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Discovering the Relationship between Lactic Acid Bacteria and Biogenic Amines during the Processing and Storage of toast Cheese

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Abstract

The study investigated the changes of growth of microorganisms (total aerobic mesophilic bacteria (TAMB), mold and yeast, total lactic acid bacteria, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, and Bifidobacterium lactis), biogenic amine formation, moisture content, pH, texture, nitrogen content and values of 2-thiobarbituric acid reactive substance of toast cheese stored at four different temperatures (5 °C, 15 °C, 25 °C and 37 °C). Additionally, the relationship between type of lactic acid bacteria and biogenic amines during processing and storage of toast cheese was searched. Storage time had significant effect (p<0.05) on microbial level of toast cheese. Also, microbial counts mainly increased (p<0.05) with increasing storage temperature. Total lactic acid bacteria (LAB) count increased from 3.52±0.27 log cfu/g up to 6.69±0.01 log cfu/g with storage time and temperature. TAMB count ranged from 4.39 to 6.91 log cfu/g during storage, whereas mold and yeast count increased up to 4.15±0.14 log cfu/g within 45 days of storage, then it decreased to about 2.92±0.03 log cfu/g. pH values of the samples reduced (p<0.05) from 6.61±0.02 to 5.05±0.02 during the storage period. Statistical analysis correlated the relationship between the formation of biogenic amine with type of LAB, as biogenic amine formation could increase due to the growth of lactic acid bacteria. Longer fermentation and storage periods could increase the biogenic amine formation that could be higher level than their acceptable levels. It would be possible to reduce the amounts of biogenic amines by controlling the growth of LABs.

Keywords: Toast Cheese, Biogenic Amines, Lactic Acid Bacteria, Biochemical constituent, Nitrogen contents

1.INTRODUCTION

Kashar cheese, one of the three most produced types of cheese in Türkiye, can be produced using different processes (Tekinşen 2000). A kind of processed cheese has been sold under the name of "fresh kashar". However, it does not have same physical, chemical and organoleptic properties as fresh kashar cheese (Akarca and Tomar, 2019). This processed cheese has been named "Toast Cheese" by the cheese communique which was issued in the Turkish Codex (Anonymous 2015). Toast cheese is defined as obtained by heat treatment and deicing salt (such as citrates and phosphates) with the use of hard and semi-hard or

sometimes soft types of cheese (Uçuncu 2004; Akarca and Tomar, 2019). Toast cheese has high salt content with low moisture content. For this reason, it has a long shelf life compared to other cheeses. However, due to its high pH value and low lactic acid content, toast cheese is very delicate to microbial spoilage.

Biogenic amines are biologically active nitrogenous compounds formed generally by the decarboxylation of free amino acids. Lactic acid bacteria can tolerate the cheese by causing microbial decarboxylation of the amino acids to form biogenic amines. One of the most common foodborne diseases caused by

biogenic amines is the presence of a high level of histamine. There have been several outbreaks of histamine poisoning by the consumption of cheeses, especially Swiss cheese and cheddar cheese, which contain high levels of histamine (Vale and Gloria, 1998). Consuming high amounts of biogenic amines containing cheese can lead to various physiological effects. There is a hypertensive crisis called the "Cheese Reaction". Its prominent symptom is severe headache after eating foods high in tyramine. Toast cheese contains high amounts of protein which makes it ideal substrate for the production biogenic amines. concentrations of free amino acids resulting from proteolysis, availability of amino acid decarboxylase producing microorganisms, appropriate temperature, pH, cofactors and water activity are all increase the amine production in cheese (Benkerroum, 2016). Presence of high level of certain biogenic amines in foods can cause some foodborne diseases. In some cases, presence of biogenic amines in foods is related with the poor hygienic conditions of food production and/or the contamination of food. However, in the case of fermented foods, biogenic amine formation can be related with the lactic acid bacteria used as a starter culture (Ladero et al, 2008). It was reported that biogenic amine formation in fermented foods may also related with the type of starter culture used; pure or mixed, amino negative or positive, probiotic bacteria, etc. (Ercan et al, 2013).

There are some studies relating the formation of biogenic amines with the type of lactic acid bacteria used (Ladero et al, 2008; Priyadarshani and Rakshit, 2011; Pachlova et al, 2016). As far as we know, there was a lack of information about the types of lactic acid bacteria and their relationship with biogenic amine formation during the storage of toast cheese. Therefore, this study was conducted to understand the relation between type of lactic bacteria and

biogenic amine formation and to figure out the problems that are encountered with toast cheese during manufacturing and after storage also by focusing the physical, chemical and microbiological changes. Thus, as a result of this study, steps to increase the quality of toast cheese will be determined. The objectives of this study were (1) to determine the effect of storage conditions on physical, chemical and microbiological changes of toast cheese, (2) to investigate effect of storage temperature and time on the forming of biogenic amines and (3) the discover relation between microorganisms especially type of lactic acid bacteria and biogenic amine formation during the ripening and storage of toast cheese.

2. MATERIALS AND METHOD

2.1. Materials

The chemicals; 1,7-diaminoheptane, sodium bicarbonate, perchloric acid, plate count agar (PCA), potato dextrose agar (PDA), De Man, Rogosa and Sharpe (MRS) agar, M17 agar, lactose, raffinose, maltose, fructose, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA) were obtained from Sigma-Aldrich whereas malondialdehyde bis (dimethyl acetal) (MDA) and perchloric acid from Merck GaA.

