/100g and the average was determined as 2195.75mg/100g. It was determined that the average Na content (2378.64 mg/100g) of white cheese samples treated with high temperature was higher than that of the control white cheese samples (Table 3).

			Dipoping								
Past.	Starter	CaCl <sub>2</sub> %	Ripening times	Mineral substances (mg /100g)							
Norm	culture		(day)	Κ	Na	Р	Ca	Mg			
	lic	0.03	2. (fresh)	122.72	1431.34	589.20	706.07	57.53			
	ophi		60.	89.99	2595.94	710.92	455.51	43.76			
in.	Thermophilic	0.04	2. ( fresh )	142.61	1655.09	743.58	856.33	56.37			
5 m	LT L		60.	94.41	2881.19	738.49	556.66	41.31			
85 °C / 5 min.	ు	0.03	2. ( fresh )	109.95	1335.02	858.91	959.08	57.87			
85	Mesophilic		60.	82.53	3559.64	635.41	512.63	43.57			
	lesoj	0.04	2. ( fresh )	156.37	2017.67	1378.73	1168.96	57.19			
	2		60.	89.15	3366.78	696.37	569.55	40.56			
85°C / 15 min.	lic	0.03	2. ( fresh )	131.51	1478.11	948.27	849.89	54.40			
	lihqc		60.	82.09	2707.76	891.82	656.85	39.73			
	Thermophilic	0.04	2. ( fresh )	146.67	1528.81	1369.31	1182.88	51.59			
	Th		60.	88.60	3111.13	794.98	630.81	39.21			
	0	0.03	2. ( fresh )	145.21	1540.45	815.26	811.49	58.94			
	phili		60.	91.44	3606.61	621.34	539.18	41.79			
	Mesophilic	0.04	2. ( fresh )	156.46	1770.64	1376.04	1062.17	58.71			
	Z		60.	95.79	3287.79	929.31	679.14	45.41			
65°C/30	Maaanhili	0.015	2. ( fresh )	159.21	1422.80	1025.68	1008.21	60.38			
min. (control)	Mesophilic		60.	87.16	2968.70	855.10	509.01	43.77			

 Table 3. Mineral substance analysis results of cheese samples

# 3.3. Fatty Acids Analysis Results

The fatty acid analysis results of white cheese samples are given in Table 4. Fatty acids (especially short-chain ones; C4, C6, C8) are the main factors in the formation of cheese flavor (Scott, 1981). However, the excess of short-chain fatty acids creates a rancid taste defect and each one provides a separate undesirable aroma. Fatty acids are the most important and effective taste and aroma elements

of many cheese types. In cheeses, fatty acids with 4 or more carbon atoms are formed as a result of lipolysis of milk fat and/or breakdown of amino acids during ripening (Urbach, 1993). Lipolysis occurs according to the unique lipase enzyme or microbial lipase enzyme of milk. However, the majority of fatty acids (C4-C20) are produced by lipolysis of triglycerides, and some (C2-C6) are produced by the degradation of lactose and amino acids (Curioni & Bosset, 2002).

Butyric acid (C4) plays an important role on the aroma of semi-hard cheeses (Woo et al. 1984). The butyric acid amount of white cheese samples treated with high temperature varied between 1.522-1.982% and the average was determined as 1.743% (Table 4). The interaction of pasteurization time x CaCl<sub>2</sub> is shown in Figure 7. The average butyric acid rate was determined as  $1.671\pm0.032\%$  in the application using mesophilic culture and as  $1.812\pm0.032\%$  in thermophilic culture applications.



Figure 7. Pasteurization time x CaCl<sub>2</sub> addition interaction on butyric acid ratio of cheese samples

The butyric acid rate of white cheeses produced using 0.03%  $CaCl_2$  was higher than the butyric acid rate of white cheeses supplemented with 0.04%  $CaCl_2$ ., It was determined that the butyric acid rate of white cheeses treated with 0.03%  $CaCl_2$  was lower than that of cheeses treated with 0.04%. The % butyric acid content of control white cheese samples was 1.779% on the  $2^{nd}$  day of ripening (fresh) whileit was determined as 1.398% on the 60<sup>th</sup> day. The average butyric acid content determined in the high-temperature treated white cheese samples (1.743%) was found to be higher than that of the control samples (1.589%). Akalın et al. (1998) determined the butyric acid ratio in cheeses produced with different production methods as 2.48% and Dağdemir and Özdemir (2008) as 2.164-2.645%, which are higher than the results of this research.

