Characterization of aroma-active compounds, sensory properties, and proteolysis in Ezine cheese

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ABSTRACT

Ezine cheese is a white pickled cheese ripened in tinplate containers for at least 8 mo. A mixture of milk from goat, sheep, and cow is used to make Ezine cheese. Ezine cheese has geographical indication status. The purposes of this study were to determine and compare the changes in basic composition, aroma, and sensory characteristics, and proteolytic activity of Ezine cheese stored in tinplate containers and plastic vacuum packages during storage. Aroma-active compounds were determined by thermal desorption gas chromatography olfactometry. To evaluate the proteolytic activity, casein and nitrogen fractions were determined. The results indicated that compounds identified at high intensities were dimethyl sulfide, ethyl butyrate, hexanal, ethyl pentanoate, (Z)-4-heptenal, 1-octen-3-one, acetic acid, butyric acid, and *p*-cresol. Characteristic descriptive terms were cooked, whey, creamy, animal-like, sour, and salty. The level of proteolysis increased in Ezine cheese during storage. Ezine cheese can be ripened in small-size packaging after 3 mo of storage. Approximately 6 mo is sufficient to produce the characteristic properties of Ezine cheese.

Key words: Ezine cheese, aroma, proteolysis, packaging type

INTRODUCTION

Ezine cheese is a full-fat, white pickled cheese variety. Ezine cheese is produced from the mixture of goat milk (at least 40%), sheep milk (45–55%), and cow milk (at most 15%) provided from the towns and villages located in the north and west of the Mount Ida region of Turkey. Ezine cheese has geographical indication (TPE, 2006) status. In general, the cheese is ripened in tinplate containers for at least 8 mo. Cheese milk is

pasteurized and no starter culture is used for production of the cheese.

Cheese flavor originates from enzymatic, chemical, or microbial transformations of protein, fat, lactose, and citrate in milk (Law, 1984; Fox et al., 1995). Lactose, lipid, and protein catabolisms are main sources of generation of aroma compounds in cheese. These pathways are activated by endogenous enzymes in milk, coagulating enzymes, and microbial enzymes used to manufacture or ripen cheese.

Massouras et al. (2006) determined volatile flavor compounds of Teleme cheese made from sheep and goat milk by using the headspace technique. Teleme cheese is also a white pickled cheese variety and starter culture is used to produce this cheese. Teleme cheese is salty and sour. It can be produced from sheep, goat, cow milk, or any mixture thereof (Massouras et al., 2006). In this study, a total of 21 major compounds were identified, including aldehydes, alcohols, ketones, and acids.

Proteolysis is the major biochemical process in the development of cheese flavor. Degradation of casein by milk coagulating enzymes and proteinases and peptidases from lactic acid bacteria leads to the generation of peptides and free amino acids. Amino acids do not contribute to the flavor, but they are precursors of the aromatics. Degradation of amino acids to alcohols, aldehydes, acids, esters, and sulfur compounds is required for development of characteristic flavor in certain types of cheeses (Kranenburg et al., 2002). Amino acid catabolites by lactic acid bacteria isolated from Cheddar cheese were reported by Singh et al. (2003). For example, methionine/cysteine is a precursor of methanethiol, which has a cabbage-like aroma note. Methionine is also precursor of methional with boiled potato aroma. In addition, *p*-cresol is produced by degradation of tyrosine (Singh et al., 2003). Acetaldehyde is a major aroma compound of fermented dairy foods and threonine aldolase converts threonine into acetaldehyde (Ott et al., 2000).

Proteolytic activity of cheese is caused by enzymes from coagulant, milk, and bacteria (starter, nonstarter, or secondary starter) (Fox, 1989). Hayaloglu et al. (2005) investigated the effect of certain starter cultures

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on proteolytic profile and ripening characteristics of Turkish White brined cheese during a 3-mo ripening period. The results of that study showed that cheeses manufactured with a starter culture had a higher concentration of soluble N. Urea PAGE patterns also showed that hydrolysis of caseins accelerated during ripening. Specifically, α_{S1} -casein was extensively degraded after 30 d of storage. However, β -casein was degraded slowly during maturation.

Ezine cheese has its own characteristic taste and aroma different from other white pickled cheeses. No starter culture is used to produce this cheese. It is produced in a specific region and widely consumed in Turkey. It has economical importance for the region. Chemical composition and sensory attributes of Ezine cheeses (22 samples) collected from local producers were characterized (Karagul-Yuceer et al., 2007). A descriptive sensory evaluation technique was used to determine flavor profile. Major descriptive terms generated by panel members were FFA, cooked, creamy, whey-like, animal-like, salty, and sour. In general, Ezine cheese is ripened in tinsplate containers (17–20 kg capacity). However, at the end of the ripening period, cheeses can be repacked in smaller packages, such as tinsplate or plastic vacuum packaging, for retail purposes.

Characteristic aroma-active compounds and effects of packaging type and size on some properties of Ezine cheese were not determined. The objectives of this study were to determine and compare the changes in basic composition, aroma and sensory characteristics, and proteolytic activity of Ezine cheese stored in tinsplate containers and plastic vacuum packages over 12 mo.

MATERIALS AND METHODS

Cheese Making Procedure

Cheese was produced in a local cheese plant in May. The mixture of goat milk, sheep milk, and cow milk (1,500–2,000 L) was pasteurized at 67°C for 30 min. After coagulation of milk by rennet enzyme at 32 to 34°C, cheese gel was cut and whey was drained. Then, cheese curd was molded and immersed in brine (14–16%). After that, cheese molds were put in tinsplate containers (20 kg capacity) and brine was added. After finishing a 3-mo ripening period in the tinsplate container, cheeses were repacked in 1-kg plastic vacuum packaging or 1-kg tinsplate containers. Three groups of cheeses (**C**: 20-kg tinsplate coated with lacquer, **T**: 1-kg tinsplate coated with lacquer, and **V**: 1-kg plastic vacuum pack) were analyzed in every 3-mo period. Duplicate cheese samples were used for each period. Cheeses were ripened at 2 to 4°C for 12 mo.

Composition of Cheese

Procedures described by Bradley et al. (1992) were followed to determine titratable acidity (lactic acid, %), pH, dry matter (%), total protein (%), salt (%), and ash (%) in the cheeses. Fat content was determined by the Gerber-van Gulik method (NEN 3059, 1969).