Toast cheese samples were kindly produced by Kahkecioğlu Cheese factory in Gaziantep city, Türkiye. Two batches, about 64 cheeses each of 250 grams, were prepared. About 16 samples were put into an incubator at 5°C, 16 samples at 15°C, 16 samples at 25°C and 16 samples at 37°C. At each sampling time, 2 samples were taken, and duplicate analyses were done in each sample. Therefore, each data point was the average of 4 measurements.

2.2. Microbiological Analysis

The enumeration of microorganisms, as a log cfu/ml, in toast cheese made on samples from 5°C, 15°C, 25°C and 37°C corresponding to storage time. All samples were serially diluted up to 10-7 with peptone water (0.1% v/v) and

pour plate technique, 0.2 ml of inoculum, was applied to all of the microbial counts, then the plates were incubated at the desired conditions. All operations were done as duplicated on petri dishes.

Lactic acid bacteria counts were carried out according to the method proposed by Dave and Shah (1998). Streptococcus thermophilus were counted by the pour-plate technique into M-17 agar enriched with 1% lactose (M17-lactose) and petri plates were incubated in anaerobic incubator (Sanyo, Japan) at 5% CO2 at 30°C for 72 hrs. For the counting of L. delbrueckii subsp. bulgaricus, applicable dilutes were inoculated by pour-plated into MRS agar enriched with 1% of incubated under anaerobic fructose and condition (5% CO2) at 30°C for 72 hrs. Bifidobacterium lactis was enumerated on MRS agar that enriched with 1% of raffinose were used with spread-plated technique and incubated anaerobically with 5% CO2 at 30°C for 72hrs. Total aerobic mesophilic bacteria were enumerated with PCA in spread-plate technique at 37°C for 48hrs. Mold and yeast were counted with PDA by spread-plate technique at 37°C up to 5 days.

2.3. Determination of Biogenic Amines

The biogenic amines were determined by the use of a chromatographic method (Şahin-Ercan et al., 2019). The HPLC consisted of a Shimadzu gradient pump (Shimadzu LC 20AB, Shimadzu Solvent Delivery Module, Kyoto, Japan), a Shimadzu auto-injection unit (Shimadzu SIL2OAHT, Kyoto, Japan), a Shimadzu UV detector (Shimadzu SPD 20A, Kyoto, Japan), and a RP-18 guard. Spherisorb ODS2 was 200 µm and 4.6 mm, 200 mm was the column of the HPLC. A solution of ammonium format (0.4 M) prepared from ultra-pure water (Millipore Elix 10UV and Milli-Q, Millipore S.A.S. 67120 Molsheim, France) and acetonitrile was filtered through a filter of 0.45 µm Millipore (Billerica, MA).

Using a Waring blender, two grams of sample were homogenized with 10 ml of 0.4 M perchloric acid. The sample was filtered and centrifuged at 1790xg for 10 min. The extraction was repeated with another 10 mL of 0.4 M perchloric and acid, the complained supernatants were made up to 25 mL with 0.4 M perchloric acid. One milliliter of extract was piped into a glass stopped test tube, adding 200 μL of 2 N NaOH and 300 μL of saturated sodium bicarbonate solutions. To each sample, two milliliters of dansyl chloride (10 mg/mL) solution was added and incubated at 40°C for 45 minutes. Residual dansyl chloride was removed by adding 100 μL ammonia (25 %). The solution was adjusted to 5 mL with acetonitrile after 30 min. The solution was centrifuged at 1790xg for 5 min. The supernatant was filtered (0.45 μ m) and then 20 μL of clear solution was injected onto the HPLC. The dansyl derivative of standards was diluted to 1 mL with 0.4 M perchloric acid and they were introduced to HPLC at the same condition to draw standard curve (Şahin-Ercan et al., 2019).

2.4. Determination of Moisture Content and pH

For determination of the moisture content of toast cheese, an oven method was used. To determine the moisture content, about 5 g of samples were put into the oven at 105°C up to the constant weight reached.

The pH 211 Microprocessor pH meter was used to determine the pH values of toast cheese samples. 10 grams of homogenized sample were mixed with 90 ml of distilled water and the pH of the sample was recorded when the value stabilized (Mei et al., 2006).

2.5. Determination of Texture

The texture of toast cheese, stored at four different temperatures, at 5°C, 15°C, 25°C and 37°C, were measured to find effects of storage on the hardness and chewiness values. For texture analysis, samples were stored at room

temperature at least 2 hours before being analyzed so that cuts (3cm \times 3cm \times 2cm) and seized for equilibration to room temperature ($^{\sim}20^{\circ}$ C).

Hardness and chewiness analyses were performed using a TAX-T2 texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). This test was preformed especially requirement in the system like, pretest speed as 1.00 mm/sec, test speed as 1.00mm/sec, target mode strain, time 25.00%, duration time 5sec, target type auto force and trigger as 0.005kg and tare mode auto (Mei et al., 2006)...

