

Formation of biogenic amines during fermentation and storage of tarhana: a traditional cereal food

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Abstract

Tarhana is one of the most important traditional fermented foods in Turkey, which is produced by lactic acid bacteria (LAB) and yeast fermentation. To date, no information has been published regarding the biogenic amine formation during the processing of tarhana. The aim of the present study was to investigate some biogenic amine contents of tarhana samples during fermentation and storage periods. Three different types of tarhana were produced by different formulations and total dry matter, pH, total acidity, LAB counts and biogenic amine contents of these samples were determined during fermentation and storage periods. The acidity and LAB populations of tarhana samples increased significantly during the fermentation period and then decreased by drying procedure and became nearly constant during the storage period. Tyramine, putrescine and cadaverine were the major biogenic amines, and the changes in the concentrations of these biogenic amines had the same trend as those in acidity and LAB counts. Spermidine, spermine and histamine were found irregularly and at very low concentrations. There were significant correlations between acidity and tyramine, putrescine and cadaverine concentrations, and also between acidity and LAB counts.

Keywords: lactic acid bacteria, putrescine, tyramine

1. Introduction

Dietary amines are traditionally classified as polyamines which are endogenous components of living cells (putrescine, spermidine and spermine) and biogenic amines formed by non-specific decarboxylation reactions (putrescine, cadaverine, tryptamine, 2-phenylethylamine, histamine, tyramine and agmatine) (Oliveira *et al.*, 2005). Biogenic amines in fermented or spoiled foods and beverages are generated by microbial decarboxylation of amino acids during fermentation or are formed by the enzymes of raw material. Amines have important metabolic roles in living cells. Polyamines are essential for growth; other amines like histamine, tyramine and serotonin are involved in nervous system functions and the control of blood pressure (Lonvaund-Funel, 2001; Özdestan and Üren, 2009).

Biogenic amines are of importance because of their potential toxicity and they have been responsible for many

cases of food poisoning (Hornero-Méndez and Garrido-Fernández, 1997; Lange *et al.*, 2002; Lonvaund-Funel, 2001). The estimation of biogenic amines is important not only from the toxicological point of view, but also they can be used as indicators of degree of freshness or spoilage of foods (Alberto *et al.*, 2002).

Tarhana was first produced by Turkish people in Middle Asia and afterwards it spread out to different parts of the world (Özdemir *et al.*, 2007). Tarhana is a fermented cereal food and one of the oldest traditional Turkish soups taking an important part of the Turkish diet, with high nutritional quality and long shelf-life properties without any deterioration. In general, tarhana is produced in four steps: dough mixing, fermentation, drying and grinding. Tarhana dough is prepared by mixing wheat flour, yoghurt and other ingredients such as bakers' yeast (sour dough or tarhana may be used instead of bakers' yeast), tomato, onion, paprika, salt, spices and seasonings (Bilgiçli *et al.*, 2006; Daglioglu, 2000; Tamer *et al.*, 2007).

The nutritional properties, aroma and flavour of tarhana dough are improved by fermentation. This results from the appropriate balance between the metabolism of the homo/hetero fermentative lactic acid bacteria (LAB) and the metabolism of yeast, which represent the naturally occurring microorganisms in tarhana. The cereal and milk are predominantly fermented by the LAB. The association of LAB and yeast during fermentation contributes to the production of metabolites, which impart pleasant tastes and flavours to food (Erbaş *et al.*, 2006).

There is not a standard procedure for the production of tarhana, the amounts and types of ingredients and fermentation conditions may vary from place to place in Turkey, affecting chemical composition, nutritional content and sensory attributes of tarhana. The ratio of yoghurt to wheat flour is usually 1:1; in some regions the content of yoghurt may be reduced or increased. Various spices are used as flavouring agents (mint, thyme, dill and tarhana herb) in different parts of Turkey (Değirmencioglu *et al.*, 2005). After mixing ingredients, tarhana dough is fermented for 1 to 15 days. Then it is sun-dried in homemade production or oven-dried for commercially produced tarhana. Several types of tarhana can be classified depending upon processing method and raw materials used (Tamer *et al.*, 2007).