Analysis of Aroma Compounds

Aroma compounds (Tables 2 and 3) were provided by Aldrich Chemical Co. (St. Louis, MO), Bedoukian Research Inc. (Danbury, CT), Merck (Darmstadt, Germany), Fluka (Seelze, Germany), and Aromsa (Gebze, Kocaeli, Turkey).

Sample Preparation

Thermal Extraction. To isolate aroma compounds in cheese, a thermal extractor (TE 2, Gerstel GmbH and Co. KG, Mülheim an der Ruhr, Germany) was used. Three grams of cheese, homogeneously prepared, was put in a glass extraction tube and aroma compounds were purged under an ultra-pure nitrogen gas stream at constant flow on Tenax TA (Gerstel GmbH and Co. KG) absorbent. Tenax TA is a porous material and it contains approximately 60 mm or 180 mg of 2,6-diphenylene oxide polymer. The thermal extraction process was applied at 30°C for 15 min by using a heater (Aux Controller 163, Gerstel GmbH and Co. KG).

Thermal Desorption. Volatiles adsorbed on the Tenax TA were thermally desorbed (TDS 2, Gerstel GmbH and Co. KG) and cryo-focused at –150°C before injection (Cooled Injection System CIS, Gerstel GmbH and Co. KG) to perform gas chromatography-olfactometry (**GCO**) analysis. A similar extraction and desorption procedure was used for the characterization of nutty flavor in Cheddar cheese (Avsar et al., 2004).

GCO

The GCO system consisted of an HP 6890 GC (Agilent Technologies, Wilmington, DE) equipped with a flame-ionization detector (**FID**), a sniffing port, and cooled injection system (**CIS**). Helium was used as a carrier gas. Inlet pressure was 20 psi and total flow was 25.1 mL/min. A polar capillary column (HP-Innowax 30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m film thickness; J&W Scientific, Folsom, CA) and a nonpolar column (HP-5 30 m length $\times$ 0.32 mm i.d. $\times$ 0.25 μ m film thickness; J&W Scientific) were used for sniffing. After the analytical column, effluent was split 1:1 between the FID and olfactory port using deactivated fused

silica capillaries (90 cm length $\times$ 0.25 mm i.d.). Oven temperature was programmed from 30 to 240°C at a rate of 10°C/min, with initial and final hold times of 3 and 20 min, respectively. The FID and sniffing port were maintained at the temperatures of 320°C and 200°C, respectively. The CIS was programmed from -150 to 280°C at a rate of 12°C/s. Final hold time was 3 min. Equilibrium time was 0.05 min. Postpeak intensity scale was used for determination of aroma intensity. Two sniffers quantified the odor intensities using a 10-point scale anchored on the left with “not” and on the right with “very.” Odorants were identified by comparing retention indices and odor quality of unknowns with those of authentic standards analyzed at the same experimental conditions. Retention indices were calculated by using n-alkane series (Van den Dool and Kratz, 1963). Sniffers had 80 h of experience with GCO technique, scale using, and odor description.

Sensory Analysis

A roundtable discussion with a 9-member panel (4 females and 5 males, ages ranged from 24 to 39 yr) was conducted to identify the descriptive flavor terms for the cheeses (Meilgaard et al., 1999). Panelists were introduced to the flavor terms developed by Karagul-Yuceer et al. (2007) for Ezine cheese. The same descriptive terms were used by the panel members. Sensory evaluation was started in the third month of storage and samples were evaluated every 3 mo over the 12-mo storage period. Panelists quantified the attributes using 15-point product-specific scales anchored on the left with “not” and on the right with “very” (Meilgaard et al., 1999). The panel received about 50 h of training during generation and definition of descriptive terms. Panelists were presented with water, unsalted crackers, and expectoration cups to cleanse the palate between samples. Cheeses were presented in 3-digit-coded styrofoam plates. Duplicate samples were served in the different sessions. All panelists in a session evaluated the same cheeses in a randomized order.

Electrophoretic Analysis

Sample Preparation. A homogeneous sample (0.5 g) was transferred into a 50-mL Falcon tube. Ether was used to extract the fat from the cheese. Sample buffer was prepared by mixing 7.5 g of Tris, 490 g of urea, and 4 mL of HCl acid (37.5%) and made up to 1 L with distilled water. The 0.5-g sample was dissolved in 25 mL of buffer. After mixing, the sample was kept in a 55°C waterbath for 10 min, and then it was centrifuged

at $3,000 \times g$ at 10°C for 10 min (Sigma, Gottingen, Germany).

The central portion (2 mL) was transferred into a 15-mL centrifuge tube, 60 μ L of 2-mercaptoethanol and 40 μ L of bromphenol blue (0.1%) were added, and then mixed. Casein standards (β , bovine milk, min. 90%; and α , bovine milk, min. 70%; Sigma-Aldrich) were dissolved in sample buffer. Samples and standards were applied into gel with a 10- μ L syringe (Hamilton, Bonaduz, Switzerland).

Urea PAGE Analysis. The procedure for urea PAGE of casein samples was similar to the method reported by Andrews (1983) with some modification. The assays were carried out with a Protean LI XI vertical slab unit and a Powerpac Basic power supply (Bio-Rad Laboratories Ltd., Watford, UK).

The slab gels consisted of a 4% stacking gel and a 12% separating gel. Separating gel buffer was prepared by dissolving 3.2 g of Tris and 19.3 g of urea in 100 mL of distilled water, and then the pH was adjusted to 8.8 with 0.1 N HCl using a pH meter. The solution was made up to 50 mL with distilled water. Then, 3.75 mL of 40% acrylamide/bisacrylamide (37.5/1) solution, 8.75 mL of separating buffer, 6 μ L of tetramethylethylenediamine, and 25 μ L of ammonium persulfate solution (10% wt/vol) was transferred into an Erlenmeyer flask, shaken, and immediately poured into the gel apparatus. Distilled water (0.5–1 mL) was placed on the gel solution for a uniform surface. After polymerization, water was removed by drying paper.

Stacking gel buffer was prepared by dissolving 0.41 g of Tris and 15 g of urea in 100 mL distilled water, and then the pH was adjusted to 8.4. The solution was made up to 50 mL. Then, 0.5 mL of 40% acrylamide/bisacrylamide (37.5/1) solution, 4.5 mL of stacking gel buffer, 5 μ L of tetramethylethylenediamine, and 20 μ L of ammonium persulfate were added. After shaking, this solution was poured on the separating gel. After polymerization, the comb was removed.