2.5. Determination of Nitrogen Contents of Toast Cheese

Total nitrogen and nitrogen fractions of toast cheese protein were determined during the storage period. The total nitrogen content of toast cheese was determined by the kjeldahl method (IDF, 1993) (Kjeltec 2200). About 1 g of the sample, 7 g of potassium sulfate (K2SO4), 1 spatula of copper sulfate (CuSO4) and 12 ml of concentrated sulfuric acid were added to it and two small mixing balls were placed in the digestion tubes. Then, the combustion process was carried out at 400°C for 40 minutes. After the combustion process was completed, the samples were sufficiently rested and distillation analysis was performed on the kjeltec 2200 device. The sample was titrated with 0.1 N HCl until the color changed from blue to red. The protein content of cheeses was calculated by multiplying the total nitrogen content by 6.38. The water soluble nitrogen (WSN) content of toast cheese was determined by the method proposed by Ürkek (2008). About 20 g of toast cheese was mixed with 100 ml of deionized water. Then the samples were homogenized in a warming blender for 2 minutes. Homogenized samples were kept for 1 hour at 40°C. Then the samples were centrifuged at 3000xg for 30 minutes at 4°C. 3 mL of the filtrate was taken

and its nitrogen content was determined by the kjeldahl method.

Trichloroacetic acid soluble nitrogen (TCASN) content was determined by the method of Bütikofer et.al (1993). 25 ml of WSN extract was added to 25 ml of 240g/kg TCA solution. Suspension was held at room temperature for 2 hours and then filtered (Whatman No. 40). 3 mL of the filtrate was taken and its nitrogen content was determined by the kjeldahl method.

2.6. Determination of 2-Thiobarbituric Acid Reactive Substances (TBARS) of Toast Cheese

Two grams of homogenized toast sample were taken and TBARS was 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 a glass-stoppered test tube. TBA reagent (5 mL) was added and the mixture heated in a boiling water bath for 35 min. After cooling, the absorbance of the sample was read against the appropriate blank at 538 nm (Şahin-Ercan et al, 2019). The concentration range for standard preparing the curve using malondialdehyde bis (dimethyl acetal) were adjusted from 5x10-9 to 5x10-8 mol MDA.

2.6. Statistical Analysis

The results were analyzed by statistical analysis SPSS version 14.0 for Windows (SPSS Inc., Chicago, IL, USA). The two-way analysis of variance (ANOVA) and Duncan's multiple range test were performed to determine how the temperature and storage time affect the microbial growth, pH, moisture content, TBARS, nitrogen fractions and textural attributes. Pearson two-way correlation test was conducted to search the relationship between biogenic amine formation and microbial growth.

3. RESULTS AND DISCUSSION

3.1. Microbiological analysis

Growth of Lactic Acid Bacteria (LAB) in toast cheese was recorded during 105 days of storage kept at four different temperatures (5°C, 15°C, 25°C and 37°C) and their results are shown in Figure 1. The two-way ANOVA results showed that increasing the storage time temperatures increased (P<0.05) the growth of S. thermophilus (Figure 1a), Lactobacillus delbrueckii subsp bulgaricus (Figure 1b) and Bifidobacterium lactis (Figure 1c). When the toast cheese samples were stored at 5°C, 15°C and 25°C, same increment trend for the growth of S. thermophilus was observed, but the highest increment was at 37°C (Figure 1a). Akarca and Tomar (2019) reported that LAB count of toast cheese, collected from Afyonkarahisar province, varied between 4.06 and 6.08 log CFU/g. Öksüztepe et al. (2009) also found that increasing storage time increased LAB count in kashar cheese. There are also different studies for kashar cheese, such as reported by Çetinkaya and Soyutemiz (2006). They found that the count of S. thermophilus decreased from 8.24 log cfu/g to 3.10 log cfu/g after 90 days. Akarca and Tomar (2019) found that Lactococcus/Streptococcus spp. count of toast cheese ranged between 3.88 and 5.75 log CFU/g.

The growth of TAMB was followed within 105 days, and the results are shown in Figure 1d. Increasing the storage temperature increased (P<0.05) the total aerobic mesophilic bacteria count. Whereas, generally increasing the storage time up to 45 days increased the TAMB. However, after that time, TAMB count decreased in all samples at all temperatures. Akarca and Tomar (2019) reported that TAMB count of toast cheese changed between 3.59 and 7.36 log CFU/g. Sert et al. (2007) reported that TAMB count in kashar cheese increased with increasing ripening time to about 4.29-4.57

log cfu/g. Sert et al. (2007) reported that TAMB count in kashar cheese could increase from poor hygiene manufacturing up to 7.70-8.47 log cfu/g.

Mold and yeast count of samples stored at 5, 15, 25 and 37°C increased (P<0.05) for the first 45 days of storage and then gradually decreased with increasing storage time (Figure 1e). The highest and lowest mold and yeast counts were found for the samples stored at 25C and at 45th for 105th days of storage, respectively. Our results were similar to those found in kashar cheese by Gül and Dervisoğlu (2013). Akarca and Tomar (2019) observed that mold and yeast count of toast cheese varied between 2.58 and 7.78 log CFU/g. Moreover, similar results reported by Öksüztepe et al. (2009) show that the creamed or uncreamed cottage cheese, packaged with aluminum at 5°C reduces mold and yeasts and keeps a fresh flavor. Sert et al. (2007) reported that the addition of starter culture in toast inhibits mold and yeast growth on the surface of the cheese. Aljewic and Cichosz (2015) correlate the decrease in mold and yeast population with a high population of starter lactic acid bacteria in cheese.

