The effect of storage temperatures and packaging methods on properties of Motal cheese

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ABSTRACT

The effects of storage temperature (+4°C and −18°C) and packaging method (nonvacuum and vacuum) on biogenic amines in Motal cheese during storage periods were investigated. In addition, dry matter, titratable acidity, total nitrogen, water-soluble nitrogen, trichloroacetic acid-soluble nitrogen, phosphotungstic acid-soluble nitrogen, free amino group (proteolysis), electrophoretic patterns of casein, and amounts of lactic acid bacteria and coliforms were determined. Storage period had a significant effect on all of the biogenic amines. When compared with vacuum packaging, normal packaging had higher amounts of putrescine, cadaverine, histamine, and tyramine. Coliforms were not found at detectable levels (<100 cfu/g) in all cheese samples. Results of urea-PAGE analysis of cheese samples were in good agreement with biogenic amine results and other proteolysis parameters.

Key words: biogenic amine, microbiological and chemical properties, Motal cheese

INTRODUCTION

Turkey is the largest producer of milk and dairy products in the region. Cheese is an important product of all the dairy products produced in Turkey. There are more than 100 varieties of cheese in Turkey, but they are limited to about 30 different cheese types when grouped according to their similarities. Three major cheese types are produced in Turkey: white pickled cheese, Kaşar cheese, and Tulum cheese. Tulum cheese, a special cheese produced in Turkey, is not well known elsewhere. It is preferred for its characteristically natural moldy taste and flavor. During the ripening period, natural contaminating molds grow and contribute to the ripening process. Tulum is a semi-hard cheese that can be made from whole, semi-skim, or skim milk from sheep, goats, cows, and buffaloes, or a mixture of these milks. It has a crumbly texture and a strong flavor.

In total, 12.243 million tonnes of milk is produced in Turkey per year (Anonymous, 2008), and 2% of the milk is processed to Tulum cheese (Anonymous, 2001). Motal cheese is a type of Tulum cheese. Two types of cheese are used in the making of Motal cheese: white cheese and Civil cheese. The white cheese is generally produced using sheep milk in the sheep milking period, whereas Civil cheese uses cow's milk. The cheese may be packaged into animal skin, and butter or cream may be added to it depending on the preference of the producer. Butter is added to the cheese mixture (equal amounts of each cheese) at a ratio of approximately 1 kg per 18-L tin of cheese (approximately 5% of total cheese) (Coşkun et al., 1998; Andiç, 1999; Kamber, 2008).

The ripening time of Motal cheese is at least 4 mo and the ability to shorten the ripening time without any loss of flavor characteristics would be very advantageous. However, the shelf life of Motal cheese under refrigeration conditions is very short because of microbial activity and mold contamination. As a result of excessive ripening, some of the cheeses in the dairy plant or in the consumer's refrigerator can deteriorate and the cheese is discarded. Therefore, exploring the feasibility of extended storage of Motal (Tulum) cheeses in frozen storage, and the evaluation of effects of such storage on chemical, biochemical, and microbiological characteristics is of great importance (Coşkun et al., 1998; Andiç, 1999; Kamber, 2008). In contrast to those of many other cheese varieties, the textural properties of Motal cheese are not adversely affected by freezing, which provides another advantage for frozen storage of Motal cheese.

Frozen foods have an excellent overall record of safety, and illnesses associated with frozen foods are rare. However, few researchers have studied the effect of freezing on fully ripened and partially ripened cheeses, even though this preservation method is routinely used in many cheese factories. The most noteworthy of these studies have focused on partial aspects of the sensory, functional, or chemical characteristics of Gorgonzola and Provolone (Ottogalli and Rondinini, 1974), Mozzarella (Oberget et al., 1992; Califano and Bevilacqua, 1999; Chaves et al., 1999), Cheddar (Lück, 1977; Sen

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and Gupta, 1987; Kasprzak et al., 1994), and Manchego-type cheese (Prados et al., 2006), resulting in the conclusion that frozen storage is suitable for cream cheese, fully ripened Manchego-type cheese, unripened Camembert, and brick cheese, but not for Gouda or Cheddar cheese.

Cervantes et al. (1983) concluded that freezing (1-wk storage) and thawing did not affect the quality of Mozzarella cheese as assessed by compression, beam bending, and sensory evaluation. Recent studies on the effect of freezing rates and frozen storage duration on the sensorial (Tejada et al., 2000), chemical, and microbiological characteristics (Tejada et al., 2002) of ripened Los Pedroches cheese and the sensorial, microbiological, and compositional characteristics of Manchego-type cheese (Prados et al., 2006) concluded that these cheeses could be stored at -20°C for approximately 6 mo without any significant alteration of the characteristics studied. In studies of the changes in organic acid composition of plain soft goat milk cheese, Park et al. (2004) reported that a 6-mo-long frozen storage appears to be feasible for off-season marketing of soft goat milk cheese.

The major factors that govern the formation, accumulation, and types of amines are probably the availability of amino acids (and hence proteolysis of cheese) and the presence of bacteria able to decarboxylate amino acids (Chang et al., 1985; Stratton et al., 1992). Numerous bacteria, both intentional and adventitious, are capable of amine production. These are *Escherichia coli*, *Enterobacter aerogenes*, *Streptococcus mitis*, *Streptococcus faecium*, *Streptococcus lactis*, *Clostridium perfringens*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus arabinosa*, *Lactobacillus fermentum*, *Lactobacillus buchneri* and *Leuconostoc* spp. (Chang et al., 1985; Stratton et al., 1992). Knowledge of the biogenic amine levels in Motal cheese is necessary to make an assessment of the health hazard arising from the consumption of these products, especially by susceptible individuals.

The aim of this study was to determine the effect of frozen storage and different packaging methods on the formation of biogenic amines and on some chemical, biochemical, and microbiological properties of Motal cheese during the storage period. Also, this study had the wider objective of introducing Motal cheese to the world.

MATERIALS AND METHODS

Motal Cheesemaking

Whole sheep and cow milk was supplied from a local dairy plant (Van, Turkey). Commercial animal rennet was obtained from Mayasan Company (İstanbul, Turkey). Cheese samples were manufactured at the Food

Engineering Laboratory of Agriculture Faculty of Yüzüncü Yıl University (Van, Turkey). Whole raw sheep milk was coagulated with rennet for 75 min. After coagulation, the curd was cut into 8- to 10-mm cubes with a wire knife and pressed for 90 min. The curd was salted at a level of 2%. After salting, the curd was held for an additional 48 h. At the end of this period, the obtained white cheeses were well packed in plastic bags with brine containing 7% NaCl. The samples were ripened at $5 \pm 1^{\circ}\text{C}$ for 90 d.