Electrophoresis running buffer was prepared by dissolving 3 g of Tris and 14.6 g of glycine in 1,000 mL of distilled water, then the pH was adjusted to 8.4. Electrophoresis was performed at room temperature at 100 V until the end of the stacking gel. Then voltage was increased to 110 V until the tracking dye reached the bottom of the gel. The gel was stained with Coomassie Brilliant Blue R250 and dried with a gel air drying system (Bio-Rad Laboratories Ltd.). Image analysis was performed by PC scanner (HP Scanjet 2400, Agilent Technologies). Casein band density was determined by using a Gel-Pro Analyzer (Version 4, MediaCybernetics, Bethesda, MD).

Nitrogen Fractions

Water-Soluble Nitrogen. Grated cheese samples (10 g) were mixed with 40 mL of distilled water and blended with an electric hand-held homogenizer (Biospec Products Inc., Bartlesville, OK) for 3 min. The mixture was held at 40°C for 1 h in a waterbath (ST-402, Nüve, Ankara, Turkey) and then centrifuged (Sigma 2-16K, Sigma Zentrifugen, Osterode am Harz, Germany) at $3,000 \times g$ for 30 min at 4°C. The upper fat layer was removed and the supernatant filtered through Whatman #42 filter paper (Kuchroo and Fox, 1982). Ten milliliters of filtrate was taken and the nitrogen content was determined by the micro-Kjeldahl method (IDF, 1993).

Trichloroacetic Acid Soluble Nitrogen (12%). Trichloroacetic acid soluble nitrogen (TCASN) was prepared by mixing 25 mL of water-soluble extract with an equal amount of 24% trichloroacetic acid solution. The mixture was held at room temperature for 1 h and then filtered through Whatman #42 filter paper (Polychroniadou et al., 1999). Twenty-five milliliters of filtrate was taken and the nitrogen content was determined by the micro-Kjeldahl method (IDF, 1993).

Phosphotungstic Acid Soluble Nitrogen (5%). Phosphotungstic acid soluble nitrogen (PTASN) was prepared by mixing 5 mL of water-soluble extract with 3.5 mL of 3.95 M H₂SO₄ and 1.5 mL of 33.3% phosphotungstic acid solution. After the mixture was held overnight at 4°C, it was filtered through Whatman #42 filter paper (Jarrett et al., 1982). The nitrogen content of the filtrate was determined by the micro-Kjeldahl method (IDF, 1993).

Statistical Analysis

The effect of storage periods and packaging materials on sensory descriptors and nitrogen fractions of the cheeses were analyzed by factorial ANOVA model [1]. Tukey's HSD multiple comparison test was used for mean separations:

$$Y_{ijk} = \mu + S_i + P_j + (SP)_{ij} + e_{ijk}, \quad [1]$$

where Y_{ijk} is the observed or measured value of the cheese k in storage i and packaging j , μ is the general population mean, S_i is the effect of storage periods ($i = 1, 2, 3, 4$), P_j is the effect of packaging materials ($j = 1, 2, 3$), $(SP)_{ij}$ is the effect of storage by packaging interaction, and e_{ijk} is the random error term.

The nonmetric multidimensional scaling method was used to investigate the relations between some sensory and aroma characteristics. Multidimensional scaling is a technique for finding a configuration of points in

low-dimensional space that represents multivariate data. Essentially, the purpose of multidimensional scaling is to provide a visual representation of the pattern of proximities (i.e., similarities or distances) among a set of objects (Kruskal, 1964; Karagul-Yuceer et al., 2007). Stress coefficient and R^2 were used to determine goodness-of-fit. All statistical analyses were performed using SPSS for Windows (version 15.0; SPSS, 2006).

RESULTS AND DISCUSSION

Composition of Cheese

There were no significant differences among the cheeses in terms of dry matter content (%) during the 12-mo storage period ($P > 0.05$). However, packaging type significantly changed the dry matter ($P = 0.025$). The highest dry matter content was observed in sample C (48.76%) and the lowest one was in sample V (47.13%). This might be related to the packaging material and size. A significant interaction was determined between packaging material and storage ($P = 0.016$) in terms of salt content. Salt content of the cheeses ranged between 2.99 and 3.92%. No significant differences were observed in cheeses T and V during storage. However, sample C had the lowest salt content (2.99%) at the third month. In addition, no significant differences among the cheeses were observed after 3 mo of storage.

Table 1 shows the changes in lactic acid (%), pH, fat (%), and ash (%) contents of the cheeses over 12 mo. Acidity of cheeses in mo 9 to 12 was higher than the values at mo 3 and 6. No significant differences were observed in the fat contents of the cheeses during storage (Table 1). The highest ash content was observed at 12 mo (Table 1). Ezine cheeses were in the normal ranges in terms of acidity and composition (TS 591, 1995). These results were supported by the findings of Hayaloglu et al. (2005) for Turkish White brined cheese.

Aroma-Active Compounds and Sensory Evaluation

In total, 35 aroma-active compounds were identified in Ezine cheese during 12 mo of storage (Tables 2 and 3). These compounds belonged to different chemical families including aldehydes, ketones, esters, acids, sulfur compounds, and alcohol. Table 2 shows the aroma-active compounds, odor properties, and aroma intensities of the compounds determined in cheese samples before packaging on the first day of cheese production. Aroma intensities of cheeses stored in different packaging materials over 12 mo of storage can be seen in Table 3. Only 3 different odorants were identified in the cheese samples before packaging (Table 2). These

Table 1. Lactic acid, pH, fat, and ash during 12 mo of storage

Month	Composition/property (mean $\pm$ SE)			
	Lactic acid (%)	pH	Fat (%)	Ash (%)
3	0.67 $\pm$ 0.04 ^b	5.52 $\pm$ 0.02 ^b	24.67 $\pm$ 0.35 ^a	4.91 $\pm$ 0.05 ^b
6	0.62 $\pm$ 0.03 ^b	5.72 $\pm$ 0.02 ^a	24.75 $\pm$ 0.93 ^a	4.97 $\pm$ 0.05 ^b
9	0.86 $\pm$ 0.02 ^a	5.18 $\pm$ 0.01 ^c	24.83 $\pm$ 0.12 ^a	5.09 $\pm$ 0.04 ^{ab}
12	0.90 $\pm$ 0.02 ^a	5.13 $\pm$ 0.02 ^c	23.96 $\pm$ 0.20 ^a	5.22 $\pm$ 0.05 ^a
<i>P</i> -value	0.000	0.000	0.043	0.010

^{a-c}Means in the same column followed by different superscript letters represent significant differences.

odorants were propionic acid, (E,E)-2,4 decadienal, and β -ionene. These chemicals were not identified in aged cheeses. In general, intensities of aroma compounds for first day cheese (Table 2) were lower than samples stored in different packaging material for 12 mo (Table 3). Specifically, dimethyl sulfide with sulfur/corn-like aroma note, diacetyl with buttery note, and ethyl butyrate with bubble gum odor quality had very high aroma intensity on d 1 (Table 2).