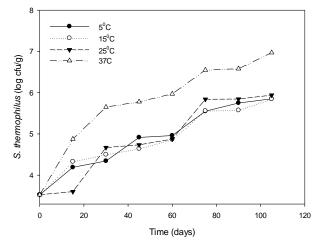
3.2. Determination of Biogenic Amines

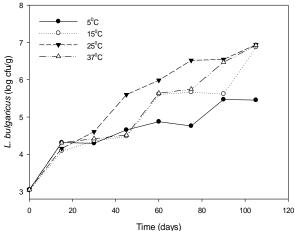
Changes in histamine (HI) level in toast cheese were analyzed within 15-day intervals during 105 days of storage at four different temperatures (5°C, 15°C, 25°C and 37°C). Increasing the storage time and temperature increased (P<0.05) histamine concentration (Table 1). During storage at 5°C, HI concentration was not changed significantly (P>0.05) with time, compared to other temperatures. During the ripening period (60, 75, 90 and 105 days), the concentration of histamine significantly (P<0.05) increased when stored at 15°C, 25°C and 37°C. These results were lower than those found by Şahin-Ercan et al. (2019) and Innocente and D'Agostin (2002).

Changes in spermidine in toast cheese were analyzed during 105 days stored at four different temperatures. The results of statistical analysis are shown in Table 1. The ANOVA results showed that increasing the storage time temperature increased (P<0.05)spermidine concentration (Table 1). During storage, the spermidine concentration of toast cheese was stored at 5°C and 15°C was not changed significantly (P>0.05). The spermidine concentration of toast cheese was stored at 25°C and 37°C during storage time were significantly (P<0.05) increased. This study confirmed those found for kashar cheese by Şahin- Ercan et al. (2019).

The results of the two-way ANOVA analysis showed that increasing the storage time and temperatures increased (P<0.05) tyramine (TYR) concentration. During storage, the TYR concentration of toast cheese stored at 5°C was not changed significantly (P>0.05) with time, compared to other storage temperatures. However, the TYR concentration of toast cheese

stored at 15°C, 25°C and 37°C during the fresh stage (0, 15, 30 and 45) days was slightly changed (P<0.05) however, during the ripening period (60, 75, 90 and 105 days) of toast cheese, its concentration dramatically (P<0.05) increased. Changes of tryptamine (TRY) in toast cheese were also analyzed for 105 days stored at four different temperatures. The ANOVA results showed that increasing the storage time and temperature increased (P<0.05) TRY level. The TRY concentration in toast cheeses that were stored at 15°C, 25°C and 37°C were significantly (P<0.05) increased, but not (P<0.05) at 5°C. The results obtained in this study were lower than those found by Şahin-Ercan et al. (2019) also similar to those found by Innocente and D'Agostin (2002). Similar trends observed for cadaverine (CAD) and spermine concertation, these results were lower than those found in kashar cheese by Şahin-Ercan, et al. (2019), also similar to those found by Innocente and D'Agostin (2002).





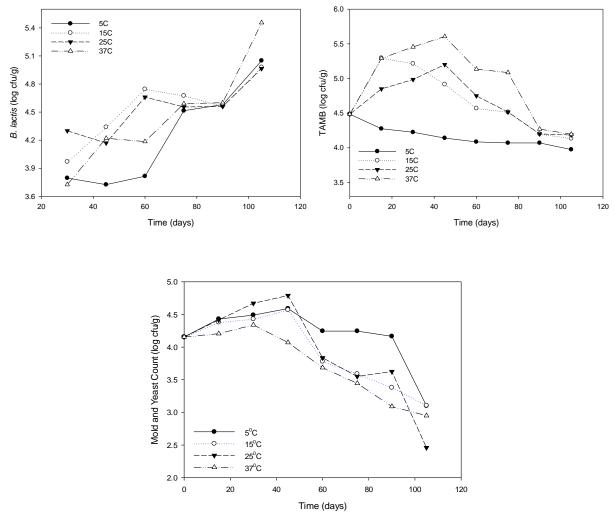


Figure 1. Effect of temperature and storage time on microbial count (a) S. thermophilus, (b) L. bulgaricus, (c) B. lactis, (d) total aerobic mesophilic bacteria, and (e) mold and yeast in toast cheese.

Pearson correlation test showed that biogenic amine formations and type of microorganisms relationships. Histamine formation increased with increasing S. thermophilus (r=0.802, p<0.01) and Lactobacillus delbrueckii subsp bulgaricus (r=0.787, p<0.01) counts whereas decreased with increasing mold and yeast counts(r= - 0.401, p<0.05), but no relation with TAMB (r= 0.227, p>0.05). Same trends were observed for other biogenic amine formations. However, Bifidobacterium lactis count was not correlated with histamine (r=0.364, p>0.05), tryptamine (r=0.371, p>0.05) and spermidine (r=0.144, p>0.05) formations. These results showed that biogenic amine formation could increase by the growth of lactic acid bacteria. From that point, increasing the

fermentation and storage periods that gave suitable conditions for growing of lactic acid bacteria, biogenic amine formation can get higher level than their acceptable levels. As it was known that upper limit of histamine for food is 100 mg per kg of foods (Eerola et al., 1997). Diamines such as putrescine and cadaverine have no adverse health effects, but may react with nitrite to form carcinogenic nitrosamines (Hernandez-Jover et al., 1997)

Table 1. Changes in biogenic amines (mg/kg) and textural attributes in toast cheese.