Skimmed raw cow milk was tested for acidity (0.45% lactic acid) at room temperature. Then, acidified skim milk was heated and coagulated. After coagulation, the curd was shaped by hand and salted at a level of 2% NaCl. After salting, the curd was held for an additional 48 h for dehydration. Finally, Civil cheeses were well packed in plastic bags with brine containing 7% NaCl. The samples were ripened at $5 \pm 1^{\circ}\text{C}$ for 90 d.

White and Civil cheese were sliced and crumbled. The Motal cheese was a mixture of Civil cheese (50%), white cheese (50%), and butter (approximately 5% of total cheese) produced in the laboratory. The Motal cheese was divided into 4 groups. Two groups were vacuum packaged in polyethylene bags, and one was stored at $+4 \pm 1^{\circ}\text{C}$ and the other at $-18 \pm 1^{\circ}\text{C}$ for 180 d. The other 2 groups were packaged without vacuum in polyethylene bags, and one was stored at $+4 \pm 1^{\circ}\text{C}$ and the other at $-18 \pm 1^{\circ}\text{C}$ for 180 d. Samples of Motal cheese were analyzed at 0, 30, 60, 120, and 180 d of the storage period.

Sampling

Samples were taken for microbial and chemical analyses. Cheeses were analyzed for biogenic amine contents (cadaverine, putrescine, tyramine, tryptamine, histamine, and phenylethylamine), lactic acid bacteria (LAB) and coliform counts, titratable acidity, total nitrogen (TN), water soluble-nitrogen (WSN), trichloroacetic acid-soluble nitrogen (TCA-SN), and phosphotungstic acid soluble nitrogen (PTA-SN). Samples for biogenic amine determination were stored at -86°C until analysis. The cheeses were grated, homogenized thoroughly, and analyzed immediately. Each analysis was performed in duplicate.

Biogenic Amine Analysis

Six aqueous standard solutions containing cadaverine dihydrochloride, putrescine dihydrochloride, tyramine hydrochloride, tryptamine hydrochloride, phenylethylamine hydrochloride, and histamine dihydrochloride, and 1,7-diaminoheptane (as internal standard) from Sigma (St. Louis, MO) were derivatized as described for the cheese samples.

Biogenic amine contents of the samples were determined according to the method of Eerola et al. (1993). Biogenic amines were extracted from 2.0-g samples with 0.4 M perchloric acid and detected as their dansyl derivatives by HPLC. The gradient-elution system was 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. The gradient-elution program was started at 50% solvent B and ended at 90% solvent B in 25 min. The system was equilibrated for 10 min before the next analysis. The flow rate was 1.0 mL/min and the column temperature was 40°C. A 20- μ L sample was injected onto the column. Peaks were detected at 254 nm using the HPLC system with a column Spherisorb ODS2 150A, 150 $\times$ 4.60 mm (Waters, Milford, MA) and a gradient pump, which included an Agilent HPLC (1100 series, G1311A Quaternary Pump, G1315A Diode Array Detector, G1313A Autosampler, G1322A Vacuum Degasser; Agilent, Palo Alto, CA), and a computer including the Agilent package program. The quantitative determinations were carried out by internal standard (1,7-diaminoheptane) method, using peak heights. Biogenic amine contents were expressed as milligrams per kilogram.

Microbiological Analysis

For each cheese sample, 10 g was weighed, diluted aseptically in 90 mL of citrate buffer (2%, wt/vol), and homogenized in a sterile polyethylene bag using a Stomacher 400 (Seward Laboratory, London, UK) for 1.5 min. For microbiological analysis, a 10-g sample was prepared by homogenizing with 90 mL of physiological saline water (0.85% NaCl) in a sterile polyethylene bag using a Stomacher 400 (Seward Laboratory) for 1 min. Further decimal dilutions were prepared from this homogenized mixture. The following incubation conditions for microbiological analysis were used: de Man, Rogosa, Sharpe agar (Merck, Darmstadt, Germany) for 3 d at 37°C for LAB, and violet red bile agar (Merck) for 2 d at 37°C for coliforms. Bacteria counts were expressed as colony-forming units per gram of sample (log cfu/g).

Physical, Chemical, and Electrophoretic Analyses

Cheese samples were analyzed for titratable acidity as lactic acid percentage according to the method described by Case et al. (1985). The cheese samples were analyzed for TN using micro-Kjeldahl digestion and distillation units (Nüve, Ankara, Turkey). The cheese sample was ground and homogenized. Cheese was digested in H₂SO₄, using CuSO₄·5H₂O (Merck) as catalyst with K₂SO₄ (Merck) as boiling point elevator, to release nitrogen from protein and retain nitrogen as ammonium salt. The initial 1-g test portion size as-

sured an adequate amount of H₂SO₄ remaining at the end of digestion. Concentrated NaOH (Merck) was added to release NH₃, which is distilled, collected in H₃BO₃ (Merck) solution, and titrated (AOAC, 1990). Total solids content was determined by weight difference using a drying oven (Nüve) according to the methods described by Case et al. (1985). Water-soluble N, TCA-SN, and PTA-SN compounds of cheeses were determined as described by Butikofer et al. (1993). The free amino group contents of samples were estimated by the trinitrobenzene sulfonic acid (TNBS) method of Polychroniadou (1988). The reaction was performed with different amounts of extract (originally 0.5 mL) and with a modified volume (0.2 to 0.5 mL) to make use of the whole linear range of absorbance according to the Lambert-Beer law. The quantity of free amino groups was expressed in glycine (Merck) equivalents. Blank and calibration tests were carried out to determine the means and standard deviations in the method (Polychroniadou, 1988; Tunçtürk, 1996). All determinations were in duplicate. The electrophoretic analysis of casein patterns was conducted with the method of Creamer (1991) with some modifications (Tarakçı et al., 2004). Electrophoresis unit was supplied by Owl (Owl Separation Systems, Portsmouth, NH) and the power unit by Consort (Consort, Turnhout, Belgium).

Statistical Analysis

The result of analyses that depended on storage temperatures, packaging methods, and storage periods were analyzed according to a complete randomized design with 2 replicates. All data were subjected to variance analyses, and differences between means were evaluated by Tukey's multiple range test (significance $P < 0.05$) using the SPSS statistic program (version 8.00, 1997; SPSS, Chicago, IL software). To gain insight into the structure of the data set, principal components analysis (PCA) was performed. Principal components analysis is a well-known mathematical transformation of the raw data; it is an exploratory technique that indicates relationships among groups of variables and second shows relationships between objects (Piggott and Sharman, 1986).