Intensity of diacetyl was very high in the fresh cheese (Table 2). Probably, diacetyl was produced by the activity of nonstarter lactic acid bacteria in fresh cheese. However, lower odor intensities were observed in cheeses C, T, and V during storage (Table 3). High salt content and low pH values of cheeses during storage may inhibit the bacterial growth. Urbach (1997) showed that diacetyl was responsible for a mild and pleasant flavor of young Cheddar cheese. Utilization of citrate by lactic

Table 2. Aroma-active compounds in Ezine cheese by postpeak intensity (10-point scale) on d 1

No.	Chemical	RI ¹			Aroma intensity, ³ (mean $\pm$ SE)
		Innowax ⁴	HP5 ⁴	Odor quality ²	
1	Methanethiol	669	<500	Sulfur/cabbage	0.25 $\pm$ 0.25
2	Acetaldehyde	700	<500	Green apple	1.38 $\pm$ 0.38
3	Dimethyl sulfide	749	<500	Sulfur/corn	4.38 $\pm$ 0.88
4	2-Methylbutanal	903	680	Sweet/fruity	1.00 $\pm$ 0.25
5	Diacetyl	979	624	Buttery	6.00 $\pm$ 0.25
6	Ethyl butyrate	1,028	809	Bubble gum	4.00 $\pm$ 0.25
7	Hexanal	1,052	786	Cut grass	3.25 $\pm$ 0.25
8	3-Methylthiophene	1,070	779	Plastic	2.50 $\pm$ 0.01
9	2-Heptanone	1,123	868	Fatty	2.38 $\pm$ 0.38
10	Heptanal	1,166	905	Green	2.00 $\pm$ 0.50
11	(Z)-4-Heptenal	1,206	878	Oxidized fat	3.38 $\pm$ 1.62
12	Ethyl hexanoate	1,232	998	Fruity/pineapple	0.50 $\pm$ 0.50
13	1-Octen-3-one	1,241	978	Mushroom	3.75 $\pm$ 2.00
14	Unknown 1	1,269	680	Buttery/sour	1.88 $\pm$ 0.88
15	Octanal	1,289	1,048	Fatty	1.75 $\pm$ 0.50
16	2-Heptanol	1,285	959	Green/metallic	2.13 $\pm$ 1.13
17	2-Acetyl 1-pyrroline	1,312	915	Popcorn	2.50 $\pm$ 1.00
18	Unknown 2	1,327	—	Cheesy	1.25 $\pm$ 0.25
19	2-Nonanone	1,355	1,082	Oxidized/sour	1.00 $\pm$ 0.01
20	Methional	1,460	896	Boiled potato	2.75 $\pm$ 0.01
21	Acetic acid	1,466	652	Vinegar	1.38 $\pm$ 0.63
22	(E)-2-Nonenal	1,483	1,140	Hay/cucumber	1.75 $\pm$ 0.25
23	Propionic acid	1,497	920	Sour, sweaty	2.50 $\pm$ 0.25
24	Butyric acid	1,603	823	Rancid/cheesy	2.95 $\pm$ 1.05
25	(E,E)-2,4-Nonadienal	1,688	1,222	Fatty/soapy	0.88 $\pm$ 0.38
26	(E,E)-2,4-Decadienal	1,803	1,292	Frier oil	1.20 $\pm$ 0.30
27	Unknown 3	1,835	1,054	Burnt	2.13 $\pm$ 0.38
28	β -Ionene	1,913	1,492	Hay	1.00 $\pm$ 0.50
29	Maltol	1,991	1,070	Burnt sugar	2.70 $\pm$ 0.70
30	<i>p</i> -Cresol	2,061	1,090	Barny	2.15 $\pm$ 1.15
31	γ -Dodecalactone	>2,300	—	Sweet/lactone	1.00 $\pm$ 0.25

¹Retention indices on Innowax and HP5 columns.

²Aroma quality determined on olfactory port.

³Mean aroma intensities (postpeak intensity, 10-point scale) given by 2 sniffers on Innowax column. Odor intensities represent the mean of each sample evaluated in duplicate by 2 sniffers.

⁴J&W Scientific, Folsom, CA.

Table 3. Aroma-active compounds in Ezine cheeses stored in tinplate container and plastic vacuum over 12 mo of storage by postpeak intensity (10-point scale)