	Time (days)	Tyramine (TYR)	Tryptamine (TR)	Histamine (HI)	Cadaverine (CAD)	Spermine	Spermidine	Hardness (g)	Chewiness
5 ℃	0	19.49±0.7aA	33.44±4.7aA	31.68±1.48aA	22.09±0.35aA	21.79±0.14abA	7.71±0.21bA	6204±288.43cA	5408.86±463.51aA
	15	21.28±0.7aA	38.82±9.8aA	33.74±0.6abA	21.92±0.05aA	21.59±0.11aA	7.41±0.09aA	5692±43.58bA	5795.44±120.1bA
	30	19.63±0.7aA	37.59±4.5aA	35.34±1.5abA	22.75±0.31aA	21.86±0.07bA	8.47±0.09cA	5606±63.94bA	5644.19±49.03bBA
	45	20.18±0.7aA	32.73±5.4aA	33.25±0.6abA	22.48±0.54aA	21.68±0.07abA	8.51±0.02cA	5316±100bA	5598.15±10.00abA
	60	20.53±0.4aA	37.40±2.2aA	35.31±2.2abA	22.30±0.00aA	27.71±0.07cA	9.96±0.02eAB	5455±397bA	5588.15±20.00abA
	<i>7</i> 5	19.15±1.4aA	38.36±16.2aA	34.31±0.47abA	22.02±0.00aA	31.86±0.12fA	9.40±0.07dAB	5340±233bA	5644.19±15.00abA
	90	19.80±1.4aA	61.56±4.5bA	34.66±0.00abA	21.90±0.14aA	31.47±0.07eA	10.50±0.03fA	4117±60aA	6255.05±25.00cA
	105	19.97±0.6aA	67.93±9.6bA	35.77±0.00bA	22.69±0.14aA	30.78±0.07dA	10.48±0.01fA	3953±200aA	6455.05±20.00cA
15 °C	0	19.49±0.7aA	37.88±11.0aA	31.68±0.00aA	22.09±0.35aA	21.79±0.14aA	7.87±0.01aA	6204±288.43cA	5408.86±463.51a
	15	19.44±0.7aA	38.90±11.1aA	34.30±0.00bA	22.54±0.00abA	22.83±0.21bA	8.38±0.10bcA	5071±27.26eA	5885.01±168.7bA
	30	19.36±1.3aA	39.92±6.68abA	34.89±0.00abA	22.96±0.00bA	21.51±0.49aA	8.60±0.04cA	4510±422dA	5870.93±33.34bA
	45	20.15±0.4abA	51.20±6.67abcA	34.99±0.00bcA	22.95±0.00bA	27.38±0.28cA	8.61±0.04cA	3425±38.12cA	5961.18±12.79bcA
	60	20.79±0.8abA	51.32±6.3abcA	34.38±0.2bcA	23.64±0.±0.00cA	30.60±0.21dA	8.19±0.01abA	3287±430bcA	5953.80±10.00bcA
	<i>7</i> 5	21.22±0.5bA	57.14±5.3cdA	37.22±2.1bcA	24.45±0.00dA	43.24±0.07eA	8.72±0.04cA	2364±269aA	6441.76d±22.00dA
	90	19.24±2.0bA	59.47±1.54cdA	35.23±0.00cdA	24.74±0.00dA	48.68±0.07fA	9.52±0.33dA	2417±347aA	6441.76±20.00dA
	105	21.19±3.9bA	71.41±3.87dA	36.27±0.00dA	24.27±0.00dA	47.54±0.07gA	11.18±0.22eA	2892±128bA	6219.61±10.00cdA
25 ℃	0	19.49±2.6aA	32.94±4.01aA	31.68±2.8aA	22.09±0.35abA	21.79±0.14aA	7.87±0.02aA	6204±288.43cA	5408.86±463.5a
	15	20.13±1.3aA	38.94±9.4aA	35.39±0.5abA	21.93±0.00aA	39.30±0.35bA	9.58±0.12bA	5790±19.76eA	5670.47±30.00bA
	30	19.52±1.52aA	58.25±9.2aA	35.82±0.2abA	22.46±0.00bA	57.76±0.14cA	9.17±0.24bA	4510±422dA	5790.64±10.33bcA
	45	20.01±0.4aA	103.89±16.8bA	35.42±0.3bA	23.76±0.00cA	78.28±0.21dA	11.14±0.12cA	3628±231cA	5796.61±30.00bcA
	60	19.46±1.4aA	126.30±6.1cA	36.57±0.4abA	24.24±0.00dA	95.92±0.07eA	12.63±0.05cA	2287±430bA	5857.50±22.00bcA
	75	24.25±2.5bA	134.04±16.4cA	36.27±0.7abA	24.52±0.21dA	141.52±0.21fA	11.92±0.08cA	1497±124aA	6209.34±10.00dA
	90	26.49±1.1bA	170.96±11.3dA	36.50±0.6abA	27.08±0.00eA	142.48±0.14gA	15.94±0.05dA	1391±373aA	6209.34±33.00dA
	105	27.79±1.42bA	182.06±11.03dA	36.81±0.00abA	28.12±0.00fA	149.89±0.07hA	16.36±0.43d	1681±243aA	6072.78±32.00cdA
37 ℃	0	19.49±2.8aA	137.88±11.0aA	31.68±0.00aA	22.09±0.35aA	21.74±0.07aA	7.90±0.06aA	6204±288.43cA	5408.86±463.51a
	15	20.62±0.8aA	141.14±6.5aA	35.40±0.53bA	22.65±0.45bA	45.93±0.07bA	13.17±0.07bA	5391±72.91eA	5715.47±128.77bA
	30	20.80±4.7bA	180.73±2.9bA	36.81±0.00bcdA	24.52±0.21cA	47.66±0.07cA	27.20±0.07cA	4406±241dA	5701.14±52.77abA
	45	20.36±3.3abA	113.08±17.9aA	36.81±1.3cdA	25.60±0.00dA	82.50±0.14dA	29.92±0.07dA	3366±268.60cA	5771.03±21.00bcA
	60	20.47±2.6abA	237.32±27.5cA	37.86±0.1dA	25.52±0.00dA	91.88±0.14eA	30.20±0.01eA	2608±334bA	6063.41±12.00cA
	75	26.05±2.1bA	189.58±0.28bA	36.23±0.00bcA	25.71±0.00dA	135.82±0.14fA	33.93±0.01gA	1444±193aA	6076.56±11.00dA
	90	27.32±1.8bA	256.88±15.6cdA	36.69±0.00cA	28.82±0.00eA	147.66±0.35gA	31.77±0.14fA	1339±329aA	6411.40±22.00dA
	105	28.80±1.9bA	285.48±9.31dA	36.28±0.00bcA	27.67±0.00fA	139.87±0.07hA	31.66±0.02fA	1402±327aA	5899.97±33.00bcA

