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Research Article

BIOGENIC AMINE CONTENTS OF FRESH AND MATURE KASHAR CHEESES DURING REFRIGERATED STORAGE

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ABSTRACT

Kashar is one of the most consumed traditional cheeses in Turkey. It is produced as fresh or mature, which differ in ripening periods. Safe consumption period of kashar was investigated during refrigerated storage. Five samples of fresh and five samples of mature kashar cheeses collected from local supermarkets in Turkey were analyzed. Changes in biogenic amines, pH and thiobarbituric acid reactive substance of fresh and mature kashar cheeses were investigated during storage. Mature kashar cheeses had higher biogenic amine concentrations than fresh kashar cheeses. During storage, total biogenic amine contents of all samples increased significantly ($p<0.05$). Total biogenic amine contents of mature kashar cheeses were higher than maximum allowed limit of 1000 mg/kg and can cause toxicity. It was found that fresh kashar cheeses were safer than mature kashar cheeses with respect to the toxic limits of biogenic amines.

Keywords: Fresh kashar, Mature kashar, Cheese, Biogenic amines

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Introduction

Kashar, a semi-hard Turkish traditional cheese, is one of the most consumed cheeses in Turkey (Koca & Metin, 2004). According to Turkish Statistical Institute, total cheese production of Turkey was 665580 tonnes in 2015, and semi-hard cheese production was 191206 tones (Anonymous, 2005). The reasons of popularity are long shelf life and flavor. It has similar characteristics with Caciocavalle, Provolone, Regusono, Kashkaval cheeses and with the 'Pasta Filata' type cheese such as Mozarella partially (Halkman & Halkman, 1991). Some researchers mentioned similarity between Cheddar and Kashar Cheese (Çetinkaya et al., 2003).

According to Turkish Standards, Kashar cheese is classified as ''fresh'' and ''old or mature'' in terms of ripening (Turkish Standards Institute, (TSI), 1999). Both types can be eaten at breakfast; however the fresh cheese is also consumed in toasted sandwiches or baked foods in the same way as Mozzarella cheese (Çetinkaya et al, 2003; Üçüncü 2004). Mature kasar cheese is traditionally produced in 27-30 cm diameter and 10-13 cm height and 6-10 kg weight. Traditionally, kashar cheese is made from raw sheep or cows' milk or their mixtures without the addition of starter cultures. The traditional method involves renneting, curd forming, curd fermentation (about pH 5.1-5.4), scalding and texturing of the curd in hot water (65-80°C) containing 6-8% NaCl, shaping of the scalded curd, pre-ripening at 15-20 °C and ripening at 2-4°C for at least 3 months (Aran, 1998). Mature Kashar cheese is consumed after long term ripening, and it is believed that the cheese gains its characteristic flavour after 6 to 12 months. During this period dry salting is applied over the surface of cheese. Production of mature kashar cheese requires more labour force and time, reduce yield due to water loss during ripening period (Sert et al., 2007).

Amino acids provide carbon, nitrogen and energy sources for bacterial cells and play an important role in the development of flavour in cheeses. Cheese is an ideal substrate for amine production. It contains the high free amino acids concentration as a result of proteolysis, availability of amino acid decarboxylase producing microorganisms, adequate temperature, pH, cofactor and water activity (Benkerroum, 2016). Other factors affecting the production of biogenic amines in cheeses include the presence of spoilage microorganisms and the synergistic effects between microorganisms. Moreover several extrinsic factors may also play an important role, namely, pasteurization of milk, salt-in-moisture levels and ripening time (Linares et al., 2013). In particular, the pH of cheese (5.0-6.5) is optimum for the activity of most decarboxylases and it has been found that the production of biogenic amines is accelerated by high temperatures during production and manufacture of cheese and by the prolonged aging process (Spizzirri et al., 2013). Biogenic amines are organic, basic, nitrogenous compounds (Şahin Ercan et al., 2013). Free amino acid decarboxylation leads to biogenic amine formation (Flasarova et al., 2016). Importance of biogenic amines in foods is mainly due to two reasons; firstly, the intake of foods containing high content of biogenic amines cause health hazard through the direct, toxic effect of these compounds and their interaction with some medicaments; secondly, they may have a role as indicators of quality and/or acceptability in some foods (Shalaby, 1996; Ruiz-Capillas & Moral, 2001; Şahin Ercan et al., 2013). The presence of low levels of biogenic amines in cheeses and other foods is not considered as serious risk. However, high amount of biogenic amine consumption may result in various physiological effects (Koehler et al., 1978). Several outbreaks of histamine poisoning have occurred following the consumption of cheese, particularly Swiss and Cheddar, containing high levels of histamine (Vale & Gloria, 1998). There is a term that is called "Cheese reaction" is a hypertensive crisis. It's characterized by a release of catecholamines from the sympathetic nervous system and the adrenal medulla, both causing an increase of the mean arterial blood pressure (≥180/120 mmHg) and heart rate by peripheral vasoconstriction, producing hypertensive crisis as more dangerous consequence. Its certain symptom is severe headache, has been observed after ingestion of foods rich in tyramine (Vale & Gloria 1998; Linares et al., 2013).

