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The effects of heat treatment on chemical, biochemical, and microbiological properties of Ezine cheese

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Abstract

In this study, Ezine cheese was manufactured in accordance with different heat treatment norms (at 65 °C for 10, 20 and 30 minutes) and ripened for 180 days. Different heat treatment norms did not affect the chemical composition of the cheeses, while statistically significantly affected the water-soluble nitrogen (WSN) and 12% trichloroacetic acid-soluble nitrogen (TSN-SN) ratios. Although pH and protein were reduced during ripening, titration acidity, moisture, WSN, TCA-SN and amino acid ratio increased. αs1-casein was more hydrolyzed than β-casein during ripening in Ezine cheese. The heat treatment time increased, while the counts of total aerobic mesophilic bacteria and *Escherichia coli* decreased.

Keywords: *Ezine cheese, Heat treatment, Ripening, Proteolysis, Microbial quality*

1.INTRODUCTION

Turkey is a country that has a rich variety of cheese. The most important cheeses include White, Kaşar, Tulum, Otlu, Dil, Mihaliç, Çerkez, Çökelek, Civil and Lor cheeses (Yasar and Guzeler, 2011). While White, Kaşar and Tulum cheeses are manufactured in each region of the country, other cheeses such as Mihaliç, Otlu and Çökelek are manufactured and consumed in local regions (Demirci and Şimşek, 1997). White cheese is a cheese that is mostly manufactured and consumed in Turkey. White cheese is traditionally manufactured from raw milk without starter culture at small dairy farm, and manufactured as pasteurized and cultured at large plant (Üçüncü, 2005). Ezine cheese's manufacturing technology is similar to that of White cheese. Ezine cheese is manufactured by traditional method and starter cultures are not used in the production to better preserve the characteristics of the cheese.

Ezine cheese is a whitish light yellow, semi-hard type of full-fat cheese with few and small pores, which is manufactured by mixing minimum 40% goat milk, 45% sheep milk, and maximum 15% cow milk based on the season of the milks from the sheep, goats, and cows feeding on the natural vegetation and water resources in Ezine, Bayramiç and Ayvacık located in North and Western regions of Mount Ida. The Mount Ida, where the manufacturing is performed, is a high rainfall region that has a rich vegetation including hundreds of alliaceous plants, primarily sage, wooly mint, bee balm, thyme, oregano, and marjoram, and is also rich in oxygen (TPE, 2006). The most important property of Ezine cheese that differentiates it from White cheese is that goat, sheep, and cow milks are

used together in manufacturing of Ezine cheese, and ripened not later than 8 months. In addition, Ezine cheese has a characteristic flavor and aroma (Karagul Yuceer et al., 2009).

In cheese production, the basis for heat treatment of milk is to significantly reduce all the pathogenic microorganisms and other microorganisms, and to make the cheese manufactured to be safe for the consumers (Celik and Turkoglu, 2007). Heat-treated milk is directly related to the final quality of cheese. Heat treatment affected sensory and chemical properties of Urfa cheese (Atasoy et al., 2008). Milk's pasteurization affects both extent and characteristics of the proteolysis of Cheddar cheese. The increase in the pasteurization temperature helps increase denaturation of whey proteins and interaction with casein (Lau et al., 1991). The populations of starter or non-starter lactic acid bacteria of half-fat Cheddar cheese were not affected by increasing the milk pasteurization temperature during ripening. The reduced-fat Cheddar cheese pH diminished significantly on increasing pasteurization temperature over 270 day ripening period (Rynne et al., 2007). Heat treatment of milk significantly affects the sensory characteristics of cheese, especially flavor and aroma of the cheese (Mendia et al., 1999).

There have been limited researches on Ezine cheese (Karagul Yuceer et al., 2007; Işleten et al., 2007; Karagul Yuceer et al., 2009; Tuncel et al., 2010). There is no any study on the impact of different heat treatment norms on the characteristics of Ezine cheese. The premises manufacturing Ezine cheese has the standard heat treatment norm. There may emerge unsolicited flavor, odor, gas and texture defects in Ezine cheese sometimes because of the microorganisms in the raw milk. The objective of this study was to evaluate the effects of different heat treatment norms (at 10, 20, 30 minutes at 65 °C) on the chemical, biochemical and microbiological, characteristics of Ezine cheese during ripening (on 1st, 60th, 120th, and 180th days).