No.	Chemical	RI ¹		Odor quality ²	Aroma intensity ³											
					3 mo			6 mo			9 mo			12 mo		
		Innowax ⁴	HP5 ⁴		C	T	V	C	T	V	C	T	V	C	T	V
1	Methanethiol	669	<500	Sulfur/cabbage	1.87	3.70	2.87	1.12	2.12	1.37	2.25	2.75	2.50	1.85	3.12	2.87
2	Acetaldehyde	700	<500	Green apple	2.50	2.75	2.12	1.95	3.50	2.00	3.37	3.50	2.87	2.87	1.87	2.75
3	Dimethyl sulfide	749	<500	Sulfur/corn	3.50	2.00	1.85	2.90	3.75	2.37	3.37	3.50	3.87	2.57	3.25	3.07
32	Acetone	806	543	Sour milk	ND ⁵	1.50	1.20	0.37	ND	0.25	1.37	1.12	1.12	0.37	0.75	0.50
4	2-Methylbutanal	903	680	Sweet/fruity	0.75	0.50	1.37	1.70	1.45	1.00	0.37	1.00	2.00	2.12	1.37	0.75
5	Diacetyl	979	624	Buttery	1.70	3.45	1.77	1.60	2.87	2.62	2.50	3.12	2.27	2.15	2.37	1.62
6	Ethyl butyrate	1,028	809	Bubble gum	2.63	2.25	2.33	3.00	3.75	3.37	3.63	4.50	4.25	2.87	2.50	3.37
7	Hexanal	1,052	786	Cut grass	2.95	2.15	2.25	2.50	2.20	2.45	3.12	2.87	2.45	2.00	2.00	0.50
8	3-Methylthiophene	1,070	779	Plastic	1.37	4.75	3.00	2.75	3.37	1.50	2.75	3.50	3.45	2.57	3.22	2.25
9	2-Heptanone	1,123	868	Fatty	0.20	2.50	1.50	0.25	1.62	0.50	ND	ND	ND	0.90	1.00	0.37
10	Heptanal	1,166	905	Green	0.65	2.87	1.15	1.50	1.00	1.00	1.50	1.62	3.87	1.00	2.00	2.00
33	Ethyl pentanoate	1,191	939	Ester/fruity	2.63	3.65	1.70	2.50	3.37	2.52	3.95	4.25	3.37	3.50	5.00	3.25
11	(Z)-4-Heptenal	1,206	878	Oxidized fat	3.00	5.25	2.25	2.00	3.62	1.32	4.12	3.25	4.12	2.12	3.62	3.00
12	Ethyl hexanoate	1,232	998	Fruity/pineapple	ND	1.25	2.25	ND	ND	ND	2.25	0.87	3.25	1.50	2.62	1.37
13	1-Octen-3-one	1,241	978	Mushroom	3.25	5.25	4.75	3.85	4.87	1.87	3.32	4.37	4.75	3.37	4.87	3.37
14	Unknown 1	1,269	680	Buttery/sour	ND	3.02	ND	1.75	1.75	1.65	2.00	0.75	0.58	ND	ND	ND
15	Octanal	1,289	1,048	Fatty	1.37	0.50	0.50	ND	1.90	0.50	2.00	2.25	2.50	2.62	3.25	2.25
16	2-Heptanol	1,285	959	Green/metallic	3.45	3.50	1.12	3.00	2.75	2.50	3.50	3.25	4.12	3.75	5.12	3.00
17	2-Acetyl-1-pyrroline	1,312	915	Popcorn	2.00	3.37	2.75	2.15	2.00	1.45	2.75	3.40	2.62	0.75	1.75	1.00
18	Unknown 2	1,327	—	Cheesy	3.25	3.25	3.37	3.50	4.50	3.75	1.75	4.25	4.75	4.00	4.45	3.65
19	2-Nonanone	1,348	1,082	Fatty/floral	2.75	0.87	2.37	2.50	3.50	1.62	2.75	3.12	4.40	3.87	3.00	3.25
20	Methional	1,460	896	Boiled potato	1.75	3.50	2.12	1.50	2.25	1.37	3.37	2.25	2.12	4.00	5.25	3.37
34	Nonanal	1,406	1,111	Dirty/fatty	1.87	1.62	1.87	0.87	ND	0.87	1.00	1.87	1.62	0.87	2.50	1.75
21	Acetic acid	1,466	652	Vinegar	3.75	4.12	2.50	5.37	3.62	5.25	2.75	3.37	3.75	5.75	3.25	4.62
22	(E)-2-Nonenal	1,483	1,140	Hay/cucumber	2.25	0.37	1.20	0.62	1.00	1.40	2.50	2.13	2.15	0.70	ND	3.20
24	Butyric acid	1,603	823	Rancid/cheesy	3.37	4.37	2.75	4.50	4.75	5.00	4.25	5.00	4.25	4.87	5.37	5.50
25	(E,E)-2,4 Nonadienal	1,688	1,222	Fatty/soapy	ND	3.00	1.12	1.47	ND	0.37	1.50	1.87	0.75	ND	2.00	1.75
27	Unknown 3	1,835	1,054	Burnt	1.75	3.00	2.75	4.87	3.87	4.25	3.12	2.75	4.62	2.25	2.75	3.62
29	Maltol	1,949	1,070	Burnt sugar	2.87	2.75	1.90	2.50	1.90	3.02	2.25	2.00	1.75	3.00	3.00	2.00
35	Unknown 4	1,967	868	Vitamin	2.00	2.50	2.25	ND	2.25	ND	ND	ND	ND	ND	ND	ND
30	<i>p</i> -Cresol	2,061	1,090	Barney	3.63	1.53	2.63	2.85	2.87	2.70	2.35	2.70	2.20	2.37	2.63	3.25
31	γ -Dodecalactone	>2,300	—	Sweet/lactone	ND	1.50	0.25	0.70	0.75	0.25	2.25	2.00	1.37	2.37	3.00	3.00

¹Retention indices on Innowax and HP5 columns.²Aroma quality determined on olfactory port.³Mean aroma intensities (postpeak intensity, 10-point scale) given by 2 sniffers on Innowax column. Odor intensities represent the mean of each sample evaluated in duplicate by 2 sniffers. Cheese stored in C = 20-kg tinplate packaging; T = 1-kg tinplate packaging; V = 1-kg plastic vacuum packaging.⁴J&W Scientific, Folsom, CA.⁵ND = not determined.

Table 4. Sensory attributes of Ezine cheese over 12 mo of storage¹

Month	Mean intensity of sensory attributes									
	Cooked	Whey	Creamy	FFA	Goaty	Animal-like	Fermented	Sour	Salty	Umami
3	2.42 ^a	2.01 ^a	1.95 ^b	0.75 ^d	1.17 ^b	1.22 ^c	1.31 ^c	2.14 ^b	8.80 ^b	0.57 ^b
6	1.76 ^b	2.05 ^a	2.99 ^a	1.86 ^a	1.68 ^a	1.95 ^a	1.98 ^a	3.17 ^a	10.54 ^a	1.03 ^a
9	2.00 ^b	1.51 ^c	2.00 ^b	1.00 ^c	1.33 ^b	1.44 ^b	1.58 ^b	2.18 ^b	10.47 ^a	1.14 ^a
12	2.14 ^{ab}	1.73 ^b	2.18 ^b	1.58 ^b	1.78 ^a	1.61 ^b	1.77 ^{ab}	2.65 ^b	9.99 ^a	1.18 ^a
<i>P</i> -value	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

^{a-d}Means in the same column followed by different superscript letters represent significant differences.

¹Attributes were scored on a 15-point universal Spectrum intensity scale where 0 = absence of the attribute and 15 = extremely high intensity of the attribute (Meilgaard et al., 1999).

acid bacteria plays a key role in generation of diacetyl, which is an important flavor compound of butter, buttermilk, and some young cheeses (Starrenburg and Hugenholtz, 1991).

Sulfur-containing compounds including methanethiol, dimethyl sulfide, and methional were identified in Ezine cheese samples. Intensities of methanethiol and methional increased in samples during storage. Specifically, samples C and T had the highest methional intensities at the mo 12 of storage (Table 3). Methional is the first product of Strecker degradation of methionine (Ballance, 1961; Tressl et al., 1989). Methanethiol and dimethyl sulfide were major contributors of Cheddar cheese flavor (Milo and Reineccius, 1997) and Swiss Gruyere cheese flavor (Rychlik and Bosset, 2001).