To the best of our knowledge, any study about biogenic amine contents of fresh and mature kashar cheeses during storage period has not been found in the literature. The aim of this study was to determine the changes in some quality (pH, protein, ripening index (RI), total nitrogen (TN), water soluble nitrogen (WSN), salt and moisture content) and safety (thiobarbituric acid reactive substance and biogenic amines) parameters of cheeses during storage period and also to compare fresh and mature kashar cheeses.

Materials and Methods

Sampling

Ten randomly purchased commercial kashar cheeses produced in Turkey were analysed. Five of them were fresh (S1, S2, S3, S4 and S5) and the other five were mature kashar (S6, S7, S8, S9 and S10) cheeses. Kashar cheeses were stored at 4 ±1°C for three weeks and samples were taken for analysis at 0, 1, 2 and 3th weeks of storage. Kashar cheeses were analyzed for biogenic amines (cadaverine, histamine, phenylethylamine, tyramine, tryptamine, putrescine and spermidine), pH, moisture content and TBARS values. Initial protein, WSN, TN and salt contents were also determined. The kashar cheeses were grated, homogenized using the Waring blender thoroughly and analyzed immediately. Each analysis was performed at least in dublicate.

Chemicals

1,1,3,3-Tetraethoxypropane (TEP) and 2-thiobarbituric acid were obtained from Sigma (St. Louis, MO); β-phenylethylamine hydrochloride, histamine dihydrochloride, cadaverine dihydrochloride, spermidine, putrescine dihydrochloride, tryptamine hydrochloride and tyramine hydrochloride were obtained from Sigma (St. Louis, MO) and were used as biogenic amine standards; sodium hydroxide, 25% ammonium and sodium bicarbonate were from Merck (Darmstadt, Germany), acetone from Reidel De Haen (Germany), dansyl chloride from Sigma Co. (St. Louis, MO), ammonium acetate from Merck (Darmstadt, Germany), and perchloric acid from JT Baker (Holland). All chemicals except acetonitrile were analytical grade (extra pure) and acetonitrile was HPLC grade.

Determination of Cheese Characteristics

Moisture content, total nitrogen, water soluble nitrogen, ripening index, salt contents and pH of cheese samples were determined in triplicate runs. Moisture content of cheeses was determined according to AACC, 1995 Approved Methods. The cheese samples were analysed for TN using Kjeldahl method (AOAC, 1990) with digestion, distillation and titration steps. The protein content of cheeses was calculated by multiplying the total nitrogen content by 6.38. WSN of kashar cheese samples were determined as described by Butikofer et al, (1993). RI of samples was calculated from the ratio of water soluble nitrogen to total nitrogen (Butikofer et al., 1993). Salt content was determined by the Mohr method (AOAC, 1990). pH value of kashar cheese was determined using a pH meter (Jenway 3010; Jenway Ltd., Essex, UK) equipped with an electrode (J95, 924001, Jenway Ltd., Essex, UK).

Determination of TBARS (2-Thiobarbituric Acid Reactive Substances) Values

TBARS of samples were determined by the spectrophotometric method (Bozkurt & Erkmen, 2004). Two grams of homogenized kashar samples were taken and TBARS were extracted twice with 10 mL of 0.4 M perchloric acid. Extracts were collected and made up to 25 mL with 0.4 M perchloric acid and centrifuged for 5 min at 1790g. After centrifugation, 1 mL of supernatant was pipetted into glass stoppered test tube. TBA reagent (5 mL) was added and the mixture was heated in a boiling water bath for 35 min. After cooling the absorbance of sample was read against the appropriate blank at 538 nm. A standard curve was prepared using 1,1,3,3-tetraethoxypropane (TEP). TBARS values were determined as mg malondialdehyde (MA)/kg sample.