2. MATERIALS AND METHOD

2.1. Materials

Bovine, ovine, and goat milks were used for cheese production. Milks were obtained from the vicinity of Ezine (Çanakkale, Turkey). Calf rennet (1/16 000 MCU/ml), salt was supplied by Mayasan Gıda Sanayi ve Ticaret A.S. (Istanbul, Turkey), Billur Tuz San. A.S. (Izmir, Turkey), respectively. Aluminum tin cups (Mumcu Teneke A. S., Balıkesir, Turkey) were used during ripening..

2.2. Methods

2.2.1. Cheese production

Ezine cheese was produced by traditional method. The productions were performed in triplicate. Sheep, goat, and cow milks at +4 °C collected from the region as specified to be milk source for Ezine cheese by Turkish Patent Institute (2006) were mixed by 45% sheep milk, 40% goat milk and 10% codayw milk at Local Manufacturing Plant (Ezine, Çanakkale, Turkey). Figure 1 shows the production flow chart used processing the samples.

The milk mixture was filtered and divided into 3 equal parts. The milk mixture was separately pasteurized at 65 °C for 10, 20, and 30 minutes. The milk mixture was cooled down to 33 °C coagulation temperature and coagulated by adding calf rennet for 90 minutes. At the end of coagulation, the curd was cut into small cubes (1 cm3) and rested in whey for 10-15 min. The whey was drained for 1 hour (without pressing). The curd was taken under pressure for 2 hour. Cheese was cut into blocks about 7x7x7 cm using a knife. The blocks were placed brined (15%) for about 12 hours. Aluminum tin cups were filled with 1 kg of cheese and brine (7%) and closed hermetically. The cheeses were ripened in tin cups + 4 °C for 180 days. Ezine cheese samples were taken after 1, 60, 120, and 180 days ripening. The Ezine cheeses were coded as T (at 65 °C for 10 min), Y (at 65 °C for 20 min) and Z (at 65 °C for 30 min).

Sheep, goat and cow milks were mixed (45%+45%+10%)
↓
Pasteurization at 65 °C for 10, 20 and 30 min (T: 10 min.; Y: 20 min.; Z: 30 min. pasteurization)
↓
Cooling down to 33±1 °C
↓
Coagulation with rennet for 90 min
↓
Cutting in small cubes (1 cm^3)
↓
Curd was taken under pressure
↓
Cutting into blocks (7x7x7 cm)
↓
Blocks were placed brined (15%) for about 12 hours
↓
Aluminum tin cups were filled with 1 kg of cheese and brine (7%)
↓
The cheeses were ripened in tin cups + 4 °C for 180 days

Figure 1. Flow diagram of manufacture of Ezine cheese

2.2.2. Chemical Analysis

2.2.3. Milk and Cheese composition

In the raw milk and different heat treated milks, the titratable acidity, total solid, fat (Anon., 1994), protein (IDF, 1993) were determined.

Ezine cheese was analyzed in duplicate for total solid (IDF, 1982), fat (Anon., 1994), protein (IDF, 1993), salt (Anon., 1983), ash (AOAC, 2000), and titratable acidity (lactic acid %) (Anon., 1994). pH was measured with pH meter (Sartorius, PB-11, Göttingen, Germany).

2.2.4. Proteolysis

The water-soluble nitrogen (WSN) (Kuhcroo and Fox, 1982), 12% thrichloroacetic acid-soluble nitrogen (TSN-SN) (Polychroniadou et al., 1999), 5% phosphotungusticacid-soluble nitrogen (PSN-SN) (Jarrett et al., 1982), and total free amino acid content (Folkertsma and Fox, 1992) of Ezine cheese were determined.