Caproic acid (C6) plays an important role on the aroma of semi-hard cheeses (Woo et al. 1984). The caproic acid content in white cheese samples treated with high temperatures varied between 1.576-2.005% and average was determined as 1.718% (Table 4). The average caproic acid content in control white cheese samples was determined as 1.772%. While the caproic acid content was 1.892% at the beginning of ripening, it decreased to 1.652% on the 60th day. The average caproic acid content of white cheese samples treated with high temperature was found to be lower than the control sample. Akalın et al. (1998) reported the caproic acid rate in white cheeses as 2.48% and Dağdemir and Özdemir (2008) reported it as 2.093-2.689%, which is higher than the results of this research.

The caprylic acid (C8) rate in white cheese samples treated with high temperatures varied between 0.883-1.207% and average was determined as 1.032% on (Table 4). While the caprylic acid rate in the trial cheeses produced by adding 0.03% CaCl<sub>2</sub> was  $0.99\pm0.3\%$ , it was determined as  $1.07\pm0.3\%$  in those treated with 0.04% CaCl<sub>2</sub>. As the caprylic acid content of control samples on day 2 (fresh) was 1.166%, the content on day 60 was determined as 1.087% and the average was 1.127%. The value found in the control white cheese samples was higher than the white cheese samples treated with high temperatures (Table 4). Akalın et al. (1998) reported the caprylic acid rate in white cheese samples produced using different production methods as 2.77%, and Dağdemir and Özdemir (2008) reported it as 1.102-1.709%.

The capric acid (C10) rate in white cheese samples treated with high temperatures varied between 1.940% and 2.570%, and the average was determined as 2.248% (Table 4). The capric acid content of control white cheese samples varied between 2.569-2.483% and was determined to be 2.526% on average during storage. The capric acid ratio determined in control white cheese samples was higher than in samples treated with high temperature (Table 4). Akalın et al. (1998) reported the capric acid rate in white cheeses as 6.04% and Dağdemir and Özdemir (2006) reported capric acid as 3.276-2.272%, which is higher than the research findings.

The rate of lauric acid (C12) in white cheese samples subjected to high temperatures varied between 2.492% and 3.156%, and the average was determined as 2.776%. The lauric acid content of control white cheese samples varied between 3.090% - 3.107% and the average was determined to be 3.099%. The lauric acid rate determined in control white cheese samples was found to be higher than in white cheese samples treated with high temperatures (Table 4).

In control white cheese samples, the ratios of myristic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acid were 11.635-11.699%, respectively; 34.770-34.976%; 10.645-10.701%; 26.236-26.748%; 0.228-0.747%; 0.375-0.725% respectively; It was determined that the ratios varied between 0.229-0.242%. Mallatou et al. (2003) reported that in the cheeses they produced using cow, sheep and goat milk, the total amount of free fatty acids was significantly higher in those produced from cow's milk and that palmitic and oleic acid rates were at the highest level in all cheeses during ripening. Guizani et al. (2006) reported that lipolysis occurred during 30 days of ripening in semi-hard cheeses produced from pasteurized milk and that palmitic, oleic, myristic, capric and lauric acids were the most important fatty acids. The rate of free fatty acids in cheeses varies according to temperature, number of lipolytic microorganisms enzymes, water content (Park, 2001), ripening time and different production methods (Larrayoz et al., 1999; Poveda &

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Cabezas, 2006). Free fat acids decrease with the increase of the pasteurization temperature (Rynee et al., 2004; Buffa et al., 2001). The degree of lipolysis is determined by the amount of free fatty acids that increases during ripening (Contarini & Toppino, 1995; Mallatou et al., 2003). One study reported that due to high temperature application, the fat content of cheese samples decreased, the water content increased, and no significant change was observed in the free fatty acid ratio (Park, 2001). In addition, since the high salt content in cheeses is a limiting factor for enzyme and microorganism activity, the degree of lipolysis decreased and therefore no significant change was observed in the rate of free fatty acids (Escriche et al., 2000; Mallatou, et al., 2003).