Heat-generated aroma-active compounds were also identified in Ezine cheeses. These volatiles were maltol, diacetyl, and 2-acetyl-1-pyrroline (Tables 2 and 3). Scanlan et al. (1968) also identified diacetyl and maltol as heat-generated aroma compounds in milk. On d 1, maltol intensity was 2.70 (Table 2). Maltol intensities of cheeses were between 1.75 and 3.02 during storage (Table 3). However, the intensity of 2-acetyl-1-pyrroline with popcorn-like aroma note was 2.5 in cheese on d 1 (Table 2). At the end of storage (mo 12), C, T, and V samples had lower intensities of popcorn aroma than in other months (Table 3).

Some other compounds with high intensities were hexanal, (Z)-4-heptenal, 1-octen-3-one, and butyric acid in Ezine cheese on d 1 (Table 2). Hexanal with cut grass aroma was an important contributor of Ezine cheese aroma. Odor intensity was higher for d 1 cheese (Table 2) than for other cheeses during storage (Table 3). Hexanal is a secondary oxidation product of linoleic acid (Hammond and Hill, 1964). Hexanal was also identified in other pickled cheeses including Teleme (Massouras et al., 2006) and Feta (Bintsis and Robinson, 2004). Mushroom-like aroma was generated by 1-octen-3-one in Ezine cheeses at high intensities (Tables 2, 3). The intensity of this aroma varied in

cheeses stored in different packaging material during storage. In general, sample T had higher intensities of mushroom odor than samples C and V over the 12-mo storage period. Certain oxidation conditions may generate mushroom flavors; 1-octen-3-one was identified as being responsible for mushroom flavor in skim milk and butterfat (Stark and Forss, 1964) and skim milk powder (Karagul-Yuceer et al., 2001).

Garde et al. (2005) determined aroma compounds in Hispanico cheese, produced with and without bacteriocin-producing lactic culture, by using the dynamic headspace technique connected to GC-MS. They identified wide varieties of aroma compounds in the cheese including acetaldehyde, hexanal, heptanal, nonanal, ethyl butyrate, ethyl hexanoate, acetone, 2-heptanone, and 2-nonanone. We identified the same compounds in Ezine cheese.

Bintsis and Robinson (2004) investigated the effects of adjunct cultures on the aroma compounds of Feta-type cheese. Aroma compounds of the cheese samples were identified using headspace trapping onto Tenax TA and analyzed using thermal desorption combined with GC-MS. The flavor compounds isolated were alcohols, aldehydes, ketones, esters, and other miscellaneous compounds. The use of different adjunct cultures had a major effect on the volatile composition of cheese samples. High amounts of ethanol were identified in the cheeses. Acetaldehyde, hexanal, heptanal, octanal, and nonanal were some of the aldehydes in Feta cheeses. In addition, acetone, diacetyl, 2-heptanone, and 2-nonanone were major ketones of Feta cheese. Even though starter culture was not used in Ezine cheese, the same aldehydes and ketones were identified in this cheese, too.

Halloumi cheese is also ripened in brine (about 10% NaCl). Kaminarides et al. (2007) investigated the changes in aroma compounds and sensory characteristics of Halloumi cheese kept in brine for 45 d. For isolation of volatiles, the static headspace system was used. Acetaldehyde, acetone, diacetyl, acetic acid, and

Table 5. Goaty and animal-like flavor intensities¹ of Ezine cheeses stored in different packaging material

Package ²	Mean intensity of sensory attributes	
	Goaty	Animal-like
C	1.51 ^{ab}	1.66 ^a
T	1.38 ^b	1.47 ^b
V	1.59 ^a	1.54 ^{ab}
<i>P</i> -value	0.017	0.011

^{a,b}Means in the same column followed by different superscript letters represent significant differences.

¹Attributes were scored on a 15-point universal Spectrum intensity scale where 0 = absence of the attribute and 15 = extremely high intensity of the attribute (Meilgaard et al., 1999).

²Cheese stored in C = 20-kg tinplate packaging, T = 1-kg tinplate packaging, V = 1-kg plastic vacuum packaging.

butyric acid were some of the volatiles determined at high intensities in the cheese during storage. These results agreed with our findings for Ezine cheese.

The results of sensory analysis were presented in Tables 4, 5, and 6. Significant differences were observed among the intensities of some descriptors over storage. Specifically, young cheese flavors including cooked and whey had higher intensities at mo 3 of storage. However, FFA, goaty, animal-like, and fermented scores were higher at mo 6 (Table 4). Basic taste scores such as sour, salty, and umami were lower at mo 3. Similar results were also found by Karagul-Yuceer et al. (2007) for Ezine cheeses that had different ripening periods.

Effect of packaging material was found significant on the intensities of goaty ($P = 0.017$) and animal-like flavor ($P = 0.011$) intensities (Table 5). Goaty flavor score was higher in the cheese stored in plastic vacuum packaging (V) than in 1-kg tinplate container (T; Table 5). Animal-like flavor of sample C was higher than that of sample T. Size and material of packaging might have an effect on flavor migration, permeation, or sorption (Risch, 2000).

Significant interactions were observed between packaging material and storage period for sulfur ($P = 0.000$), bitter ($P = 0.000$), sweet ($P = 0.041$), and bite ($P = 0.047$) terms (Table 6). Sulfur aroma as an important descriptor for aged cheeses showed higher intensities for all C, T, and V samples after 6 mo of storage (Table 6). In general, sulfur intensities of samples C and T did not show significant differences. However, vacuum-packed cheeses at 6 and 12 months had lower sulfur intensities than the others (Table 6). Bitter, sweet, and bite intensities were also determined in the cheeses at very low intensities (Table 6). The main acidic compounds in Ezine cheese are acetic, butyric, and propionic acids (Tables 2 and 3). Acetic acid is produced by citrate and sugar metabolism in dairy foods (Cogan, 1995) or oxidative deamination of certain amino acids including glycine, alanine, and serine (Adda, 1982). It was shown that acetic acid characterizes pickled cheese with a harsh, but not rancid flavor (Abd El-Salam et al. 1993). The bite characteristic in cheeses may be related to this acidic compound. Butyric acid was also identified in other types of brined cheeses as a major volatile compound (Massouras et al., 2006; Kaminarides et al., 2007).