2.2.5. Urea-PAGE Electrophoresis Analysis

The cheese samples were analyzed according to Hayaloglu et al. (2005) after ripening for 1, 60, 120, and 180 days. Electrophoresis gels were produced using vertical gel electrophoresis unit (Thermo Scientific Owl™ P8DS Gel System, USA). Gels were stained using Coomassie Brilliant Blue G250. 10 mg cheese sample was dissolved in 1 mL buffered solution. It was kept in water bath at 55 °C for 10 minutes. Samples were centrifuged at 3000 rpm for 10 minutes at 4 °C (Sigma, Postfach, Germany). Samples were injected in 10 ml during analysis. Electrophoresis unit was installed by the above named company. TEMED (N,N,N',N'-Tetramethyl ethylenediamine) and ammonium persulfate were added to start polymerization and transferred to the unit. It was waited about 30 minutes for the completion of polymerization. Loading gel was transferred to unit after separation gels polymerization to complete until it has been. It was waited about 20 minutes for the completion of polymerization. Unit was filled with electrode buffer and samples were transferred to the unit. 200 V Electricity was supplied to the gels for about 120 minutes. Gels were transferred to staining solution when walking was complete. Gels were allowed to be left in staining solution for one night. Then, they were washed with distilled water.

2.2.6. Urea-PAGE Electrophoresis Analysis

10 ml milk and 10 g cheese sample were separately transferred to stomacher bag containing 90 ml 0.1% peptone water under aseptic conditions (10-1 dilution). It was homogenized for 90 seconds in Stomacher (BagMixer, Interscience Co., France). Other decimal dilutions were prepared in the same method (AOAC, 2000). Total aerobic mesophilic bacteria (TAMB) were enumerated on Plate Count Agar (PCA Merck 105463, Germany) at 37 °C for 24 h (FDA, 2000). Lactic acid bacteria counts were determined on Man Rogasa Sharpe (MRS Merck 110660, Germany) at 30 °C for 48 h. (Marshall, 1992). Counts of coliform were counted on Florocult Violet Bile Agar (VRBA Merck 104030, Germany) at 37 °C for 24 h (FDA, 2000). Same

plates were examined under UV light at the end of the 24-hour to identify *Escherichia coli*. Counts of yeast and mould were determined on Yeast Extract Glucose Chloramphenicol Agar (YGCE, Merck 11600) at 25 °C for 3-5 days.

2.2.7. Statistical Analysis

All statistical analyses were performed using SPSS 15.0 program for Windows (SPSS Inc., Chicago, IL, USA). The impact of ripening and heat treatment on quality was analyzed using bifactorial ANOVA Model. General comparisons were made, when there were trivial interactions. The results were expressed as Mean and Standard deviation. Tukey's multiple comparison tests were used to determine the differences between the averages.

3. RESULTS AND DISCUSSION

3.3.1. Chemical composition and microbiological analysis of Ezine cheese milk

The milks supplied from the producers were mixed by 45% ewe milk, 40% goat milk, and 10% cow milk, and the milk was prepared for Ezine cheese. The chemical compositions and microorganism counts of the Ezine cheese milks are given in the Table 1. As it can be seen at the Table 1, it was found out that the heat treatment norms applied to the milk did not affect the chemical composition of the Ezine cheese milks ($p>0.05$). The increased heat treatment norms applied to the milk reduced the microorganism counts of the Ezine cheese milk. Different heat treatment norms affected coliform and yeast-mould counts of milks ($p<0.05$). Goat milk is supplied from 5 villages, ewe milk from 6 villages, and cow milk from 4 villages within the regions allowed for the Ezine cheese production. The milks are the mixtures of the milking at the evening and in the morning. As especially the goat and ewe milk producers make traditional production and hand milking, the hygienic quality of the milk is low.

3.2. Chemical composition of Ezine cheese

Chemical compositions of Ezine cheese manufactured in accordance with different heat treatment norms are given in Table 2. When examined in general, different heat treatment norms did not statistically affect the chemical compositions of Ezine cheese ($p>0.05$). While pH value of Ezine cheese decreased significantly during ripening ($p<0.05$), titration values increased significantly ($p<0.05$). In the early ripening period, pH decrease in Ezine cheeses is much faster. The reason for this may be degradation of lactose remaining in the cheese at the beginning. Similar results reported by Tuncel et al. (2010) for in Ezine cheese. Hayaloglu (2007) found that titration acidity of White cheese increases during storage.