Determination of Biogenic Amines

The chromatographic method (Eerola et. al., 1993) was used for the determination of the biogenic amines. The HPLC consisted of a Shimadzu gradient pump (Shimadzu LC 20AB, Shimadzu Solvent Delivery Module, Kyoto, Japan), a Shimadzu auto injection unit (Shimadzu SIL20AHT, Kyoto, Japan), a Shimadzu UV detector (Shimadzu SPD 20A, Kyoto, Japan) and a RP-18 guard column. The HPLC column was Spherisorb ODS2, 200 µm and 4.6 mm×200 mm. Ammonium formate solution (0.4 M) prepared by ultra-pure water (Millipore Elix 10UV and Milli-Q, Millipore S.A.S. 67120 Molsheim, France) and acetonitrile were filtered through a 0.45 µm millipore filter (Billerica, MA). Ammonium formate and acetonitrile were used as the LC mobile phases. A gradient elution program was used with mobile phases of acetonitrile (solvent A) and 0.4 M ammonium formate (solvent B), starting with 50% solvent A and 50% solvent B and finishing with 90% solvent A and 10% solvent B after 35 min. The flow rate was 1.0 mL/min.

Two grams of sample was homogenized in 10 mL of 0.4 M perchloric acid using a Waring blender. The sample was centrifuged for 10 min at 1790g and filtered. The extraction was repeated with a further 10 mL of 0.4 M perchloric acid solution and the supernatants were combined and made up to 25 mL with 0.4 M perchloric acid. One millilitres of extract was pipetted into glass stoppered test tube and 200 µL of 2 N NaOH and 300 µL of saturated sodium bicarbonate solutions were added. Two millilitres of dansyl chloride (10 mg/mL) solution was added to each sample and incubated for 45 min at 40°C. Residual dansyl chloride was removed by adding 100 µL of 25% ammonia. After 30 min, the solution was adjusted to 5 mL with acetonitrile, centrifuged for 5 min at 1790*g*, the supernatant filtered (0.45 µm) and 20 µL then injected onto the HPLC. The standard solution of the dansylated derivatives was diluted to 1 mL with 0.4 M perchloric acid to give concentrations from 0.5 to 10 µg/mL.

Statistical Analysis

The results were analyzed statistically using the SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA test) and Duncan's multiple range test were performed. Values of $p < 0.05$ were used to indicate significant differences.

Results and Discussion

Cheese Characteristics

Table 1 shows the results obtained from the analysis of fresh and mature kashar cheeses at the beginning of the storage period. Salt content of cheeses were changed beetwen 1.3-5.0%. The Turkish Food Codex (No: 2015/6) states that fresh and mature kashar cheeses should have salt values up to 3.0 and 4.0 %, respetively (Anonymous, 2005). Salt content of fresh kashar cheeses detected in this study is not suited to the Turkish Food Codex. It was reported that kashar cheeses contain an average of 2.54-5.24% salt Sert et al. (2007). On the other hand, salt contents of fresh kashar cheese were significantly higher (p<0.05) than the mature kashar cheeses. This could be due to the differences in production of mature and fresh kashar cheeses. Also, moisture content range of both mature and fresh kashar cheese were in the range of 37.9-46.1% which is suited to the reported range of 29.18-57.29% (Sert et al., 2007). According to the results, moisture contents of mature kashar cheeses (37.9-41.6%) was lower than fresh kashar cheeses (43.3-46.1%). This could be due to dry salting process over the surface of mature kashar cheeses during the ripening period (6-12 months). Results showed that generally higher values of TN, WSN, RI and protein were obtained for mature kashar cheeses (Table 1). Obtaining higher values of TN, WSN, RI and protein in mature kashar cheeses could be due to longer proteolsis. Proteolysis is the most complex and important biochemical event that occurs in most cheeses during ripening. It has direct influence on flavour and texture as softening of cheese during ripening (McSweeney, 2004). The evolution of the WSN/TN could be interpreted as the level of proteolysis (Sert et al., 2007) which correspounds to RI.