The following averages were measured in tarhana: moisture 10.2%, protein 16%, carbohydrates 60.9%, fat 5.4%, crude fiber 1%, salt 3.8% and ash 6.2% (Daglioglu, 2000; Erbaş *et al.*, 2006; İbanoğlu *et al.*, 1995). Tarhana is a good source of minerals (K, Ca, Mg and Zn) with a favourable bioavailability. Tarhana is also a valuable source of B vitamins, organic acids and free amino acids; it is healthy for children, the elderly and patients. It is generally consumed as soup at lunch and dinner. Tarhana soup is highly flavoured and thick creamy and it can be easily digested (Özdemir *et al.*, 2007). Sengun *et al.* (2009) identified LAB isolated during processing of tarhana. A total of 226 Gram-positive and catalase-negative isolates were obtained.

There are some other products similar to tarhana such as kishk in Syria, Jordan and Egypt (Youssef, 1990), kushuk in Iraq (Alnouri *et al.*, 1974), tahonya/talkuna in Hungary and Finland (Hafez and Hamada, 1984), trahana in Greece and atole in Scotland (Tamime *et al.*, 2000). There are many studies about tarhana. However, no information was published regarding the biogenic amine formation during the processing of tarhana. Since tarhana is a fermented food containing protein and free amino acids which might be used by decarboxylase-positive microorganisms, the formation of various biogenic amines might be expected. The aim of the present study was to investigate the formation of biogenic amines during fermentation and storage of tarhana.

2. Materials and Methods

Materials

For tarhana dough, wheat flour (with a moisture content of 13%, ash content of 0.55%, crude protein content of 10%, on dry basis), yoghurt that was produced using a starter culture (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*), strained yoghurt that was produced using homemade yoghurt instead of a starter culture, tomato, red pepper, table salt, onion, tarhana herb, mint and dill were purchased from the local markets in İzmir, Turkey.

Apparatus

Chromatographic separations were performed by using an Agilent 1100 High Performance Liquid Chromatograph (HPLC, Agilent, Santa Clara, CA, USA) equipped with a diode array detector, a gradient elution pump and auto sampler injection system. The chromatography column used was a Hichrom C₁₈ (10 µm particle size, 300 mm × 3.9 mm i.d., Hichrom Ltd., Theale, UK) thermostatted at 20 °C. An HI 221 microprocessor pH meter (Hanna Instruments, Romania) was used for pH measurements. For the determination of total dry matter contents, an oven (Dedeoğlu, Turkey) was used.

Chemicals

Cadaverine dihydrochloride, tryptamine, 2-phenylethylamine, spermidine trihydrochloride, spermine, histamine dihydrochloride, tyramine and agmatine sulphate were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Methylamine hydrochloride and 1,7-diaminoheptane (internal standard, IS) were obtained from Merck (Schuchardt, Germany). Putrescine dihydrochloride was obtained from Fluka (Steinheim, Germany). Of the other reagents, sodium hydroxide, benzoyl chloride, sodium chloride, anhydrous sodium sulphate, trichloroacetic acid and distilled water were supplied from Merck (Darmstadt, Germany), whereas methanol, acetonitrile and diethyl ether (all HPLC grade) were from Lab-Scan (Dublin, Ireland), hydrochloric acid was from J.T. Baker (Deventer, Holland), sodium acetate trihydrate was from Riedel-de Haën (Seelze, Germany) and phenolphthalein was from Panreac Química S.L.U. (Barcelona, Spain). Man Rogosa and Sharpe (MRS) agar was obtained from Lab M (Lancashire, UK) and peptone water was from Difco (Detroit, MI, USA). The standard solution of biogenic amines and internal standard solution were prepared following the method of Özdestan and Üren (2009).