RESULTS AND DISCUSSION

Biogenic Amine Analysis

Biogenic amine statistical analysis results of the Motal cheese samples are shown in Table 1. Putrescine, cadaverine, and tyramine were the major amines in Motal cheese, whereas phenylethylamine concentration was very low in all samples. The results show that the level of biogenic amines increased slightly from 0 to 30

d, changed significantly from 30 to 60 d, and showed a 3- or 10-fold increase from 60 to 180 d, except for tryptamine and phenylethylamine in Table 1. This may be explained by the suitable temperature during storage period and the abundance of substrate (FAA) after 60 d. This trend was different from the results reported by Schneller et al. (1997), who found that total biogenic amines level remained more stable after 3 mo, even in cheeses with high biogenic amine content ($>3,000$ mg/kg).

Storage temperatures had a very significant effect ($P < 0.01$) on the biogenic amines except for phenylethylamine (Table 1). These effects were determined in putrescine, cadaverine, histamine, and tyramine at a storage temperature of $+4^{\circ}\text{C}$, which were statistically different from that of the other storage temperature ($P < 0.01$). Biogenic amine amounts increased during storage at $+4^{\circ}\text{C}$, whereas the amounts remained almost unchanged at -18°C (Table 1). This finding is because microbiological activity is slowed at -18°C and biogenic amines cannot be formed by microorganisms (Joosten and Van Boekel, 1988).

Storage period had a significant effect ($P < 0.01$) on all biogenic amines. The cheeses stored at $+4^{\circ}\text{C}$ were found to contain more biogenic amines except phenylethylamine and tryptamine compared with cheeses stored at -18°C . Tyramine, histamine, cadaverine, and putrescine concentrations were increased in the samples stored at $+4^{\circ}\text{C}$ for 180 d. This may be explained by the appropriate temperature for microorganism growth and the availability of substrate (FAA) formed during storage period. However, LAB are still viable and may be producing biogenic amines even at the end of this period. In addition, nonstarter lactobacilli and enterococci have been implicated in the production of high levels of biogenic amines in most cheese types (Joosten and Northolt, 1987; Roig-Sagués et al., 2002; McSweeney, 2004).

Packaging method had a significant effect ($P < 0.01$) on cadaverine, histamine, tyramine, and phenylethylamine concentrations in the samples stored at $+4^{\circ}\text{C}$ at 180 d. The non-vacuum-packaged cheeses had higher amounts of putrescine, cadaverine, histamine, and tyramine ($P < 0.01$) compared with vacuum-packaged cheeses. In cheese packaged using both methods and stored at $+4^{\circ}\text{C}$, casein was found to be strongly degraded by the enzymes, which increased the content of FAA in cheese. These FAA were then used in production of carbon dioxide and amines by bacteria (Darwish, 1993)

Tyramine

Tyramine is one of the biogenic amines that can cause some health disorders in sensitive people. From 0 to 180

d of ripening, tyramine was the major biogenic amine in Motal cheese (329.41 mg/kg at 180 d); its level increased during storage ($+4^{\circ}\text{C}$ in vacuum and non-vacuum packaging). At 180 d it was within the concentrations found by Schneller et al. (1997) in semi-soft cheeses but lower than that reported by Valsamaki et al. (2000). The results show that the level of tyramine increased slightly from 0 to 60 d and then increased almost 5-fold until 180 d in vacuum- and non-vacuum-packaged samples stored at $+4^{\circ}\text{C}$ (Table 1). There were no changes in tyramine concentration during the storage period at -18°C in vacuum- and non-vacuum-packaged samples (Table 1). Tyramine levels were apparently affected by the storage conditions.

Öner et al. (2004) reported that tyramine levels were found to be higher than tryptamine levels in Tulum cheeses. Tyramine content was detected in one sample at a level of 329.00 mg/kg and in the others in the range of 30.40 to 186.53 mg/kg by these researchers. Only one sample did not include tyramine (Öner et al., 2004). Tyramine levels were found to be 212.5 mg/kg by Silvana et al. (1998). In this study, we found almost the same results as these authors.

Durlu-Özkaya et al. (2000) investigated some biogenic amines in Tulum cheese and found that tyramine was the dominant biogenic amine in the stored samples. The level of tyramine ranged from 109.6 to $1,575.5$ mg/kg. The tyramine content in dairy products has been studied in Spanish cheeses by Pechaneck et al. (1983) and they found amounts of tyramine between 50.69 and 735.69 mg/kg in various cheese samples. These amounts are higher than those in the current study. Enterococci members of the LAB may cause the formation of tyramine (Leuschner et al., 1999).

In most cases, consumption of food containing biogenic amines does not lead to illness because amine-destroying enzymes in the digestive tract prevent the uptake of these potentially hazardous compounds into the bloodstream. The tyramine levels detected in this work were <800 mg/kg, which is the upper limit that can be consumed without noticeable effects (Joosten, 1988; Nout, 1994).

Histamine

Histamine production draws the attention of many researchers because the most frequent foodborne intoxications caused by biogenic amines involve this amine. Symptoms of clinical illness have been associated with consumption of 100 to 180 mg of histamine (Joosten and Van Boekel, 1988). Histamine concentration in our Motal cheeses (0 d) was found to be approximately 27.65 mg/kg and increased to 219.63 mg/kg at 180 d ($+4^{\circ}\text{C}$ nonvacuum packaging). Histamine levels were

Table 1. Overall effect of storage temperature, packaging method, and storage period on the biogenic amines (mg/kg) of Motal cheese (values are means $\pm$ SD)