Changes of pH and TBARS Values

Changes of pH and TBARS values during the storage periods of kashar samples are given in Table 2. The pH values of fresh and mature kashar cheese samples were not affected significantly (p> 0.05) and remained almost constant during refrigerated storage. TBARS values is used as a marker of lipid oxidation. Degradation of polyunsaturated fatty acids results in malonaldehyde formation. Lipid oxidation could cause destruction of valuable nutrients, off-flavours and production of toxic compounds (Medeiros et al., 2014). Consequently, TBARS value is a critical parameter especially during storage period of foods that's why it is detected in this study. It could cause adverse sensorial results which affects consumer acceptance. TBARS values were affected significantly (p<0.05) by storage. As the storage period prolong, TBARS values of all cheese samples increased. Initial range of TBARS value of fresh kashar cheeses were 0.03-0.18 mg/kg and increased to 0.20-0.39 mg/kg at the end of the storage period. This range was 0-0.15 mg/kg and 0.23-0.57 mg/kg for mature kashar cheeses at the beginning and end of the storage period, respectively. Lipid oxidation leads through formation of hydroperoxides to short chain aldehyde, ketones and other oxygenated compounds. They are considered to be responsible for the development of rancidity, cause undesirable flavour and related to heart disease and cancer (Botsoglou et al., 1994). Change in the TBARS values of mature kashar cheeses were higher than that in the fresh kashar cheeses during the storage period. It was reported that if the TBARS value is higher than 1 mg/kg, generally off-odors are formed and it is considered as the begining of organoleptic perception of lipid oxidation (Wu et al., 1991). None of the samples exceed this limit during 2 weeks refrigerated storage period.

Biogenic Amines Content

Changes in Histamine Concentration

Changes of histamine concentration and their statistical analysis are given in Tables 3 and 4, for fresh and mature kashar cheeses, respectively. Histamine concentration changed significantly (p<0.05) with storage time. Initial histamine concentration range of fresh and mature kashar cheeses are 29.0-76.4 mg/kg (Table 3) and 52.8-1334.4 mg/kg (Table 4), respectively. At the end of the storage period, this range changed as 45.4-145.9 mg/kg and 270.15-3042.9 mg/kg for fresh and mature kashar cheeses, respectively. Mean of initial histamine concentration of fresh kashar cheeses (60.8 mg/kg) was similar to histamine concentration (63.5 mg/kg) in Gouda cheese (Silvana et al., 1998), but mean of initial histamine concentration of mature kashar cheeses (525.5 mg/kg) were high.

During the storage period, histamine concentration increased (p<0.05) for both fresh and mature kashar cheeses (Table 3 and 4). Substrate availability could be an accelerating factor for histamine production in cheese (Joosten, 1998). Also, the use of raw milk or post-contamination in cheese may result in high levels of histamine formation (Stratton et al., 1992). It was reported that histamine intake of 8-40 mg, 40-100 mg and higher than 100 mg may cause slight, intermediate and intensive poisoning, respectively (Nout, 1994). According to the results, consumption of fresh kashar cheeses samples at the beginning of the storage period may cause slight or intermediate poisoning with respect to the their histamine levels. However, consumption of mature kashar cheeses at the beginning of storage period may cause intensive poisoning.

Table 1. Initial characteristics of kashar cheese samples before storage

Sample	TN (%)	WSN (%)	Protein (%)	Salt (%)	RI (%)	Moisture (%)
S1	4.2±0.2cd	0.9±0.04a	26.0±1.5bcd	4.2±0.2a	21.4±0.3a	43.3±0.7a
S2	3.7±0.2abc	0.9±0.05a	23.4±1.5abc	4.8±0.5ab	24.3±3.2ab	45.6±0.1c
S3	3.4±0.1a	0.8±0.05a	21.4±0.6a	4.3±0.5a	23.5±2.1ab	45.1±0.2c
S4	4.2±0.5cd	1.1±0.05b	26.2±3.0cd	4.6±0.4ab	26.1±4.2b	44.0±0.5b
S5	3.8±0.1abc	0.8±0.0a	23.8±0.3abc	5.0±0.1b	21.1±0.3a	46.1±0.4c
S6	3.5±0.3a	1.7±0.1cb	22.3±1.8a	3.3±0.2c	48.5±5.7c	41.6±0.1c
S7	4.3±0.1ab	1.9±0.01c	26.9±0.3b	1.9±0.4ab	44.1±1.2bc	40.5±0.1c
S8	3.9±0.3a	1.3±0.0a	25.1±2.1ab	2.5±0.1b	33.3±1.9a	39.9±0.8a
S9	4.5±0.1b	1.9±0.01c	30.2±0.6c	1.9±0.2ab	42.2±2.3b	39.4±0.1a
S10	4.5±0.1b	1.5±0.02b	28.4±0.6c	1.3±0.1a	33.3±5.1a	37.9±0.9b

Different small letters indicate statistical difference at α = 0.05 level in each column.

S1- S5 are fresh kashar and S6-S10 are old kashar cheeses.

Table 2. Changes of pH and TBARS values of fresh and mature kashar cheeses during storage.