Different small letter indicates statistical difference at α =0.05 level in each column. Different capital letters indicate statistical difference at α =0.05 level among products in each temperature.

3.3. Texture profile

The hardness and chewiness values of toast cheese during storage at four different temperatures (5°C, 15°C, 25°C, 37°C) were determined and their results are given in Table 1. Increasing the storage time and storage temperature decreased (P<0.05) hardness levels. The hardness value of toast cheese stored at 5°C for fresh stage 0, 15, 30 and 45 days was slightly different (P<0.05), compared with other storage and times temperatures. Results given in Table 1, confirmed that the hardness of toast cheese decreased with increasing the storage time. Similar results were reported by Lawrence et al (1987); Guinee et al (2000); and Zisu and Shah, (2005). Results obtained in this study were lower than those found by Eroglu et al. (2016) as the hardness of the cheese sample at the beginning of the ripening period was in the range of 7223-8679g.

The ANOVA results show that increasing the storage time with temperatures (P<0.05) increased chewiness values. The chewiness value of toast cheese stored at 5°C for 0, 15, 30, 45, 60 and 75 days was slightly significant (P<0.05) compared with other storage times and stored temperatures. Increasing storage periods at all temperatures affect the chewiness of toast cheese sample. Increasing storage time and stored temperatures, the chewiness of samples increased. The results obtained in this study had similar findings to those found in kashar cheese by Koca and Metin (2004) and Akalın et al (2002)

3.4. Change in pH value and Moisture content

The pH value is an important factor affecting the quality of fermented products. Especially in cheese, it is important for milk coagulation,

proteolysis, texture and aroma (Fuentes et al, 2015). Changes in the pH values of toast cheese with storage time at different temperatures are shown in Figure 2. Results showed that increasing the storage time and temperature decreased (P<0.05) pH values. The pH value of samples stored at 5°C up to 45 days of storage were not significantly changed (P>0.05) compared to other storage time and temperatures. It is known that the pH decreases due to the lactic acid produced by the lactic acid bacteria in fermented products. Akarca and Tomar (2019) reported that pH value of toast cheese, collected from Afyonkarahisar province, varied between 4.64 and 5.11. Guinee et al (2000) reported that the pH value of cheddar cheese was constant in the first 30 days of storage. Our results agreed with Sert (2007)'s results such that during at 37° for 15 days of storage of sample having starter culture of lactic acid bacteria causes reduction in the pH of toast cheese.

Moisture content of toast cheese at different temperatures (5°C, 15°C, 25°C and 37°C) during the storage period of 105 days, 8 times with 2week periods followed (Figure 3). It was found that storage time and temperature significantly changed the moisture content of toast cheese (P<0.05). Significant decrease in moisture content values was observed in samples stored at 25°C and 37°C (P<0.05), while no significant change was detected in the toast cheese samples stored at low temperatures. Also, the interaction between storage temperature and storage time significantly affected the moisture content values (P<0.05). One of the reasons for moisture content decrease could be due to the leakage of water from cheese samples at high temperatures, which was also observed in our study