Amine	Storage temperature and packaging method	Storage period (d)				
		0	30	60	120	180
Tyramine	−18°C; vacuum	149.02 $\pm$ 5.80 ^{a,A}	146.07 $\pm$ 9.52 ^{b,A}	151.00 $\pm$ 20.22 ^{b,A}	146.47 $\pm$ 9.40 ^{b,A}	152.24 $\pm$ 32.07 ^{c,A}
	−18°C; nonvacuum	153.66 $\pm$ 16.36 ^{c,a,A}	145.40 $\pm$ 9.81 ^{b,A}	149.77 $\pm$ 7.82 ^{b,A}	153.56 $\pm$ 19.33 ^{b,A}	157.23 $\pm$ 24.52 ^{c,A}
	+4°C; vacuum	154.54 $\pm$ 21.34 ^{a,C}	177.93 $\pm$ 11.23 ^{a,b,C}	250.44 $\pm$ 4.58 ^{a,BC}	289.38 $\pm$ 18.14 ^{b,AB}	364.87 $\pm$ 47.01 ^{b,A}
	+4°C; nonvacuum	152.00 $\pm$ 41.15 ^{a,C}	187.91 $\pm$ 7.66 ^{a,C}	236.80 $\pm$ 21.50 ^{a,C}	447.07 $\pm$ 65.11 ^{a,B}	643.31 $\pm$ 44.15 ^{a,A}
Histamine	−18°C; vacuum	30.54 $\pm$ 2.19 ^{a,A}	28.10 $\pm$ 9.09 ^{a,A}	28.76 $\pm$ 5.20 ^{b,A}	30.25 $\pm$ 3.80 ^{b,A}	27.83 $\pm$ 9.69 ^{c,A}
	−18°C; nonvacuum	27.69 $\pm$ 6.00 ^{a,A}	25.96 $\pm$ 4.80 ^{a,A}	27.42 $\pm$ 5.39 ^{b,A}	28.18 $\pm$ 5.73 ^{b,A}	30.27 $\pm$ 5.56 ^{c,A}
	+4°C; vacuum	30.48 $\pm$ 5.00 ^{a,C}	41.37 $\pm$ 5.60 ^{a,C}	69.31 $\pm$ 5.46 ^{a,BC}	102.27 $\pm$ 14.21 ^{a,B}	150.61 $\pm$ 14.71 ^{b,A}
	+4°C; nonvacuum	27.65 $\pm$ 9.43 ^{a,B}	40.50 $\pm$ 3.72 ^{a,B}	69.45 $\pm$ 10.41 ^{a,B}	189.90 $\pm$ 16.97 ^{a,A}	219.63 $\pm$ 27.77 ^{a,A}
Cadaverine	−18°C; vacuum	40.58 $\pm$ 1.00 ^{a,A}	43.20 $\pm$ 11.36 ^{c,A}	43.80 $\pm$ 5.84 ^{c,A}	37.80 $\pm$ 6.15 ^{b,A}	42.70 $\pm$ 10.49 ^{c,A}
	−18°C; nonvacuum	43.38 $\pm$ 3.52 ^{a,A}	41.53 $\pm$ 4.51 ^{c,A}	43.05 $\pm$ 6.36 ^{c,A}	42.03 $\pm$ 4.60 ^{b,A}	43.70 $\pm$ 8.77 ^{c,A}
	+4°C; vacuum	54.11 $\pm$ 5.99 ^{a,D}	239.68 $\pm$ 20.41 ^{a,C}	250.16 $\pm$ 6.69 ^{b,C}	507.87 $\pm$ 25.89 ^{a,B}	666.72 $\pm$ 39.72 ^{b,A}
	+4°C; nonvacuum	46.92 $\pm$ 15.20 ^{a,C}	144.65 $\pm$ 12.85 ^{b,C}	371.19 $\pm$ 7.98 ^{a,BC}	559.17 $\pm$ 34.80 ^{a,B}	1,166.85 $\pm$ 188.34 ^{a,A}
Putrescine	−18°C; vacuum	29.36 $\pm$ 1.03 ^{a,A}	31.49 $\pm$ 8.10 ^{c,A}	32.75 $\pm$ 3.92 ^{c,A}	31.58 $\pm$ 2.81 ^{b,A}	31.71 $\pm$ 10.61 ^{b,A}
	−18°C; nonvacuum	31.33 $\pm$ 3.32 ^{a,A}	30.22 $\pm$ 3.12 ^{c,A}	32.39 $\pm$ 4.64 ^{c,A}	31.41 $\pm$ 4.98 ^{b,A}	32.41 $\pm$ 3.42 ^{b,A}
	+4°C; vacuum	43.66 $\pm$ 6.90 ^{a,C}	230.09 $\pm$ 14.89 ^{a,B}	201.13 $\pm$ 3.45 ^{b,B}	379.11 $\pm$ 14.13 ^{a,A}	438.19 $\pm$ 56.34 ^{a,A}
	+4°C; nonvacuum	36.12 $\pm$ 8.29 ^{a,C}	144.12 $\pm$ 12.57 ^{b,BC}	317.70 $\pm$ 11.95 ^{a,AB}	316.50 $\pm$ 82.87 ^{a,AB}	525.20 $\pm$ 110.59 ^{a,A}
Phenylethylamine	−18°C; vacuum	9.64 $\pm$ 0.45 ^{a,A}	10.15 $\pm$ 2.74 ^{a,A}	10.41 $\pm$ 3.53 ^{a,A}	11.18 $\pm$ 1.37 ^{a,A}	11.37 $\pm$ 2.12 ^{b,A}
	−18°C; nonvacuum	9.63 $\pm$ 0.95 ^{a,A}	10.24 $\pm$ 2.50 ^{a,A}	10.07 $\pm$ 2.43 ^{a,A}	10.51 $\pm$ 2.16 ^{a,A}	14.53 $\pm$ 3.55 ^{ab,A}
	+4°C; vacuum	9.40 $\pm$ 0.21 ^{a,B}	7.52 $\pm$ 1.36 ^{a,B}	7.71 $\pm$ 1.63 ^{a,B}	13.92 $\pm$ 4.14 ^{a,AB}	21.06 $\pm$ 1.39 ^{a,A}
	+4°C; nonvacuum	8.90 $\pm$ 3.11 ^{a,A}	7.51 $\pm$ 2.12 ^{a,A}	10.07 $\pm$ 1.70 ^{a,A}	7.71 $\pm$ 2.01 ^{a,A}	9.37 $\pm$ 1.03 ^{b,A}
Tryptamine	−18°C; vacuum	128.80 $\pm$ 19.23 ^{a,A}	148.45 $\pm$ 26.40 ^{a,B}	158.84 $\pm$ 12.52 ^{a,B}	138.29 $\pm$ 19.40 ^{a,B}	104.28 $\pm$ 8.24 ^{a,B}
	−18°C; nonvacuum	152.11 $\pm$ 18.27 ^{a,A}	92.22 $\pm$ 3.31 ^{a,B}	111.14 $\pm$ 6.83 ^{b,AB}	109.52 $\pm$ 14.40 ^{a,AB}	79.10 $\pm$ 10.32 ^{a,B}
	+4°C; vacuum	139.59 $\pm$ 29.15 ^{a,A}	104.86 $\pm$ 7.34 ^{a,AB}	110.41 $\pm$ 5.01 ^{b,AB}	98.41 $\pm$ 3.70 ^{a,AB}	77.42 $\pm$ 10.51 ^{a,B}
	+4°C; nonvacuum	114.32 $\pm$ 21.54 ^{a,A}	123.69 $\pm$ 5.37 ^{a,A}	78.49 $\pm$ 10.05 ^{b,A}	125.00 $\pm$ 18.81 ^{a,A}	78.72 $\pm$ 3.39 ^{a,A}

^{a-c}Different lowercase letters within a column and amine indicate significant differences between cheese samples ($P < 0.05$).^{A-D}Different uppercase letters within a row (sample) and amine indicate significant differences between ripening periods ($P < 0.05$).

slightly increased from 0 to 30 d, then increased from 30 to 60 d, and almost doubled from 60 to 180 d at +4°C in vacuum- and non-vacuum-packaged samples (Table 1). There were no changes in histamine concentration during storage at -18°C in vacuum- and non-vacuum-packaged samples (Table 1). Histamine levels were obviously influenced by the storage conditions.