Sample	pH			TBARS		
	0	1st week	2nd week	0	1st week	2nd week
S1	5.68±0.28a	5.89±0.21a	5.76±0.24a	0.13±0.04a	0.26±0.00b	0.33±0.04b
S2	5.73±0.26a	5.81±0.18a	5.76±0.24a	0.05±0.00a	0.13±0.04ab	0.21±0.00b
S3	6.05±0.15a	6.16±0.18a	6.11±0.28a	0.05±0.00a	0.13±0.04ab	0.23±0.04b
S4	5.96±0.30a	6.07±0.19a	6.06±0.25a	0.03±0.01a	0.28±0.04b	0.36±0.00c
S5	5.39±0.27a	5.57±0.21a	5.54±0.19a	0.18±0.04a	0.28±0.04b	0.39±0.04c
S6	5.48±0.24a	5.54±0.23a	5.60±0.16a	0.05±0.00a	0.13±0.04ab	0.23±0.04b
S7	5.37±0.27a	5.48±0.22a	5.75±0.19a	0.10±0.00a	0.33±0.04b	0.41±0.00c
S8	5.34±0.24a	5.45±0.22a	5.60±0.19a	0.13±0.04a	0.23±0.04b	0.33±0.04c
S9	5.52±0.24a	5.76±0.18a	6.80±0.26a	0.15±0.00a	0.39±0.11b	0.57±0.00c
S10	5.50±0.18a	5.90±0.20a	5.94±0.21a	0.00±0.00a	0.15±0.00b	0.26±0.07b

Different small letters indicate statistical difference at α = 0.05 level in each column.

S1- S5 are fresh kashar and S6-S10 are old kashar cheeses.

Changes in Phenylethylamine Concentration

The level of phenyethylamine concentration was changed significantly ($p<0.05$) during the storage period (Tables 3 and 4). Concentrations of phenylethylamine of fresh and mature kashar cheeses increased simultaneously. From a good manufacturing practice point of view, a level of 30 mg/kg of phenylethylamine concentration is regarded as acceptable (Nout, 1994). In this study, phenylethylamine concentration exceeded a concentration of 30 mg/kg even at the beginning and end of the storage period for both fresh and mature kashar cheeses. It was reported that phenylethylamine was not detected in kashar (Andıç et al., 2011), but was found in 19 of 30 herby cheeses samples (Andıç et al., 2010a) and never exceeded a concentration of 30 mg/kg in motal cheeses (Andıç et al., 2010b).

Changes in Tyramine Concentration

Tyramine, one of the toxicologically important biogenic amines, is formed in foods by the action of tyrosine decarboxylase produced by bacteria associated with the foods (Silla-Santos, 1996). The allowable maximum level of tyramine in food is 100–800 mg/kg. Concentrations of 1080 mg/kg of tyramine are toxic for humans (Shalaby, 1996). Changes in tyramine concentrations and results of statistical analysis during the storage period are given in Tables 3 and 4 for fresh and mature kashar cheeses, respectively. During the storage period tyramine concentration increased significantly ($p< 0.05$) for both fresh and mature kashar cheese samples. The levels of tyramine conentrations in fresh kashar cheeses were acceptable during the storage period. However, mature kashar cheeses had higher tyramine concentration compared to fresh kashar cheeses. Tyramine concentration of mature kashar cheese sample S9 was found very high (>800 mg/kg) before and during storage period. This could be due to the presence of high amount of tyrosine and tyrosine decarboxylase activity and poor sanitation during processing. It was reported that any food with free amino acids, especially tyrosine and phenylalanine, are subject to biogenic amine formation if poor sanitation and low quality foods are used or if the food is subjected to temperature abuse or extended storage time (Schirone et al., 2011). High amounts of tyramine were found in Spanish traditional cheeses (Roig-Sagues et al., 2002). It was reported that despite the high concentrations of the precursor amino acid tyrosine in cheeses, they do not provide evidence of tyramine in their biogenic amine inventory (Pintado et al., 2008). During fermentation and ripening, the environmental factors that affect the activity of decarboxylating enzymes may be more important than precursor availability (Schirone et al., 2011). Tyramine levels in Tulum cheese ranged and was almost from 109.6 to 1575.5 mg/kg (Durlu-Özkaya, 2000) was almost higher than the tyramine range of fresh and mature kashar cheeses (except S9) in this study. It was reported that the tyramine range of herby cheese changed as 18-1125.5 mg/kg (Andıç et al., 2010a), 212.5 mg/kg in Brazilian cheese (Vale & Gloria, 1998) and 329.0 mg/kg in Turkish tulum cheese (Öner et al., 2004). So it can be concluded that consumption of kashar cheeses was generally safe with respect to their tyramine levels.