The moisture, fat in dry matter and salt ratios of Ezine cheese statistically increased during ripening ($p<0.05$). The reason for this may be that the compounds in the cheese pass to the brine and the salt in the brine passes to the cheese because of the contact of cheese put in the brine with the brine. Hayaloglu et al. (2005) has found that the moisture amount increased in Turkish White-Brined cheese during ripening. Öner et al., (2006) has pointed out that the salt amount increased in an artisanal Turkish White Cheese during ageing. The protein ratio of Ezine cheese decreased statistically during ripening ($p<0.05$). The similar results were determined by Öner et al., (2006) for in White cheese.

3.3. Chemical composition of Ezine cheese

The nitrogen fractions and free amino acid values of Ezine cheeses manufactured in accordance with different heat treatment norms are given in the Table 3. Although the amount of water soluble nitrogen of Ezine cheeses manufactured using different pasteurization norms was not affected at 1 day of ripening ($p>0.05$), it was significantly affected on the other days of ripening ($p<0.05$). Cinbas and Kilic, (2006) reported that the water soluble nitrogen amount were higher in the traditional production (65 °C/ 5 min.) of White Cheese compared with in the industrial production (75 °C/ 5 min.) of White cheese.

Table 1. Chemical compositions and microbiological counts of Ezine cheese milks (n=3).

Parameters	Milk Samples			
	Raw milk	Heat treated milk (at 65 ºC for 10 min.)	Heat treated milk (at 65 ºC for 20 min.)	Heat treated milk (at 65 ºC for 10 min.)
pH	6.38^{a}±0.16	6.23^{a}±0.20	6.23^{a}±0.20	6.17^{a}±0.24
Lactic acid (% l.a)	0.154^{a}±0.02	0.147^{a}±0.01	0.154^{a}±0.01	0.167^{a}±0.01
Total Solid (%)	15.61^{a}±2.93	15.63^{a}±0.97	15.42^{a}±0.20	16.16^{a}±0.17
Ash (%)	0.907^{a}±0.36	1.045^{a}±0.24	0.979^{a}±0.07	1.039^{a}±0.19
Fat (%)	5.75^{a}±0.80	5.62^{a}±0.17	5.57^{a}±0.67	5.77^{a}±0.24
Protein (%)	5.36^{a}±0.03	5.61^{a}±1.04	5.59^{a}±0.12	5.59^{a}±0.50
Total aerobic mesopfilic bacteria (log cfu/g)	6.87^{a}±2.56	5.12^{a}±1.88	4.42^{a}±2.29	3.79^{a}±2.98
***E. coli* (log cfu/g)**	3.15^{a}±1.46	2.41^{a}±1.83	1.50^{a}±2.12	1.53^{a}±0.65
Coliform bacteria (log cfu/g)	4.57^{a}±0.39	4.35^{a}±0.91	2.75^{b}±0.34	2.84^{b}±0.00
Yeast and mould (log cfu/g)	4.68^{a}±0.11	3.49^{a}±0.28	2.43^{b}±0.15	1.43^{b}±0.02

$^{a-b}$ Means in the same row with different letters are significantly different (p<0.05).

The amount of water soluble nitrogen consists of protein and large peptides, and medium and low peptides, free amino acids created with the impact of rennet mostly, and a small amount of plasmin as well as (Hayaloglu, 2007; Yasar and Guzeller, 2011). The water soluble nitrogen amount of Ezine cheese increased during ripening (p<0.05). This increase is much more apparent between 1 and 120 days of ripening. Similar results were reported by Karagul Yuceer et al., (2009); Tuncel et al., (2010) for in Ezine cheese. 12% TCA-soluble nitrogen amount in cheese usually consists of small peptides, free amino acids, ammonia, and other compounds emerging as a result of the activities of bacteria enzymes (Hayaloğlu et al., 2005; Karagul Yuceer et al., 2009). Different heat treatment norms affected the 12% TCA-soluble nitrogen amounts of Ezine cheeses on all days except for the 1 day of ripening (p<0.05). The increase in the heat treatment time decreased 12% TCA-soluble nitrogen amounts of Ezine cheeses. The pasteurization cause significant damage to microorganisms in the cheese milk. Therefore, 12% TCA-soluble nitrogen amounts of the braided cheese made of raw milk were found to be more than those made from pasteurized milk (Celik and Turkoglu, 2007). Kırmacı et al., (2014) determined the similar result in Urfa cheese. 12% TCA soluble nitrogen amounts of Ezine cheeses increased significantly in during ripening (p<0.05).