Low storage temperature (-18°C) and vacuum packaging had an obvious positive effect to prevent histamine production during the storage period (180 d), and histamine levels remained lower in the samples stored under these conditions. Joosten and Van Boekel (1988) investigated histamine in Feta cheese and the level was found to be 65 mg/kg, much lower than that found in this study. Similar results (196.5 mg/kg) were reported by Silvana et al. (1998). Low storage temperature (-18°C) and vacuum packaging were found to be the most important factor in preventing the accumulation of histamine in Motal cheese.

In our study, a substantial increase of histamine content was observed after 60 d and the increase did not stop until the end of the storage period. It is possible that the decarboxylases produced by the microflora, directly in the milk or in the first step of cheese making, can also continue their action in cheese. Thus, histidine decarboxylase activity increased during the storage period and reached maximum level at the end of the storage period. As a result of this, histamine content was low (about 70 mg/kg) during the 60 d and then quickly increased until the end of the storage period. According to Joosten and Van Boekel (1988), substrate availability could be an accelerating factor for histamine production in cheese. Also, heterofermentative mesophilic lactobacilli have been reported to be important in histamine build-up in cheese, because they combine high decarboxylase activity with the potential to grow during ripening. Histamine accumulates in cheese during ripening because of the presence of some strains of mesophilic and thermophilic lactobacilli; for example, *Lactobacillus buchneri* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Joosten and Northolt, 1987) from milk and other sources. The use of raw milk or post-contamination in cheese may result in high levels of histamine formation (Stratton et al., 1992).

Cadaverine

Our results showed that the level of cadaverine regularly increased from the beginning until the end of storage period in +4°C samples, and increased almost 10- to 20-fold for vacuum and non-vacuum-packed samples, respectively (Table 1). There was no change in cadaverine concentration during the storage period at -18°C for each packaging type.

Cadaverine has less pharmacological activity than the aromatic amines but it is probably potentiators of their toxicity (Joosten, 1988). Non-vacuum-packed samples at +4°C storage had higher cadaverine contents than vacuum-packed samples. In this study, we found that cadaverine could reach levels >1,000 mg/kg at +4°C without vacuum packaging at the end of the storage period. Similar results (1,110.0 mg/kg) were reported by Silvana et al. (1998).

In our study, an important increase in cadaverine concentration was found after 30 d. Formation of the excessive amounts of cadaverine in cheese may be used as an indicator of extended protein degradation and spoilage. *Enterobacteriaceae* are possibly responsible for cadaverine build-up, especially decarboxylase-positive strains with low death rate (Joosten and Northolt, 1987; Schneller et al., 1997). In this study, the coliform amount was found to be <2.00 log cfu/g throughout the storage period. Some mixtures of lactobacilli have been found to produce cadaverine (besides tyramine and histamine), and salt-tolerant lactobacilli cause massive formation of cadaverine (Joosten and Northolt, 1987).

Putrescine

The levels of putrescine were significantly increased from 0 to 180 d for the cheeses stored at +4°C in both vacuum and non-vacuum packages (Table 1). Putrescine concentrations were found to be very stable for cheeses in both types of package and stored at -18°C (Table 1).

In this study, we found that putrescine levels of cheeses at the end of storage period can reach to levels >500 mg/kg when stored at +4°C without vacuum packaging. Putrescine content was quite high (36.12 and 525.20 mg/kg at 0 and 180 d, respectively). Similar putrescine levels were found by Joosten and Northolt (1987); however, Schneller et al. (1997) found lower values. Joosten (1988) reported that putrescine formation ceased after 30 d, and Schneller et al. (1997) observed a decrease in putrescine content after 3 mo of ripening. In contrast, in our experiment, an important increase in putrescine concentration was found after 0 d at +4°C with or without vacuum packaging throughout the storage periods. Silvana et al. (1998) reported that putrescine level was 173.7 mg/kg. In this study, we found higher levels compared with previous reports.

Phenylethylamine and Tryptamine

Phenylethylamine concentration was not increased during the storage period (Table 1). From a good manufacturing practice point of view, a level of 30 mg/kg of phenylethylamine is regarded as acceptable (Nout,

1994). In this study, phenylethylamine never exceeded a concentration of 30 mg/kg even at the end of the storage period. The final (180 d) phenylethylamine level was comparable to those of the other biogenic amines, but the rate of its accumulation was much slower.

Öner et al. (2004) reported that tryptamine was found in low concentrations from 0.32 to 40.44 mg/kg in Tulum cheese, much lower than our findings. Also, one sample did not contain detectable amounts of tryptamine in their study. In this study, tryptamine was detected at lower amounts compared with histamine, putrescine, cadaverine, and tyramine at the end of the storage period. Tryptamine was found to be lower at the end of the storage period than the initial level (Table 1). This might be explained by its being consumed by microorganisms as a nitrogen source. Also, some researchers have suggested that the decrease of biogenic amines during ripening could be related to the activity of bacterial amine oxidases (Leuschner et al., 1998).

Some enzyme-catalyzed reactions in cellular system also accelerate during freezing, but this is believed to be caused by freeze-induced dislocation of enzymes, substrates, or enzyme activators, rather than to solute concentration. The increase in volume that accompanies ice formation, as well as freeze-concentration effects, is responsible for this dislocation (Fennema, 1996).