Table 3. Changes of biogenic amine concentration (mg/kg) in fresh kashar cheeses during storage

Time (weeks)	Kashar cheese types				
	S1	S2	S3	S4	S5
Histamine					
0	49.5±10.7aA	76.4±9.6aAB	29.0±3.2aA	75.1±0.9aAB	74.1±10.0aAB
1	76.6±4.7bcAB	94.4±0bB	38.9±2.9bA	95.4±0.7bB	106.8±0.4bBC
2	78.0±8.8cAB	105.6±6.2bBC	45.4±2.5bA	103.3±0.4bABC	145.9±0.4cC
Phenylethylamine					
0	86.11±6.5abAB	35.2±1.1abA	43.1±8.6aA	63.4±6.5aAB	54.4±2.8aaAB
1	112.5±6.3abAB	42.6±1.2abcA	46.5±1.7aA	92.3±4.7bA	84.8±6.8cA
2	122.2±12.4bC	55.7±8.6cA	57.4±4.8bA	109.6±3.6cBC	65.6±3.5bcAB
Tyramine					
0	46.9±8.2aBC	37.7±7.0abA	63.1±6.8aB	52.7±2.0abAB	90.7±26.1aCD
1	50.3±6.5aB	57.0±0.5abA	76.2±4.6abAB	60.4±0.1abA	94.5±8.5aB
2	85.7±2.1abC	61.6±3.4bA	90.0±9.6bA	79.4±8.7bA	125.2±0.4bA
Tryptamine					
0	62.3±7.2aBC	37.1±2.7aA	38.4±5.1aA	48.9±0.6aAB	47.5±0.8aAB
1	69.8±1.8aB	38.8±1.2aA	45.0±1.1bA	67.6±3.7bB	51.1±2.7bA
2	65.8±9.2bC	40.1±2.3bA	42.8±3.4bA	63.7±2.2abC	59.4±9.3abBC
Putrescine					
0	12.5±0.7aA	15.4±5.2aA	119.8±9.5aD	30.2±1.1aA	224.6±20.1aE
1	22.1±6.2abA	30.5±2.1bA	164.7±11.2bC	41.5±2.6abA	274.6±13.2bD
2	20.1±2.8bA	51.4±4.4cAB	157.6±5.8abDE	61.4±4.3cABC	264.3±18.1abF
Cadaverine					
0	100.6±36.3bBC	9.2±3.7aA	51.5±3.5aAB	97.5±3.4aBC	45.6±2.3aAB
1	93.6±11.2aC	20.1±7.3abA	66.3±0.9bB	119.6±10.8bD	46.3±1.6aB
2	122.3±4.1abC	51.4±4.3cA	65.2±0.3bB	120.6±5.6bC	49.8±0.1aA
Spermidine					
0	39.0±1.2aBC	11.5±2.3aA	28.9±1.4aAB	61.2±1.5aD	19.6±4.5aA
1	59.5±5.7bB	38.5±4.2bA	28.2±2.6aA	69.8±1.8bB	29.2±4.2bA
2	57.9±6.4abB	47.2±0.6bB	57.6±4.5bBC	61.3±1.2aC	20.9±0.3aA

Different small letters indicate statistical difference at α = 0.05 level in each column.
Different capital letters indicate statistical difference at α = 0.05 level among products at each time.
S1- S5 are fresh kashar and S6-S10 are old kashar cheeses.

Table 4. Changes of biogenic amine concentration (mg/kg) in mature kashar cheeses during storage