Another reason for the low rate of free fatty acids in this study may be due to the inactivation of the natural lipase enzyme of milk as a result of the high temperature application to milk. Koçak et al., (1987) reported that the free fatty acid ratio of cheeses produced from pasteurized milk for a long time was lower than those produced from raw milk, and this may be due to the inhibition of the natural lipase enzyme of milk as a result of heat application. McSweeney (2004) reported that pasteurization temperature significantly inactivated the natural lipoprotein lipase of milk.

### 3.4. Casein Fractions

Electrograms of the white cheese samples are given in Figures 3.6 and 3.7. Fresh ( $2^{nd}$  day) white cheese samples produced by pasteurization for a period of time and by adding 0.03% to 0.04% CaCl<sub>2</sub> after adding thermophilic culture are shown with numbers 1-4, while fresh ( $2^{nd}$  day) white cheeses produced by adding mesophilic culture are shown with numbers 5-8, control.



Figure 3.6. Urea polyacryl amide gel electrophoresis photographs applied to fresh (2<sup>nd</sup> day) white cheeses



Figure 3.7. Photographs of urea polyacryl amide gel electrophoresis applied to ripe (60<sup>th</sup> day) white cheeses

An examination of the electrograms revealed that the amount of both  $\alpha$ -casein and  $\beta$ -casein decreased during ripening. During ripening,  $\alpha$ -casein and  $\beta$ -casein bands gradually weaken, and in parallel, degradation products increase. This situation results with the increase in the amount of water-soluble protein during ripening (Table 2). In the early stages of ripening, the degradation rate of  $\alpha$ -casein is higher than that of  $\beta$ -casein (Rynne et al. 2004). Kalit et al., (2005) determined that during the smoking process of Tounj cheese, which lasted 2-3 days,  $\alpha$ s1-casein started to decompose, while  $\beta$ -casein was hydrolyzed to a lesser extent by the effect of plasmin, and they suggested that the degradation of this casein fraction could be a ripening indicator for similar cheeses. Chymosin rapidly hydrolyzes  $\alpha$ s1-casein undergoes hydrolysis during the 20-day period after production until it is placed in the cold room. Benfeldt et al. (1997) stated that  $\alpha$ s2-casein and  $\beta$ -casein are hydrolyzed by plasmin and are affected by the heat treatment applied to milk, while  $\alpha$ s1-casein and  $\beta$ -casein are probably hydrolyzed by other enzymes such as rennet, and they are not affected by the heat treatment applied to milk. During the ripening of the cheese, the salt content in the brine affects casein hydrolysis. It has been reported that as the salt content in the brine increases from 4% to 8%, the hydrolyzation of  $\beta$ -casein in cheese made from goat's milk was affected more than  $\alpha$ -casein (Thomas & Mills, 1981; Veloso et al., 2004; Psoni, et al. 2006).

Veloso et al. (2004) determined that the  $\alpha$ -case in fraction degraded more rapidly in cheeses produced from sheep's milk, but the change in the  $\alpha$ -case in fraction during the ripening of cheeses produced by mixing 20% cow's milk with sheep's milk was similar to the cheese produced from cow's milk.

Pasteurization temperature has a very important effect on the degradation of  $\alpha$ -casein and  $\beta$ -casein (Gaya et al., 1990). The degradation rate and rate of  $\beta$ -casein varies depending on the pasteurization temperature. Researchers examining the ripening of different cheese varieties reported that plasmin activity decreased due to the application of high pasteurization temperatures and the degradation rate of  $\beta$ -casein decreased relatively (Rynne et al., 2004). Veloso et al. (2004) determined that the degradation of the  $\beta$ -casein fraction in all cheeses produced from cow's milk, sheep's milk and their mixtures was not as high as that of  $\alpha$ -casein. Similar results were reported by Awad (2006) on Ras cheese and by Hayaloğlu et al. (2004) in white cheese. Benfeldt et al. (1997) determined that plasmin activity was negatively affected. The same researchers reported that less degradation was observed in cheeses produced from milk subjected to high temperatures and long-term heat treatment, , especially in the  $\beta$ -casein and  $\alpha$ s2-casein fractions during ripening due to the decrease in plasmin activity, and no difference was observed in the degradation of the  $\alpha$ s1-casein fraction, probably due to the rennet effect.