Microbiological, Physical, and Chemical Analysis

Storage temperature and storage period had a very significant effect ($P < 0.01$) on dry matter, titratable acidity, WSN, TCA-SN, PTA-SN, and LAB counts, but had no effect on TN. Coliforms were not found at detectable levels (100 cfu/g) during the storage period. Coliform counts were $<2.0 \log \text{cfu/g}$, indicating that there was no contamination. In fact, coliforms may have disappeared during the ripening and storage period because of the conditions of the medium (concentration of salt, high lactic acid concentration, and microorganism occurrence). Coşkun et al. (1998) reported coliform bacteria counts of $4.63 \log \text{cfu/g}$ in Motal cheese. According to our analyses, coliform bacteria counts in Motal cheese were lower than findings of Coşkun et al. (1998). The packaging method used, the storage temperature, and storage period had very significant effects ($P < 0.01$) on LAB counts (Table 2). Lactic acid bacteria counts in vacuum-packaged cheeses were lower than those in non-vacuum-packaged cheeses. The main factor for biogenic amine production in cheese is the presence of microorganisms with high decarboxylation activity (Joosten and Northolt, 1987; Schneller et al., 1997). The biogenic amine formation in this study cannot be linked to specific species because

of the lack of detailed microbiological analyses. Lactic acid bacteria are less active in the decarboxylation of amino acids, but in the light of the high populations reached in cheese over long ripening periods, their contribution should not be disregarded (Öner et al., 2004). Enterobacteriaceae, heterofermentative lactobacilli, and *Enterococcus faecalis* were associated with considerable production up to 600 ppm of biogenic amines including phenylethylamine (Nout, 1994). Lactobacilli were found to be the major group in Motal cheese (Andiç, 1999).

The mean nitrogen content of Motal cheese samples was approximately 4.00%, and differences were not significant ($P > 0.05$) among the cheese samples (Table 2). The extent of proteolysis was evaluated by several methods including WSN. The WSN content is one of the most important changes during storage period. It can be seen in Table 2 that the values of WSN were significantly ($P < 0.01$) higher in the cheeses stored at $+4^\circ\text{C}$ than in cheeses stored at -18°C . The results show that the content of WSN was significantly increased from 0 to 180 d. Different packaging methods, storage temperatures, and storage periods had very significant effects ($P < 0.01$) on WSN, TCA-SN, and PTA-SN (Table 2).

The environmental conditions of these cheeses (low acidity, high moisture content) favor the activity of chymosin on α_{S1} -casein, resulting in the rapid production of water-soluble peptides (Romeih et al., 2002). The TCA-SN and PTA-SN increased continuously ($P < 0.01$) in all cheese samples during storage (Table 2). It is known that TCA-SN is an indication of the amount of small peptides (<20 AA residues) and AA present in cheese and its level is regarded as an index of ripening extent. Tri- and dipeptides and FAA are soluble in the PTA-SN fraction. These peptides and AA are produced mainly by the action of microorganisms (starter and nonstarter organisms) on the caseins and their peptides (Tarakçı, 2004). Nitrogen fraction (WSN, TCA-SN, and PTA-SN) content increases during ripening of cheese, as does FAA content; FAA are precursors of biogenic amines. Titratable acidity was affected by storage temperature and storing period (Table 2). In this study, we found higher acidity (0.54% lactic acid) than that of Coşkun et al. (1998) in Motal cheese (Table 2).

Proteolysis is one of the most important changes during storage period. The concentration of free amino groups increased during the storage period in all samples. It can be seen in Table 2 that the values of proteolysis level were significantly ($P < 0.01$) higher in the cheeses stored at $+4^\circ\text{C}$ than in those stored at -18°C . Also, proteolysis levels in the non-vacuum-packaged samples stored at $+4^\circ\text{C}$ were higher than those in vacuum-packaged samples at the end of the storage period. The results show that the level of proteolysis

Table 2. Overall effects of storage temperature, packaging method, and storage period on microbiological, physical, and chemical characteristics of Motal cheese (values are means $\pm$ SD)