Figure 1 shows geometrical representation of sensory attributes and aroma-active compounds produced by multidimensional scaling. In general, statistically significant differences were not determined among the samples in terms of the attributes analyzed. Therefore, differences in packaging and time were disregarded. Visual representation of similarities of aromatic terms (taste terms not included) developed by sensory panelists and aroma-active compounds (intensity >2) of cheese samples can be seen in Figure 1. In this plot, variables that highly correlated are grouped together. For example, cooked flavor, 2-acetyl-1-pyrroline, diacetyl, 1-octen-3-one, 3-methylthiophene, and methanethiol grouped together (Figure 1). Goaty, animal-

Table 6. Sensory attributes¹ of Ezine cheeses stored in different packaging material² during 12 mo of storage

Month	Mean intensity of sensory attributes											
	Sulfur (P -value = 0.000)			Bitter (P -value = 0.000)			Sweet (P -value = 0.041)			Bite (P -value = 0.047)		
	C	T	V	C	T	V	C	T	V	C	T	V
3	0.84 ^{C,a}	0.70 ^{B,a}	0.86 ^{B,a}	0.28 ^{B,a}	0.35 ^{B,a}	0.34 ^{B,a}	0.88 ^{AB,a}	0.96 ^{A,a}	1.06 ^{A,a}	0.13 ^{C,a}	0.17 ^{B,a}	0.28 ^{B,a}
6	1.81 ^{A,a}	1.30 ^{A,b}	1.30 ^{A,b}	1.49 ^{A,a}	0.88 ^{A,b}	0.86 ^{A,b}	1.07 ^{A,a}	1.12 ^{A,a}	0.95 ^{A,a}	1.02 ^{A,a}	0.63 ^{A,b}	0.74 ^{A,b}
9	1.24 ^{B,a}	1.54 ^{A,a}	1.42 ^{A,a}	0.23 ^{B,a}	0.22 ^{B,a}	0.30 ^{B,a}	1.03 ^{A,a}	0.93 ^{A,a}	0.93 ^{AB,a}	0.66 ^{B,a}	0.31 ^{B,b}	0.35 ^{B,b}
12	1.46 ^{AB,a}	1.43 ^{A,a}	1.10 ^{AB,b}	0.37 ^{B,a}	0.36 ^{B,a}	0.44 ^{B,a}	0.79 ^{B,ab}	0.96 ^{A,a}	0.70 ^{B,b}	0.24 ^{C,a}	0.18 ^{B,a}	0.23 ^{B,a}

^{A-C}Means in the same column followed by different uppercase superscripts represent significant differences.

^{a,b}Means in the same row followed by different lowercase superscripts represent significant differences.

¹Attributes were scored on a 15-point universal Spectrum intensity scale where 0 = absence of the attribute and 15 = extremely high intensity of the attribute (Meilgaard et al., 1999).

²Cheese stored in C = 20-kg tinplate packaging, T = 1-kg tinplate packaging, V = 1-kg plastic vacuum packaging.

Figure 1. Geometrical representation of sensory attributes (only aromatics) and aroma-active compounds (intensity >2) produced by multidimensional scaling (stress = 0.643, R² = 0.916). CKD = cooked; WHY = whey; CRM = creamy; FFA = free fatty acid; GOAT = goaty; ANML = animal-like; FRM = fermented; SLFR = sulfur; METETH = methanethiol; ASETAL = acetaldehyde; DMS = dimethyl sulfide; DIAC = diacetyl; EBUTY = ethyl butyrate; HXL = hexanal; THIOP = 3-methylthiophene; HPTNL = heptanal; PENTNAT = ethyl pentanoate; ZHPTNL = (Z)-4-heptenal; OCT3ONE = 1-octen-3-one; HPTNOL = 2-heptanol; PYROLIN = 2-acetyl-1-pyrroline; UNK2 = unknown 2; NONANO = 2-nonanone; MTHAL = methional; ACTIC = acetic acid; BTY = butyric acid; UNK3 = unknown 3; MLTL = maltol; CRSOL = *p*-cresol; LACTON = γ -dodecalactone.

like, fermented, and FFA descriptors developed by the sensory panel were closely related to *p*-cresol, which has a barny aroma note determined by GCO.

Urea PAGE Analysis

The effect of different packaging types on proteolysis is shown in Table 7 and Figure 2. It was observed that both β -casein and cow-origin α_S -casein slowly degraded and 88.7% of β -casein and 70.3% of α_S -casein were not hydrolyzed by the end of 12 mo of ripening

(Table 7). Degradation of cow-origin α_S -casein stopped between mo 6 and 9 and continued after mo 9. However, degradation of sheep- and goat-origin α_S casein was notably faster than other caseins. Approximately half of α_S -casein was hydrolyzed at the end of mo 6. Degradation slowed in the following 3 mo and 25.3% of α_S -casein remained at the end of the storage period. Degradation of β -casein was observed to be similar in all cheeses in different packaging materials. Additionally, the degradation amount was 1 and 1.2% lower in T and V, respectively, compared with C. The lowest

Table 7. Residual β -casein and α_S -casein in Ezine cheeses during 12 mo of storage¹

Month	Casein types ² (%)								
	β			α_S -Cow			α_S -Ewe-goat		
	C	T	V	C	T	V	C	T	V
0 ³	100	100	100	100	100	100	100	100	100
3	94.6	94.6	94.6	93.3	93.3	93.3	80.2	80.2	80.2
6	93.7	93.9	93.5	82.1	82	89.6	50.3	50.8	54.7
9	92.4	92.8	91.8	82	75.9	88.3	29.4	26	88.3
12	88.7	89.7	89.9	70.3	73.1	82.9	25.3	23.5	39.5

¹Cheese stored in C = 20-kg tinplate packaging; T = 1-kg tinplate packaging; V = 1-kg plastic vacuum packaging.
² α_S -Cow = cow-originated α_S -casein; α_S -Ewe-goat = sheep- and goat-originated α_S -casein.
³Month 0 represents the sample on d 1.