# 4. Conclusions and Recommendations

The high-grade heat treatment of milk and the addition of starter culture and CaCl2 are applications that significantly increase yield in the production of white cheese. The degree of ripening of cheese in this study increased in parallel with the storage period. The reason for the low free fatty acid content in the cheeses treated with a high temperature may be due to the inactivation of the natural lipase enzyme of the milk as a result of the high heat treatment applied to the milk and the inability of the starter to generate sufficient lipolytic activity. An increase in the amount of Na and a decrease in the amounts of K, P, Ca and Mg minerals was observed in the cheese samples during the ripening period.

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# Table 4. Fatty acid analysis results of white cheese samples

Past. Norm	Starter culture	CaCl <sub>2</sub> %	Ripening	Fatty acids (%)											
			times (day)	Butyric (C4)	Caproic (C6)	Caprylic (C8)	Capric ( C10)	Lauric (C12)	Myristic (C14)	Palmitic (C16)	Stearic ( C18)	Oleic (18:1)	Linoleic (18:2)	Linoleni c (18:3)	Arachidi c (C20)
	lic	0.03	2.	1.982	1.615	0.946	2.167	2.718	10.774	33.727	11.785	28.018	1.284	0.436	0.244
	Thermophilic		60	1.829	1.723	0.988	2.162	2.669	10.574	32.935	12.017	28.850	0.320	0.668	0.241
min.	erm	0.04	2.	1.850	1.758	1.065	2.293	2.799	10.802	33.309	11.588	27.691	1.844	0.426	0.238
15 m	Th		60.	1.774	1.760	1.051	2.258	2.767	10.749	32.867	12.046	28.951	0.256	0.653	0.249
	ు	0.03	2.	1.694	1.787	1.095	2.332	2.842	11.047	32.416	12.575	27.123	1.168	0.477	0.258
85°C	Mesophilic		60.	1.689	1.576	1.009	2.278	2.789	10.815	31.843	11.585	29.576	0.323	0.865	0.239
	lesoj	0.04	2.	1.522	1.674	1.057	2.350	2.921	12.076	36.756	11.524	22.510	1.231	0.529	0.289
	A		60.	1.659	1.669	1.020	2.219	2.715	10.445	31.745	9.055	27.617	0.243	0.636	0.200
	ic	0.03	2.	1.816	1.604	0.883	1.940	2.492	10.669	33.871	11.591	28.716	1.296	0.437	0.225
	lihqc	0.05	60.	1.606	1.628	0.995	2.140	2.625	10.181	31.710	11.871	28.227	0.259	0.658	0.265
min.	erm	Ultranophilic 0.03 0.04	2.	1.791	1.762	1.083	2.340	2.846	10.894	32.319	11.678	28.945	1.204	0.447	0.233
15 m	Th		60.	1.850	1.712	1.021	2.211	2.732	10.585	32.654	12.046	28.920	0.263	0.695	0.248
C /	Mesophilic	0.03	2.	1.617	1.661	0.983	2.159	2.700	10.707	32.503	11.840	30.055	0,752	0.425	0.230
85°C			60.	1.664	1.724	1.046	2.283	2.792	10.704	32.336	11.880	29.115	0.267	0.875	0.249
		0.04	2.	1.913	2.005	1.207	2.570	3.156	12.284	34.025	8.830	27.077	0.840	0.601	0.293
			60.	1.607	1.679	1.034	2.249	2.761	10.629	32.103	11.820	29.081	0.857	0.868	0.248
65°C / 30	Mesophi	0.015	2.	1.779	1.892	1.166	2.569	3.107	11.635	34.770	10.645	26.236	0.747	0.375	0.229
min.	lic	0.015	60.	1.398	1.652	1.087	2.483	3.090	11.699	34.976	10.701	26.748	0.228	0.725	0.242

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