Item	Storage temperature and packaging method	Storage period (d)				
		0	30	60	120	180
DM (%)	−18°C; vacuum	51.83 $\pm$ 0.95 ^{a,A}	51.61 $\pm$ 0.02 ^{a,A}	50.92 $\pm$ 0.14 ^{a,B}	51.68 $\pm$ 0.01 ^{a,A}	50.96 $\pm$ 0.06 ^{ab,B}
	−18°C; nonvacuum	51.81 $\pm$ 0.39 ^{a,A}	50.90 $\pm$ 0.19 ^{b,C}	50.83 $\pm$ 0.24 ^{a,C}	51.67 $\pm$ 0.16 ^{a,AB}	51.10 $\pm$ 0.04 ^{a,BC}
	+4°C; vacuum	52.15 $\pm$ 0.19 ^{a,A}	51.60 $\pm$ 0.20 ^{a,B}	50.87 $\pm$ 0.08 ^{a,C}	51.24 $\pm$ 0.15 ^{a,BC}	50.90 $\pm$ 0.05 ^{b,C}
	+4°C; nonvacuum	52.17 $\pm$ 0.22 ^{a,A}	50.31 $\pm$ 0.10 ^{b,B}	50.53 $\pm$ 0.08 ^{a,B}	50.13 $\pm$ 0.01 ^{a,B}	49.44 $\pm$ 0.01 ^{c,C}
Titratable acidity (%)	−18°C; vacuum	0.89 $\pm$ 0.01 ^{ab,A}	0.85 $\pm$ 0.01 ^{ab,A}	0.86 $\pm$ 0.06 ^{a,A}	0.86 $\pm$ 0.05 ^{b,A}	0.88 $\pm$ 0.01 ^{b,A}
	−18°C; nonvacuum	0.90 $\pm$ 0.02 ^{a,A}	0.82 $\pm$ 0.02 ^{b,A}	0.87 $\pm$ 0.01 ^{a,A}	0.88 $\pm$ 0.01 ^{b,A}	0.91 $\pm$ 0.06 ^{b,A}
	+4°C; vacuum	0.96 $\pm$ 0.02 ^{a,A}	0.96 $\pm$ 0.05 ^{a,A}	0.94 $\pm$ 0.00 ^{a,A}	0.99 $\pm$ 0.04 ^{ab,A}	1.05 $\pm$ 0.09 ^{ab,A}
	+4°C; nonvacuum	0.82 $\pm$ 0.00 ^{b,C}	0.91 $\pm$ 0.02 ^{ab,C}	0.94 $\pm$ 0.02 ^{a,C}	1.11 $\pm$ 0.005 ^{a,B}	1.23 $\pm$ 0.04 ^{a,A}
Total N (%)	−18°C; vacuum	3.81 $\pm$ 0.08 ^{a,A}	4.00 $\pm$ 0.04 ^{a,A}	3.96 $\pm$ 0.20 ^{a,A}	4.01 $\pm$ 0.17 ^{a,A}	4.08 $\pm$ 0.15 ^{a,A}
	−18°C; nonvacuum	3.95 $\pm$ 0.13 ^{a,A}	4.05 $\pm$ 0.09 ^{a,A}	4.05 $\pm$ 0.08 ^{a,A}	4.02 $\pm$ 0.18 ^{a,A}	4.10 $\pm$ 0.01 ^{a,A}
	+4°C; vacuum	4.01 $\pm$ 0.03 ^{a,A}	4.04 $\pm$ 0.09 ^{a,A}	3.96 $\pm$ 0.05 ^{a,A}	4.05 $\pm$ 0.22 ^{a,A}	4.06 $\pm$ 0.11 ^{a,A}
	+4°C; nonvacuum	4.10 $\pm$ 0.31 ^{a,A}	3.94 $\pm$ 0.11 ^{a,A}	4.03 $\pm$ 0.19 ^{a,A}	3.96 $\pm$ 0.05 ^{a,A}	4.10 $\pm$ 0.07 ^{a,A}
Water-soluble N (%)	−18°C; vacuum	0.28 $\pm$ 0.001 ^{a,C}	0.28 $\pm$ 0.001 ^{b,BC}	0.29 $\pm$ 0.001 ^{b,B}	0.29 $\pm$ 0.001 ^{c,AB}	0.30 $\pm$ 0.003 ^{b,A}
	−18°C; nonvacuum	0.28 $\pm$ 0.001 ^{a,C}	0.28 $\pm$ 0.001 ^{b,C}	0.29 $\pm$ 0.002 ^{b,B}	0.30 $\pm$ 0.001 ^{c,A}	0.30 $\pm$ 0.001 ^{b,A}
	+4°C; vacuum	0.28 $\pm$ 0.001 ^{a,E}	0.30 $\pm$ 0.001 ^{b,D}	0.32 $\pm$ 0.002 ^{b,C}	0.35 $\pm$ 0.003 ^{b,B}	0.41 $\pm$ 0.005 ^{b,A}
	+4°C; nonvacuum	0.28 $\pm$ 0.001 ^{a,D}	0.47 $\pm$ 0.01 ^{a,C}	0.56 $\pm$ 0.03 ^{a,B}	0.96 $\pm$ 0.004 ^{a,A}	0.99 $\pm$ 0.001 ^{a,A}
Trichloroacetic acid-soluble N (%)	−18°C; vacuum	0.11 $\pm$ 0.005 ^{a,D}	0.14 $\pm$ 0.006 ^{c,C}	0.17 $\pm$ 0.006 ^{c,B}	0.18 $\pm$ 0.008 ^{c,AB}	0.20 $\pm$ 0.003 ^{c,A}
	−18°C; nonvacuum	0.11 $\pm$ 0.005 ^{a,D}	0.15 $\pm$ 0.001 ^{c,C}	0.17 $\pm$ 0.001 ^{c,B}	0.19 $\pm$ 0.008 ^{c,A}	0.21 $\pm$ 0.002 ^{c,A}
	+4°C; vacuum	0.12 $\pm$ 0.001 ^{a,E}	0.23 $\pm$ 0.005 ^{b,D}	0.27 $\pm$ 0.006 ^{b,C}	0.30 $\pm$ 0.002 ^{b,B}	0.33 $\pm$ 0.01 ^{b,A}
	+4°C; nonvacuum	0.12 $\pm$ 0.001 ^{a,D}	0.28 $\pm$ 0.003 ^{a,C}	0.44 $\pm$ 0.002 ^{a,B}	0.81 $\pm$ 0.03 ^{a,A}	0.86 $\pm$ 0.02 ^{a,A}
Phosphotungstic acid-soluble N (%)	−18°C; vacuum	0.057 $\pm$ 0.003 ^{a,C}	0.06 $\pm$ 0.005 ^{c,C}	0.09 $\pm$ 0.002 ^{b,B}	0.10 $\pm$ 0.003 ^{b,AB}	0.11 $\pm$ 0.004 ^{c,A}
	−18°C; nonvacuum	0.054 $\pm$ 0.002 ^{a,D}	0.07 $\pm$ 0.001 ^{c,C}	0.09 $\pm$ 0.002 ^{b,B}	0.10 $\pm$ 0.005 ^{b,B}	0.11 $\pm$ 0.001 ^{c,A}
	+4°C; vacuum	0.060 $\pm$ 0.01 ^{a,D}	0.08 $\pm$ 0.001 ^{b,CD}	0.10 $\pm$ 0.001 ^{ab,BC}	0.11 $\pm$ 0.001 ^{b,B}	0.16 $\pm$ 0.001 ^{b,A}
	+4°C; nonvacuum	0.053 $\pm$ 0.005 ^{a,D}	0.10 $\pm$ 0.001 ^{a,C}	0.11 $\pm$ 0.01 ^{a,C}	0.22 $\pm$ 0.01 ^{a,B}	0.26 $\pm$ 0.01 ^{a,A}
Proteolysis (mMol)	−18°C; vacuum	128.33 $\pm$ 8.04 ^{a,B}	123.98 $\pm$ 8.25 ^{a,B}	153.42 $\pm$ 8.25 ^{b,AB}	166.98 $\pm$ 3.53 ^{b,A}	172.63 $\pm$ 8.56 ^{c,A}
	−18°C; nonvacuum	130.10 $\pm$ 8.89 ^{a,A}	132.03 $\pm$ 7.85 ^{a,A}	147.14 $\pm$ 28.44 ^{b,A}	173.64 $\pm$ 1.18 ^{b,A}	176.742 $\pm$ 16.89 ^{c,A}
	+4°C; vacuum	133.54 $\pm$ 7.37 ^{a,C}	186.20 $\pm$ 20.82 ^{a,BC}	254.51 $\pm$ 16.89 ^{a,B}	281.97 $\pm$ 41.25 ^{a,B}	408.39 $\pm$ 34.17 ^{b,A}
	+4°C; nonvacuum	124.53 $\pm$ 1.34 ^{a,D}	185.93 $\pm$ 25.93 ^{a,CD}	254.63 $\pm$ 6.68 ^{a,C}	334.45 $\pm$ 14.14 ^{a,B}	578.82 $\pm$ 30.64 ^{a,A}
Lactic acid bacteria (log ₁₀ cfu/g)	−18°C; vacuum	6.94 $\pm$ 0.00 ^{a,A}	6.75 $\pm$ 0.12 ^{b,A}	6.63 $\pm$ 0.20 ^{b,A}	6.04 $\pm$ 0.06 ^{b,B}	6.56 $\pm$ 0.01 ^{b,A}
	−18°C; nonvacuum	6.94 $\pm$ 0.01 ^{a,A}	6.33 $\pm$ 0.11 ^{c,C}	6.88 $\pm$ 0.05 ^{ab,A}	6.64 $\pm$ 0.11 ^{a,AB}	6.44 $\pm$ 0.01 ^{b,BC}
	+4°C; vacuum	6.77 $\pm$ 0.03 ^{ab,A}	6.50 $\pm$ 0.03 ^{bc,A}	6.52 $\pm$ 0.13 ^{b,A}	5.63 $\pm$ 0.21 ^{b,B}	6.57 $\pm$ 0.08 ^{b,A}
	+4°C; nonvacuum	6.55 $\pm$ 0.15 ^{b,B}	7.12 $\pm$ 0.06 ^{a,A}	7.13 $\pm$ 0.03 ^{a,A}	6.97 $\pm$ 0.11 ^{a,A}	7.09 $\pm$ 0.02 ^{a,A}