Time (weeks)	Kashar cheese types				
	S6	S7	S8	S9	S10
Histamine					
0	834.3±7.6cD	136.69±8.2cB	52.8±2.2abA	1334.4±31.4bE	269.4±23.6cC
1	1001.1±9.1dC	148.79±11.2cC	406.7±15.0eE	2035.8±58.3cG	300.8±2.5cdD
2	1007.5±10.2dG	270.15±9.5dD	362.2±0.5dE	3042.9±74.9eH	483.1±17.4eF
Phenylethylamine					
0	104.4±15.8aB	325.9±34.1bCD	355.2±20.1bD	43.9±1.7dA	280.2±4.1cC
1	263.3±12.6bcC	479.4±56.1cD	480.5±8.5cD	42.5±5.1dA	243.6±12.8bcBC
2	427.9±24.8dF	347.3±9.6bE	460.2±9.8aF	38.4±1.9cA	282.1±9.1cD
Tyramine					
0	98.0±6.1aD	289.0±5.1eE	71.2±6.4bcBC	4324.0±90.9bF	103.0±3.1bD
1	176.7±3.1bD	190.3±4.6cD	129.0±7.2cC	5771.0±17.0cE	190.3±13.8dD
2	256.4±9.2cB	253.0±0.4dB	99.8±3.3dA	6665.6±130.2dC	123.2±4.6cA
Tryptamine					
0	375.1±23.4cE	251.5±4.8eD	34.0±3.7abA	32.6±1.7bA	78.7±1.5cC
1	421.0±4.3dE	125.3±6.8dC	74.3±15.6cB	42.4±4.2bcA	76.7±11.1dD
2	552.1±2.6eF	125.3±6.8dE	62.7±0.8cC	49.1±6.1cdBC	90.1±1.2cD
Putrescine					
0	53.4±0.1aB	313.6±4.0cF	50.1±1.2cB	384.4±3.5cG	92.7±5.6bC
1	134.6±34.6abBC	326.4±5.4cE	100.5±8.4cB	440.2±2.7cF	147.7±9.0cC
2	206.1±6.1bE	255.4±21.5bF	86.7±5.6bBC	435.1±8.9cG	109.9±9.5dCD
Cadaverine					
0	98.4±7.5bcdBC	167.0±4.5cC	349.0±9.1bE	254.7±12.7aD	149.0±1.2cC
1	95.5±4.8bcC	165.0±1.7cE	441.0±9.3dG	239.3±15.6aF	150.1±7.5bD
2	179.0±5.4dD	131.0±7.4bcC	448.2±3.2dF	218.8±3.2aE	173.1±6.3dD
Spermidine					
0	48.9±0.7aCD	58.1±1.5bD	28.4±3.5abAB	132.7±1.3eF	109.5±1.5cE
1	68.3±2.7aB	87.9±6.7cC	33.6±5.1bcA	119.0±5.4dD	138.6±1.3dE
2	96.3±2.9bE	178.3±1.2eF	60.5±5.8dC	89.0±2.2cDE	79.6±6.2bD

Different small letters indicate statistical difference at α = 0.05 level in each column.
Different capital letters indicate statistical difference at α= 0.05 level among products at each time.
S1- S5 are fresh kashar and S6-S10 are old kashar cheeses.

Changes in Tryptamine Concentration

Tryptamine was detected in all kashar cheese samples. The highest tryptamine concentration was detected in sample S6 amoung all cheese samples. Tryptamine was found from 0.32 to 40.44 mg/kg in tulum cheese (Öner et al., 2004) and in the range as 0-172.6 mg/kg in herby cheese (Andıç et al., 2010a). The toxic threshold level of tryptamine is not known. During the storage period, tryptamine concentration of fresh and mature kashar cheeses increased, decreased or remain same. Decrease in tryptamine concentration could be explained as the consumption of produced biogenic amines by microorganisms as a nitrogen source. Also, some authors have suggested that the decrease of biogenic amines during ripening could be related to the activity of bacterial amine oxidases (Leuschner et al., 1999).

Changes in Putrescine Concentration

The level of putrescine concentration significantly increased (p<0.05) during storage period for all kashar cheese samples. S3 and S5 samples had higher putrescine concentration at the beginning of storage period amoung fresh kashar cheeses. S9 sample had also the highest putrescine concentration in all samples. *Enterobacteriaceae* are generally considered as microorganisms with a high decarboxylase activity, particularly in relation to the production of putrescine

(Suzzi et al., 2003). Presence of high concentrations of putrescine in S3, S5 and S9 samples could be explained by high decarboxylase activity of *Enterobacteriaceae*.

The biogenic amines most commonly found in fermented dairy products are histamine and tyramine, but putrescine is also frequently detected and can occasionally accumulate in concentrations of up to 2.5 g per kg of cheese. Risk taking into account that levels of 875 mg putrescine per kg have been detected in cheeses (Fernandez et al., 2007). None of the samples exceed this level in our study. But presence of high amount of putrescine can increase the toxic effects of other biogenic amines frequently present in fermented foods and beverages, as histamine, tyramine and phenylethylamine (Linares et al., 2013). On the other hand, high concentrations of putrescine affect the organoleptic quality of food due to its foul odour (Ladero et al., 2012).