Table 2. Chemical composition of Ezine cheese made using different heat treatment norms at 65 °C for 10 (T), 20 (Y) and 30 (Z) minutes during ripening (n=3).

Parameters	Cheeses			
	Days	T	Y	Z
pH	1	5.80±0.16^{aA}	5.99±0.02^{abA}	6.06±0.03^{aA}
	60	5.28±0.01^{aB}	5.32±0.03^{aB}	5.33±0.04^{aB}
	120	5.11±0.04^{aBC}	5.15±0.04^{aC}	5.09±0.03^{aC}
	180	4.97±0.10^{aC}	4.90±0.05^{aD}	4.99±0.03^{aD}
Titratable Acidity (lactic acid (g/100 g))	1	0.42±0.03^{aD}	0.46±0.06^{aC}	0.52±0.02^{aD}
	60	0.57±0.06^{aC}	0.60±0.05^{aC}	0.68±0.01^{aC}
	120	0.82±0.02^{aB}	0.82±0.05^{aB}	0.87±0.01^{aB}
	180	1.05±0.03^{bA}	1.05±0.05^{bA}	1.18±0.04^{aA}
Moisture (g/100 g)	1	51.97±2.68^{aB}	53.20±1.92^{aB}	53.20±1.93^{aB}
	60	54.44±0.64^{aAB}	54.78±1.14^{aAB}	54.78±1.14^{aAB}
	120	55.08±0.73^{bAB}	56.67±0.55^{aA}	56.67±0.55^{aB}
	180	56.79±0.10^{aA}	57.17±0.62^{aA}	57.16±0.62^{aA}
Fat in Dry Matter (g/100 g)	1	46.06±2.47^{aB}	46.20±3.22^{aA}	47.01±0.84^{aB}
	60	52.86±1.15^{aA}	51.99±1.59^{aA}	53.02±0.94^{aA}
	120	52.34±3.41^{aA}	51.37±1.64^{aA}	52.31±3.15^{aAB}
	180	52.42±0.19^{aA}	51.97±3.74^{aA}	52.71±2.71^{aA}
Salt (g/100 g)	1	3.54±0.17^{aB}	3.70±0.37^{aB}	3.70±0.13^{aB}
	60	4.58±0.03^{aA}	4.47±0.23^{aAB}	4.40±0.16^{aA}
	120	4.68±0.31^{aA}	4.64±0.42^{aA}	4.62±0.31^{aA}
	180	4.66±0.23^{aA}	4.64±0.35^{aA}	4.75±0.37^{aA}
Ash (g/100 g)	1	5.08±0.14^{aA}	5.14±0.52^{aA}	5.11±0.09^{aA}
	60	5.25±0.11^{aA}	5.25±0.25^{aA}	5.23±0.10^{aA}
	120	5.27±0.31^{aA}	5.34±0.21^{aA}	5.21±0.32^{aA}
	180	5.33±0.19^{aA}	5.34±0.26^{aA}	5.37±0.29^{aA}
Protein (g/100 g)	1	24.17±3.82^{aA}	23.84±3.71^{aA}	23.86±4.55^{aA}
	60	19.35±1.64^{aAB}	19.27±1.50^{aAB}	21.34±0.58^{aAB}
	120	18.63±1.70^{aAB}	18.20±1.45^{aB}	17.79±1.27^{aB}
	180	17.38±0.80^{aB}	17.83±0.58^{aB}	17.43±0.71^{aB}

The increase between 60 and 120 days of ripening is much more. Various researchers found that 12% TCA-soluble nitrogen amounts increased during ripening. (Lau et al., 1991; Yasar and Guzeler, 2011).

Nonstarter lactic acid bacteria are highly responsible for the formation of free amino acid (FAA) in cheese. FAA is formed as a result of the activity of small and medium sized peptides and lactococcal peptides that directly affect the aroma of cheese, as well (Hayaloglu et al., 2005).

Table 3. Nitrogen fractions and free amino of Ezine cheese made using different heat treatment norms at 65 °C for 10 (T), 20 (Y) and 30 (Z) minutes during ripening (n=3).