^{a-c}Different lowercase letters within a column and item indicate significant differences between cheese samples ($P < 0.05$).^{A-E}Different uppercase letters within a row (sample) and item indicate significant differences between ripening periods ($P < 0.05$).

Figure 1. Changes in urea-PAGE casein patterns of cheese samples during the 180-d storage period. 0, 30, 60, 120, 180 = days of storage; std = casein standard (50% sheep milk + 50% cow milk); lane 1 = -18°C , vacuum package; lane 2 = -18°C , nonvacuum package; lane 3 = $+4^{\circ}\text{C}$, vacuum package; lane 4 = $+4^{\circ}\text{C}$ nonvacuum package; g = γ -caseins; b = β -casein; a1 = α_{S1} -casein of sheep milk; a2 = α_{S1} -casein of cow milk.

was significantly increased from 0 to 180 d. Different packaging methods, storage temperatures, and storage periods had significant effects ($P < 0.01$) on proteolysis (Table 2).

From the appearance of the gel obtained by urea-PAGE, no remarkable difference was observed in protein bands for 2 frozen samples during ripening (Figure 1). However, decreases in both β -casein and α_{S1} -casein intensities in the samples stored at $+4^{\circ}\text{C}$ was more evident than in the samples stored at -18°C . In particular, α_{S1} -casein of non-vacuum-packaged sample was more hydrolyzed during the ripening period by the ripening process. Casein can be hydrolyzed by milk plasmin, microbial enzymes, and the clotting enzyme used (Carmona et al., 1999). In this type of cheese, activity of molds was also very high, particularly in the non-vacuum-packaged cheese stored at $+4^{\circ}\text{C}$. It can be seen from the gel that α_{S1} -casein from cow's milk is more intense than that from sheep milk. As mentioned in the Materials and Methods, Civil cheese used for Motal cheese production was produced from skim cow's milk. Thus, the ratio of casein from cow's milk was higher than that from sheep milk in the Motal cheese mass. Electrophoretic results are in good agreement with other proteolysis parameters such as WSN, TCA-SN, PTA-SN, and free amino groups. In addition, tyramine, histamine, cadaverine, and putrescine contents of cheese samples were found to be in good harmony with electrophoretic findings.

The results of PCA of the mean values of biogenic amines, dry matter, titratable acidity (lactic acid %), TN, WSN, TCA-SN, PTA-SN, proteolysis, and counts

of LAB of Motal cheese samples are depicted in a 2-dimensional plot (Figure 2).

Results from the PCA showed that principal components (PC) 1 and 2 described about 76.284% of the total variation of sample: 61.992% PC1 and 14.293% PC2. Principal component 1 was heavily loaded on titratable acidity, WSN, TCA-SN, PTA-SN, LAB, tryptamine, putrescine, cadaverine, histamine, tyramine, and proteolysis, whereas component 2 was loaded on storage period, dry matter, TN, and phenylethylamine. The PCA analysis showed that cadaverine, putrescine, tyramine, histamine, titratable acidity, WSN, TCA-SN, PTA-SN, and proteolysis were positively correlated to each other, and the correlation was very high. Figure 2 also presents the positive low correlation between TN and phenylethylamine. Results also showed that there is negative significant relationship among dry matter, tryptamine, and storage period attributes (Figure 2).

In this study, no significant correlation was found between the amounts of phenylethylamine and the other biogenic amines in the cheese samples in Figure 2. We found a high positive significant correlation between proteolysis, nitrogen fraction, and some biogenic amines. There was no correlation between LAB and biogenic amine content. Microorganisms with a decarboxylase activity can be starter microorganisms (Fernández-García et al., 2000), nonstarter lactic acid bacteria, or other spontaneous microflora (Roig-Sagués et al., 2002). However, it is difficult to find a straight correlation between microorganism counts and biogenic amine content in cheese, because amine-producing abilities of different strains of various bacteria differ widely

Figure 2. Principal component analysis biplot of biogenic amines and some microbiological-chemical properties of Motal cheese. DRYMAT = dry matter; LA = titratable acidity; TN = total nitrogen; WSN = water-soluble nitrogen; TCA = trichloroacetic acid-soluble nitrogen; PTA = phosphotungstic acid-soluble nitrogen; PROTEOL = proteolysis; LAB = lactic acid bacteria; TYR = tyramine; HIS = histamine; CAD = cadaverine; PUT = putrescine; PA = phenylethylamine; TRP = tryptamine.

(Halasz et al., 1994; Valsamaki et al., 2000; Innocente and D'Agostin, 2002).

CONCLUSIONS

The results from this study showed that storage temperature, packaging method, and storage period had significant effects on the formation of biogenic amines except phenylethylamine. Tyramine, putrescine, and cadaverine were found to be the most abundant biogenic amines in Motal cheese. Low storage temperature (-18°C) and vacuum hindered formation of putrescine, cadaverine, histamine, and tyramine. Histamine content was found to be much higher than 100 mg/kg at the end of storage, and its formation accelerated after 60 d of storage. After 60 d, total biogenic amines were $>1,000$ mg/kg in almost all cheeses stored at $+4^{\circ}\text{C}$ in both package types, but the biogenic amine contents of cheeses stored at -18°C were found to not change throughout the storage period. Storage temperature and storage period had a significant effect ($P < 0.01$) on dry matter, titratable acidity, WSN, TCA-SN, PTA-SN, proteolysis, and LAB counts; there was no effect on TN. Motal cheese could cause intoxication if exposed to extremely high contamination (especially mold) or stored for a long period at high temperatures. There-

fore, to extend the shelf life of Motal cheese, evaluation of the effects of storage conditions and packaging methods on chemical and biochemical characteristics is of great importance. Efforts should be made to understand amine formation in cheese to optimize technology and ensure low amine levels.

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