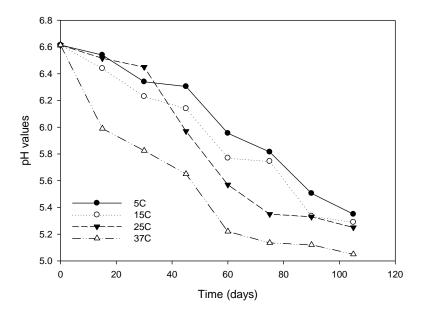


Figure 2. Change in pH value in toast cheese during the storage period

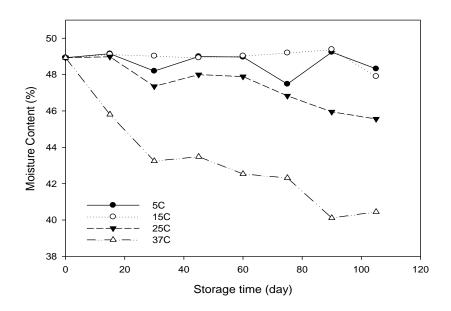


Figure 3. Change in moisture content of toast cheese with storage time and temperature

3.5. Nitrogen Contents of Toast Cheese

The total nitrogen content of toast cheese samples increased by increasing both storage time and temperature (Table 2). In the first 30-day period, total nitrogen values of toast cheese samples did not change much. However, fluctuations were observed in the following days. As a result, it was determined that the

nitrogen content increased over time (P<0.05). It was estimated that the biochemical events occurring in the cheese system, especially proteolysis, could be affected by the pH value of the cheese medium, and therefore, the protein content of cheeses with different pH values changes. In our study, the presence of different temperatures affects the pH, thus affecting the protein ratios.

Table 2. Nitrogen contents and TBARS values of toast cheeses during storage period*

		Storage Temperature							
	Days	5°C	15°C	25°C	37°C				
	0	3.04±0.28 ^{aA}	3.04±0.28 ^{aA}	3.04±0.28 ^{aA}	3.04±0.28 ^{aA}				
	15	2.98±0.43 ^{aA}	3.09±0.11°AB	3.06±0.16 ^{αAB}	3.16±0.32 ^{aB}				
.	30	3.91±0.26 ^{bA}	3.76±0.03 ^{bcdA}	3.89±0.73 ^{bA}	3.94±0.65 ^{bA}				
Total	45	3.74±0.22°A	3.58±0.63 ^{bA}	3.71±0.04° ^A	3.72±0.36°A				
Nitrogen	60	4.36±0.94 ^{dA}	3.91±0.60 ^{cdB}	4.06±0.20dBC	4,31±0.32 ^{dAC}				
(%)	75	3.74±0.50° ^A	3.71±1.01 ^{bcA}	3.97±0.54 ^{bdA}	3.65±0.52 ^{deB}				
	90	4.00±0.17 ^{bA}	4.14±0.26eA	5.04±0.51eB	4.14±0.40 ^{fA}				
	105	3.90±0.32 ^{b,cA}	3.97±0.78 ^{deAB}	4.09±0.13 ^{dB}	4.49±0.10 ^{eC}				
	0	0.24±0.01 ^{aA}	0.24±0.01 ^{aA}	0.24±0.01 ^{aA}	0.24±0.01 ^{aA}				
	15	0.78±0.03 ^{bA}	0.74±0.00 ^{bcAB}	0.67±0.01 ^{bB}	0.93±0.04 ^{bC}				
	30	0.80±0.03 ^{bA}	0.76±0.02 ^{bcA}	0.90±0.00cB	1.38±0.03°C				
WSN	45	1.03±0.15 ^{bA}	0.90±0.10°A	1.01±0.02 ^{dA}	1.76±0.03 ^{dB}				
(%)	60	1.08±0.18 ^{bA}	0.91±0.18 ^{cA}	1.10±0.07 ^{eA}	1.98±0.08 ^{eB}				
	75	0.96±0.13 ^{bA}	0.70±0.01 ^{bB} 1.54±0.01 ^{fC}		2.02±0.02 ^{eD}				
	90	0.93±0.17 ^{bA}	0.90±0.00 ^{cA}	1.45±0.01 ^{gB}	2.14±0.03 ^{fC}				
	105	1.00±0.06 ^{bA}	0.88±0.02 ^{beB}	1.51±0.00 ^{gfC}	2.20±0.03 ^{fD}				
	0	0.25±0.05 ^{aA}	0.25±0.05 ^{aA}	0.25±0.05 ^{αA}	0.25±0.05 ^{αA}				
	15	0.12±0.11 ^{bA}	0.17±0.05°A	0.20±0.00°A	0.37±0.03 ^{aB}				
	30	0.18±0.21 ^{abA}	0.20±0.01 ^{acA}	0.41±0.06 ^{bB}	0.73±0.01 ^{bC}				
TCA SN	45	$0.26\pm0.06^{\alpha A}$	0.15±0.01 ^{cB}	0.44±0.02 ^{bC}	0.82±0.02 ^{bcD}				
(%)	60	0.11±0.00 ^{bA}	0.15±0.01 ^{cA}	0.42±0.05 ^{ьв}	0.94±0.12 ^{cdC}				
	75	0.21±0.03 ^{aA}	0.38 ± 0.00^{dB}	0.70±0.00°C	1.06±0.01 ^{dD}				
	90	$0.24\pm0.03^{\alpha A}$	0.25±0.01 ^{abA}	0.65±0.02 ^{cB}	1.10±0.00 ^{dC}				
	105	0.24±0.00°A	0.31±0.00 ^{bA}	0.73±0.01 ^{cB}	1.30±0.17 ^{eC}				
	0	0.0177±0.001 ^{aA}	0.0177±0.001°A	0.0177±0.001°A	0.0177±0.001°A				
	15	0.0018±0.001 ^{bcA}	0.0023±0.004 ^{bA}	0.0057±0.003 ^{bcA}	0.0025±0.001 ^{bA}				
	30	0.0047±0.002 ^{cA}	0.0039±0.002 ^{bcA}	0.0028±0.002 ^{bcdA}	0.0060±0.002 ^{bA}				
TBARS	45	$0.0132\pm0.002^{\alpha A}$	0.0120±0.001 ^{dA}	0.0103±0.000eA	0.0120±0.003°A				
Values	60	0.0014±0.005 ^{bcA}	0.0069±0.001°A	0.0066±0.002 ^{ceA}	0.0022±0.001 ^{bA}				
	75	0.0014±0.001 ^{bA}	0.0023±0.001bA	0.0007±0.001 ^{dA}	0.0048±0.010 ^{bA}				
	90	0.0019±0.001 ^{bcA}	0.0014±0.000 ^{bA}	0.0020±0.002 ^{bdA}	0.0032±0.001bA				
	105	0.0024±0.002 ^{bcA}	0.0008±0.000bA	0.0017±0.001bdA	0.0023±0.000 ^{bA}				