Parameters	Cheeses			
	Days	T	Y	Z
Water-soluble nitrogen (% of total N)	1	5.22±1.42^{aC}	5.44±1.05^{aC}	5.19±0.88^{aC}
	60	10.66±1.62^{aB}	8.47±1.11^{bB}	7.12±0.73^{bB}
	120	12.00±0.95^{aAB}	10.33±0.76^{bAB}	10.84±0.36^{abA}
	180	14.11±0.57^{aA}	11.69±0.76^{bA}	10.47±0.47^{bA}
12% TCA-soluble nitrogen (% of total N)	1	3.02±0.98^{aB}	3.00±0.60^{aAB}	2.06±0.51^{aB}
	60	3.74±0.37^{bB}	4.26±0.36^{aA}	4.74±0.45^{aA}
	120	5.31±0.32^{bAB}	4.97±0.42^{aA}	4.77±0.42^{aA}
	180	5.75±0.39^{aA}	4.93±0.14^{bA}	4.43±0.36^{bA}
Free amino acid (mg of Leu/ g of cheese)	1	0.033±0.00^{aB}	0.034±0.00^{aB}	0.031±0.00^{aC}
	60	0.047±0.00^{aA}	0.052±0.00^{aA}	0.046±0.00^{aB}
	120	0.056±0.01^{aA}	0.058±0.01^{aA}	0.062±0.00^{aA}
	180	0.056±0.01^{aA}	0.058±0.01^{aA}	0.062±0.00^{aA}

$^{a-b}$ Means in the same row with different letters are significantly different ($p<0.05$). $^{A-C}$Means in the same column with different letters are significantly different ($p<0.05$). T (at 65 °C for 10 min), Y (at 65 °C for 20 min) and Z (at 65 °C for 30 min).

Different heat treatment norms did not affect the amounts of total free amino acids of Ezine cheeses ($p>0.05$). The free amino acid amount continuously increased during ripening and was maximized at 180 days ripening ($p<0.05$). Golge (2009) found that FAA increased in Kelle cheese during ripening.

3.4. Urea-Polyacrylamide gel electrophoresis

The electrophoretograms which obtained results of Urea-PAGE electrophoresis analysis had given in Figure 2. When Figure 2 is reviewed, it is seen that β-casein did not degrade so much during ripening. Tuncel et al. (2010) found that β-casein can be hydrolyzed 89.3% in Ezine cheese during ripening for 360 days. Hydrolysis of β-casein in cheese at a low rate is caused by that the cheese has a low pH level and high rate of salt (Kırmacı et al., 2014). The degradation of αs1-casein is more than that of β-casein in during ripening in Ezine Cheese. An important part of αs1-casein was hydrolyzed at the end of the 180-day ripening period. Karagul Yuceer et al. (2009) has reported that half of αs1-casein of Ezine cheese was hydrolyzed at the end of the 180 day ripening period and 25.3% αs1-casein remained at the end of ripening (360 day). Ozcan and Kurdal, (2012) determined that β-casein is hydrolyzed by 16.35% and αs1-casein by 55.60% in Mihaliç cheese as a result of 90-day ripening. Hayaloglu et al., (2013a) found that in Gokceada artisanal goat cheese αs1-casein is hydrolyzed more than β-casein during ripening, and that the hydrolysis of β-casein decreases as the salt concentration of cheese increases. Proteolysis in Ezine cheese was found to be lower than other types of cheese during ripening. This is caused by that the cheese milk is subject to heat treatment; however, starter culture is used. In a study about Gokceada artisanal goat cheese, it has been noted that starter cultures no added cheese's degradation

rate of αs1-caseins is less than those added mesophilic culture (Hayaloglu et al., 2013b).