Changes in Cadaverine and Spermidine Concentration

Cadaverine concentration increased significantly (p<0.05) during the two weeks of storage for all kashar cheese samples (Tables 3 and 4). Initially, cadaverine concentrations changed as 9.2-100.6 mg/kg and 98.4-349.0 mg/kg for fresh and mature kashar cheeses, respectively. During storage, cadaverine concentrations changed as 49.8-122.3 mg/kg and 131.0-448.2 mg/kg for fresh and mature kashar cheeses, respectively. Cadaverine concentration was found as relatively high levels in some samples. A wide variability of cadaverine concentration was detected and reported for different cheeses. It could be due to the differences in the manufacturing process: type of milk (sheep or cow), heat treatment of milk (such as pasteurization), ripening time, microflora and cheese mass (Andıç et al., 2010a). It was observed that sample S1 had the highest cadaverine concentration at the beginning of storage period amoung fresh kashar cheeses. Also, initial highest cadaverine concentration amoung mature kashar cheeses was detected in sample S8 (349.0 mg/kg). Cadaverine has less pharmacological activity than the aromatic amines but it is probably potentiators of their toxicity (Joosten, 1988). Also, it could be used as quality indicator for cheese making. Maximum levels of cadaverine were found as 1844.5 mg/kg in Herby cheese (Andıç et al., 2010a), 1110 mg/kg in Brazilian cheese (Vale & Gloria, 1998) and reach levels >1000 mg/kg in Motal cheese (Andıç et al., 2010b).

Spermidine in kashar cheese were also detected and it was observed that storage period was significantly (p <0.05) effective. Maximum initial spermidine concentrations were found for samples S9 (132.7 mg/kg) and S4 (61.2 mg/kg) amoung in mature and fresh kashar cheese samples, respectively. Spermidine concentration is not usually detected in cheese samples. There is no any specified toxic value for spermidine in cheese.

Total Biogenic Amine Contents of Fresh and Mature Kashar Cheeses

Total biogenic amine contents were calculated by the summation of histamine, phenylethylamine, tyramine, tryptamine, putrescine, cadaverine and spermidine contents of mature and fresh kashar cheeses and the results were given in Table 5. Initiallly, total biogenic amine contents of fresh and mature kashar cheese samples were found in the range as 222.4-556.6 and 940.6-6507.5 mg/kg, respectively. During storage, total biogenic amine contents of all samples increased significantly (p<0.05). Total biogenic amine contents of mature kashar cheese samples were higher than that of fresh kashar cheese samples. An acceptable level of 1000 mg/kg for total biogenic amine content was proposed (Silla-Santos, 1996) total biogenic amine contents of fresh kashar cheese samples never exceed this limit during storage. But, total biogenic amine contents of mature kashar cheese samples were higher than 1000 mg/kg level before and during storage period. This could be due to the long ripening period of mature kashar cheese and its consumption can cause toxicity.

Conclusion

The results of this study showed that storage period had significant effect on formation of biogenic amines in all kashar cheese samples. In general, the biogenic amine content of cheese can be extremely variable and depends on the type of cheese, the ripening time, the manufacturing process and the microorganisms present. According to the results, mature kashar cheese samples had higher biogenic amine content than fresh kashar cheeses. Concentrations of biogenic amines changed with the same trend almost in all samples during the storage period. According to the toxic limits, mature kashar cheese samples should not be consumed. Also, all mature kashar cheeses had critical toxic level of histamine. In fresh kashar cheese, there were no toxicity risk with respect to the biogenic amine. Mature kashar cheese generally prefferred due to its special flavor and taste but this study showed that it is not safe with respect to biogenic amine. Therefore, further research is needed to optimize processing technology and ensure low amine levels for mature kashar cheeses.

Table 5. Total biogenic amine contents of fresh and mature kashar cheeses

Time (weeks)	Kashar cheese types				
	S1	S2	S3	S4	S5
0	396.9±101.2aB	222.4±25.6aA	373.9±38.3aB	428.9±17.7aB	556.6±20.6aC
1	484.4±8.5aB	322.0±16.7bA	465.7±5.0bAB	546.7±29.3bBC	687.3±12.6bcC
2	552.1±41.0aBC	413.0±8.5cA	515.8±11.9bB	599.4±16.7bC	731.3±29.8bD
	S6	S7	S8	S9	S10
0	1612.5±33.2aF	1541.9±66.5bF	940.6±14.3aD	6507.5±32.5aG	1082.5±80.1aE
1	2160.5±115.9cF	1523.3±77.3bDE	1666.7±114.3cE	8691.2±6.8cG	1427.9±119.8bD
2	2725.2±42.5dG	1560.6±40.4bF	1580.5±9.7cF	10538.6±55.8dH	1340.6±32.2bE

Different small letters indicate statistical difference at α = 0.05 level in each column.
Different capital letters indicate statistical difference at α= 0.05 level among products at each time.
S1- S5 are fresh kashar and S6-S10 are old kashar cheeses.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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