^{*} Different small letter indicates statistical difference at α =0.05 level in time at each column. Different capital letters indicate statistical difference at α =0.05 level in temperature at each row

WSN values of toast cheeses generally increased during the storage period (P<0.05). When the WSN values were examined, an increase was detected until the 60th day with the samples stored at 5°C and 15°C, then a slight decrease was detected. When the samples are left to mature at 25°C and 37°C, a regular increase was observed (P<0.05). In general, the highest WSN values were determined in the last period of maturation versus in the first period of storage. Many researchers have reported that the water-soluble nitrogen ratio of kashar cheese increased (P<0.05) during ripening (Koçak et al, 1996; Koca, 2002). This could be due to the increase in the amount of nitrogen

dissolved in water as the hydrophilic properties of the peptides increase with the breakdown (Hayaloğlu, 2003).

The TCASN is a value that indicates the presence of small sized peptides and amino acids. These amino acids and peptides are formed as a result of the degradation of peptides separated from casein as a result of rennet and plasmin activity by microbial enzymes and are known as the maturity depth index in total (McSweeney, 2004). TCASN values were found to be almost the same range as the first and last storage days. In the samples stored at 15°C, a decrease was detected until the

60th day, and then there was a general increase until the end of the maturation period (P<0.05). A regular increase was observed from the first day of storage to the last day in toast cheese samples stored at 25°C and 37°C (P<0.05). Many researchers reported the TCASN ratios of kashar cheeses increase in significant level during the ripening period (Koca, 2002; Çürük, 2006). In our study, the highest TCASN value was determined in toast cheese samples stored at 25°C and 37°C.

3.5. Changes of TBARS Values of Toast Cheese

Lipid oxidation could cause destruction of valuable nutrients, off-flavours and production of toxic compounds (Mederios et al, 2014). Consequently, TBARS value is a critical parameter, especially during the storage period of foods. That's why it is detected in this study. TBARS values of toast cheese samples stored at different temperatures are given in Table 2. Storage time significantly changed the TBARS values of toast cheese (P<0.05) whereas storage temperature did not significantly affect it (P>0.05). Interaction between temperature and time did not significantly affect the TBARS values of toast cheese (P>0.05). TBARS values of all cheese samples decreased during the ripening period, which is presumed to be due to decomposition of reagents intermediate products during the storage process while the toast cheese ripens. TBARS values fluctuated throughout the maturation period, but the overall trend was decreasing in all samples

4. CONCLUSION

This study was focused on the effect of the storage on the growth of different microorganisms (S. thermophiles, Lactobacillus delbrueckii subsp bulgaricus, Bifidobacterium lactis, mold and yeast, TAMB), moisture content, pH, texture and biogenic amines content of toast cheese.

In the fresh stage, up to 45 days of storage, total LAB, TAMB and mold and yeast kept at 5°C were not significantly (P>0.05) different. From that point, increasing the storage time and temperature significantly (P<0.05) increased the growth of LAB, moisture content and biogenic amine formation. On the other hand, increasing the storage period and temperature after fresh stage caused a decrease in TAMB, mold and yeast count, pH value, hardness and chewiness. Biogenic amine formations could related with type of microorganisms. All the studied biogenic amines level increased with increasing S. thermophilus and Lactobacillus delbrueckii subsp bulgaricus counts whereas decreased with increasing TAMB and mold and yeast counts. As a result, the conditions of storage period must be adjusted to decrease the growth of LAB for decreasing the biogenic amine formation in toast cheese

Ethical Approval

None.

Declaration of Interests

The authors of this study declared no potential conflict of interests.

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Author Contribution

Concept: NHY, HB
Design: NHY, CA,HB
Data collecting: NHY, UU
Statistical analysis: UU
Literature review: NHY
Writing: NHY, UU
Critical review: CA, HB

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