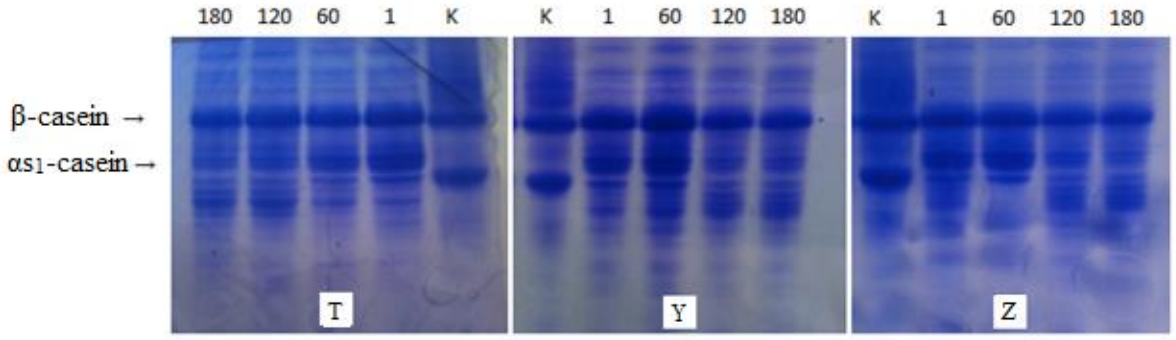

Figure 2. Urea-polyacrylamide gel electrophoretograms of Ezine cheese made using different heat treatment norms at 65 °C for 10 (T), 20 (Y) and 30 (Z) minutes during ripening K: Na-caseinate. 1, 60, 120, 180: Days of ripening period, respectively. T (at 65 °C for 10 min), Y (at 65 °C for 20 min) and Z (at 65 °C for 30 min).

3.5. Microbiological analysis

The counts of total aerobic mesophilic bacteria, lactic acid bacteria, coliforms, *E. coli*, and yeast and mould in Ezine cheese during ripening are given in the Table 4. Different heat treatment norms had statistically affected the number of total aerobic mesophilic bacteria of Ezine cheese at 60 days of ripening ($p<0.05$). Aygun et al. (2005) reported that TAMB counts of 50 samples of a traditional Turkish Carra cheese were between 4.56 and 9.89 log cfu/g. The number of aerobic mesophilic bacteria of Ezine cheese decreased during ripening. This reduction was found to be statistically significant for Z cheese which made of the milk was heat-treated for 30 minutes ($p<0.05$). Yangılar and Özdemir, (2013) stated that total aerobic mesophilic bacteria counts in Turkish White Cheese decreased during ripening. The reason for this may the increase of acidity. Öner et al. (2006) reported that the number of total aerobic mesophilic bacteria decreased in artisanal Turkish White cheese, and Çetinkaya and Soyutemiz (2006) stated that it decreased in Kaşar during ripening.

Different heat treatment norms did not affect the counts of lactic acid bacteria in Ezine cheese ($p>0.05$). The change to lactic acid bacteria counts in the T cheese (at 65 °C for 10 minutes) was statistically significant during ripening ($p <0.05$). Cambaztebe et al. (2009) reported that due to the salt and acid concentrations, the counts of lactic acid bacteria decreased in Civil cheese during ripening. Rynne et al. (2007) stated that the pasteurization time and ripening period does not affect non-starter lactic acid bacteria count in semi-fat Cheddar cheese, however, the counts of bacteria increased during ripening, especially in the first 60 days.

Different heat treatment norms and ripening did not affect the coliform bacteria count of Ezine cheese. It has been reported that while the pH of Turkish White cheese was decreasing, the count of coliform bacteria was also decreased during ripening (Dertli et al., 2003). Mirzaei (2011) reported that coliform bacteria counts were reduced from 4.69 log cfu/g to 1.66 log cfu/g for 90 days of ripening in Lighvan cheese. It is considered that the reasons for this are that Ezine cheese has coliform group of bacteria, that it is a type of bacteria which is transmitted by contamination, and that the study was carried out with such cheeses having different hygienic qualities.

Table 4 .Results of microbiological analysis of Ezine cheese made using different heat treatment norms at 65 °C for 10 (T), 20 (Y) and 30 (Z) minutes during ripening (log cfu/g) (n=3).