Figure 2. Urea-PAGE electrophoregram of the casein fractions of Ezine cheeses stored in different packaging types during 12 mo of storage. S = casein standards; α_s -casein C = cow milk α_s -casein; α_s -casein EG = α_s -casein sheep and goat milk; C = 20-kg tinplate packaging; T = 1-kg tinplate packaging; V = 1-kg plastic vacuum packaging; C0 = sample C, first day; C3 = sample C, mo 3; C6 = sample C, mo 6; C9 = sample C, mo 9; C12 = sample C, mo 12; V6 = sample V, mo 6; V9 = sample V, mo 9; V12 = sample V, mo 12; T6 = sample T, mo 6; T9 = sample T, mo 9; T12 = sample T, mo 12.

degradation of cow-, sheep-, and goat-origin α_s -caseins was determined in sample V. Di Cagno et al. (2003) reported slight degradation of α_s -casein and β -casein fragments in Canestrato Pugliese, Fiore Sardo, and Pecorino Romano cheeses made from sheep milk without adding starter culture in Italy. Slow hydrolysis of β -casein was shown in other brine-ripened cheeses (Saldamli and Kaytanli, 1998; Katsiari et al., 2000; Romeih et al., 2002). Hayaloglu et al. (2005) also found that cheese without starter had a slightly higher concentration of γ -caseins because of higher plasmin activity. In white pickled cheese, extensive plasmin activity is not expected because of the acidity and salt content (Michailidou et al., 1998; Kandarikis et al., 2001).

Moatsou et al. (2002) investigated the level of proteolysis in Feta cheeses produced by using a mixture of sheep and goat milk (4:1) in 3 different dairy plants during 4 mo of storage. Yogurt was used as a starter culture. Urea PAGE analysis showed that α_s -casein degraded very quickly. However, β -casein breakdown was almost constant. The difference in amount of degradation was related to pH and salt-in-moisture contents of cheeses.

Nitrogen Fractions

Total nitrogen contents (%) of cheeses were between 2.92 and 3.28 in the storage period. In general, there were no major changes in the total protein content of the cheeses. Tarakci and Tuncturk (2008) reported that the protein contents of white cheese were not changed during storage.

Changes in TCASN fraction of Ezine cheeses stored in different packaging material during 12 mo ($P = 0.027$) are shown in Table 8. Trichloroacetic acid soluble nitrogen contains small peptides, free amino acids,

ammonia, and other minor compounds produced by activity of bacterial enzymes (Kuchroo and Fox, 1982). Cinbas and Kilic (2006) pointed out that the TCASN fraction of white-brined cheese increased with storage time. In the present study, TCASN content of Ezine cheeses increased over the 12-mo storage period (Table 8). Sample C at mo 12 had a higher TCASN fraction than samples T and V (Table 8). Romeih et al. (2002) showed the increase in the level of TCASN in low-fat white-brined cheese during aging.

Table 9 showed the changes in water-soluble nitrogen (WSN, %) and PTASN fractions during storage. The WSN fraction includes proteins, all peptides, amino acids, and N compounds produced mainly by the residual rennet activity and microbial enzymes (Lane and Fox, 1997; McSweeney and Fox, 1997; Thakur, 2006). The WSN fraction of the cheese increased significantly during ripening period ($P = 0.001$; Table 9). Increase in levels of WSN during ripening was similar to the findings reported by Vassiliadis et al. (2009) for traditional

Table 8. Changes in trichloroacetic acid soluble nitrogen (TCASN) fraction of Ezine cheeses stored in different packaging material¹ during 12 mo of storage

Month	TCASN (%; mean $\pm$ SE)		
	C	T	V
3	2.86 $\pm$ 0.37 ^{C,a}	3.83 $\pm$ 0.09 ^{B,a}	3.23 $\pm$ 0.34 ^{C,a}
6	5.21 $\pm$ 0.47 ^{B,a}	5.63 $\pm$ 0.46 ^{A,a}	5.06 $\pm$ 0.18 ^{B,a}
9	6.40 $\pm$ 0.13 ^{B,a}	6.43 $\pm$ 0.06 ^{A,a}	5.97 $\pm$ 0.20 ^{A,ab}
12	8.83 $\pm$ 0.80 ^{A,a}	6.75 $\pm$ 0.26 ^{A,b}	7.36 $\pm$ 0.04 ^{A,b}

^{A-C}Means in the same column followed by different uppercase superscripts represent significant differences ($P < 0.05$).

^{a,b}Means in the same row followed by different lowercase superscripts represent significant differences ($P < 0.05$).

¹Cheese stored in C = 20-kg tinplate packaging; T = 1-kg tinplate packaging; V = 1-kg plastic vacuum packaging.

Table 9. Changes in water soluble nitrogen (WSN) and phosphotungstic acid soluble nitrogen (PTASN) fractions during 12 mo of storage

Month	WSN (%; mean $\pm$ SE)	PTASN (%; mean $\pm$ SE)
3	7.52 $\pm$ 0.35 ^c	1.55 $\pm$ 0.12 ^b
6	13.78 $\pm$ 0.49 ^b	1.79 $\pm$ 0.05 ^b
9	15.21 $\pm$ 1.01 ^b	2.43 $\pm$ 0.18 ^a
12	20.10 $\pm$ 0.69 ^a	2.65 $\pm$ 0.15 ^a

^{a-c}Means in the same column followed by different superscript letters represent significant differences ($P < 0.01$).

Greek Feta cheese. The PTASN fraction indicates very small peptides, amino acids, and N compounds (Thakur, 2006). The levels of PTASN increased significantly in all cheeses during storage ($P = 0.000$). Similar results were also obtained by Hayaloglu (2007) and Tarakci and Tuncurk (2008) for white-brined cheese.

The rates of increase in WSN, TCASN, and PTASN were faster, up to mo 6 of ripening. The changes in these nitrogen fractions after the 6-mo aging period were slower than in the early aging periods (Tables 8 and 9). Low pH and high moisture contents in cheeses result in the activity of chymosin on α_{S1} -casein, causing the rapid production of water-soluble peptides (Romeih et al., 2002).

CONCLUSIONS

For economic purposes, we recommend that Ezine cheese can be ripened in small-size packaging after 3 mo of storage. Approximately 6 mo is sufficient to develop the characteristic properties of Ezine cheese. Different groups of chemicals play an important role in the development of Ezine cheese aroma. The major aroma compounds are fatty acids, esters, sulfur compounds, and aldehydes. All types of cheeses contained approximately the same aroma-active compounds at different intensities. These differences may be results of packaging material/size and storage period. In general, no major differences were observed among the samples in terms of chemical and sensory properties of Ezine cheese during 12 mo of storage. The results clearly showed that the third month is the critical time for ripening. After the third month of storage, no major differences were found among the cheeses in terms of aroma-active compounds and sensory properties. Degradation of specifically α_S -casein increased after 3 mo of storage. In addition, the levels of WSN, TCASN, and PTASN increased during storage.

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