Microbial groups (log cfu/g)	Cheeses			
	Days	T	Y	Z
Total aerobic mesophilic bacteria	1	8.48±0.22^{aA}	8.40±0.29^{aA}	8.46±0.09^{aA}
	60	8.53±0.11^{aA}	8.40±0.07^{abA}	8.19±0.16^{bAB}
	120	8.47±0.15^{aA}	8.20±0.21^{aA}	7.75±0.07^{bBC}
	180	8.23±0.31^{aA}	8.02±0.15^{abA}	7.34±0.40^{aB}
Lactic acid bacteria	1	6.54±0.63^{aB}	6.69±0.37^{aA}	6.05±0.73^{aA}
	60	8.07±0.46^{aA}	7.57±0.66^{aA}	7.41±0.68^{aA}
	120	7.22±0.38^{aAB}	7.10±0.72^{aA}	7.47±0.38^{aA}
	180	6.81±0.24^{aB}	6.95±0.10^{aA}	6.82±0.42^{aA}
Coliform bacteria	1	4.09±0.83^{aA}	4.91±0.59^{aA}	4.35±1.45^{aA}
	60	3.36±1.50^{aA}	3.69±1.49^{aA}	3.12±1.19^{aA}
	120	2.42±1.24^{aA}	3.37±0.99^{aA}	3.20±0.50^{aA}
	180	2.59±0.09^{aA}	2.44±0.12^{aA}	2.76±0.25^{aA}
E. coli	1	3.44±0.16^{aA}	3.70±0.34^{aA}	3.28±0.25^{aA}
	60	1.54±053^{aB}	1.17±0.58^{aB}	1.25±0.31^{aB}
	120	0.23±0.40^{bC}	0.89±0.17^{aB}	0.00±0.00^{bC}
	180	0.23±0.40^{abC}	0.79±0.17^{aB}	0.00±0.00^{cC}
Yeast and mould	1	2.83±0.06^{aA}	2.89±0.26^{aA}	2.61±0.31^{aA}
	60	3.80±1.03^{aA}	3.27±0.15^{aA}	2.74±0.87^{aA}
	120	3.47±1.49^{aA}	3.01±1.22^{aA}	3.50±0.46^{aA}
	180	3.10±0.66^{aA}	2.98±0.78^{aA}	1.82±1.85^{aA}

$^{a-c}$ Means in the same row with different letters are significantly different (p<0.05). $^{A-C}$Means in the same column with different letters are significantly different (p<0.05). T (at 65 °C for 10 min), Y (at 65 °C for 20 min) and Z (at 65 °C for 30 min).

E. coli is a biotype of coliform which is a pathogenic microorganism. It is transmitted to the food as a result of contamination just as the coliform and *E. coli* cells are sensitive to temperature. Thus, its existence in pasteurized food indicates that heat treatment was inadequate, or that it was contaminated later (Ünlütürk and Turantaş, 2003). Different heat treatment norms statistically affected *E. coli* count of Ezine cheese at 120 and 180 days of ripening ($p<0.05$). *E. coli* could not be found in the Z cheese (at 65 °C for 30 minutes), especially at 120 and 180 days of ripening. *E. coli* counts of cheeses statistically decreased during ripening ($p<0.05$). Similar results were reported by Manolopoulou et al. (2003) for Feta cheese.

It is seen that different heat treatment norms and ripening did not significantly affect the yeast and mold counts in Ezine cheese ($p>0.05$). However, yeast and mold counts increased between 60 and 120 days of ripening period. Then, it was decreased after 120 days of ripening period. It is considered that the reasons for that each stage of ripening of cheese has yeast and mold are the water activity of cheese as well as changes to pH and salt concentrations (Mirzaei, 2011; Yangılar and Özdemir, 2013). Sengul et al. (2009) pointed out that the ripening period did not affect the yeast and mold count in Cecil cheese. The average of yeast and mould counts of Turkish White Cheese were between 5.38 and 4.65 log cfu/g that were found by Dertli et al., (2012).

4. CONCLUSION

The following results were achieved for the Ezine cheese manufactured and stored for 180 days based on the different heat treatment norms. Different heat treatment norms did not affect the composition of cheese. There had been significant increases in nitrogen fractions during ripening. αs1-casein was more degraded than β-casein during ripening. The presence of coliform bacteria and *E. coli* indicated the contamination during production. Therefore, it is important to comply with the rules of hygiene during production and storage. Consequently, heat treatment at 65 ° C for 30 minutes is recommended to produce because of lower heat treatment norms caused higher counts of coliform bacteria and *E. coli*. More studies are needed on Ezine cheese product. Especially, the contamination points should be determined and the studies should be carried out at different pasteurization temperatures.

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