Production of tarhana samples

Three different types of tarhana samples were produced by different formulations, since the amounts and types of ingredients may vary from place to place in Turkey. These formulations are presented as S1, S2 and S3 in Table 1. Onions, tomatoes, red peppers and other vegetables were chopped and cooked for 15 min and then cooled to room temperature. The cooked vegetables and yoghurt were added to the flour and then all ingredients were mixed together for 5 min to obtain homogenized dough. The tarhana dough was fermented at 30 °C for 14 days. This method was a modified form of the procedure of Erbaş *et al.* (2006). After the fermentation the dough was divided into small pieces of about 5-6 g and dried in an air-oven at 70 °C until a moisture content of about 10%. After drying, samples were milled in a hammer mill (Armfield Ltd., Ringwood, UK) and sifted through a 1 mm screen sieve. The samples were stored in cloth bags at room temperature until being analysed. Triplicate samples were taken for microbial and chemical analyses at particular days (day 0, 2, 4, 7, 10, 14, 24, 54 and 94) during fermentation and storage. The first 14 days were related with the fermentation period and the remaining days were for the storage period.

Determinations of total dry matter, pH and total acidity

The total dry matter content of samples was determined by drying the samples at 110 °C in an oven to a constant weight (İbanoğlu *et al.*, 1999). For the pH values, tarhana samples were blended with distilled water (1/4, w/w) and pH values of the suspensions were determined with a pH meter (Tamer *et al.*, 2007). The total titratable acidity of tarhana samples was determined by titration using 0.1 M NaOH solution, and expressed as lactic acid on dry base (İbanoğlu *et al.*, 1999).

Table 1. Tarhana formulations.

Ingredients (g)	Sample code		
	S1	S2	S3
Tomato	300	400	400
Red pepper	500	400	400
Onion	300	300	400
Dill	10	10	10
Mint	10	10	10
Tarhana herb	20	20	10
Semolina	-	-	200
Flour	2,000 (white)	400 (white) + 800 (whole-wheat)	1000 (white)
Yoghurt	800	650 (strained)	200 (strained)
Tarhana	5	5	5
Salt	60	60	60

Microbial analysis (lactic acid bacteria)

Microbial analyses were carried out aseptically by homogenizing 10 g of a sample in 90 ml of sterile peptone water and making serial dilutions from 10¹ to 10⁸ levels. Pour plate technique was applied using MRS agar. The plates were incubated at 30 °C for 3 days. The results were expressed as cfu/g dry weight (Turantaş *et al.*, 1999).

Biogenic amine analyses

Biogenic amine contents of tarhana samples were determined following the methods of Özdeştan and Üren (2009) and Yeğin and Üren (2008). Eight g of tarhana dough or 5 g of dried tarhana sample were suspended in 50 ml of 5 g/100 ml trichloroacetic acid solution. The resulting mixture was centrifuged at 2,150×g for 30 min, and 2 ml aliquot of the supernatant was derivatized before analysis by HPLC. Benzoyl chloride was used as derivatization agent. The internal standard and standard addition techniques were used. Biogenic amine contents of samples were expressed in mg/kg on dry base. Chromatograms were obtained for three aliquots of the same tarhana sample that underwent the whole analytical procedure. According to Özdeştan and Üren (2009) validation parameters of the method were as follows: linearity of the HPLC method was determined for methylamine, putrescine, cadaverine, tryptamine, 2-phenylethylamine, spermidine, spermine, histamine and tyramine as 133.6, 185.7, 186.0, 265.0, 272.0, 268.4, 226.6, 995.3 and 1,433 mg/kg, respectively, with a good regression coefficient from 0.9881 to 0.9995. The limits of detection were found as 0.2, 0.2, 0.3, 0.2, 0.5, 0.2, 0.5, 0.4 and 2.5 mg/kg for methylamine, putrescine, cadaverine, tryptamine, 2-phenylethylamine, spermidine, spermine, histamine and tyramine, respectively. Recoveries were calculated for each biogenic amine and varied from 72.8% to 103.4%.

Statistical analysis

Throughout the present study, all the experiments were performed in triplicate. The statistical analyses were realized with the SPSS 13.0 statistics package program. The statistical analyses of the data were achieved by using the paired t-test and Pearson correlation test. In all data analyses, a value of *P*<0.05 was considered as statistically significant.

3. Results and discussion

Three different types of tarhana formulations were produced and pH, acidity and LAB counts of these samples were determined during the fermentation and storage periods (Table 2). As can be seen in Table 2, the pH values of the S1 samples was 4.81 at the beginning of the fermentation and gradually decreased to 3.62 on the 14th day, at the end of the fermentation. Following

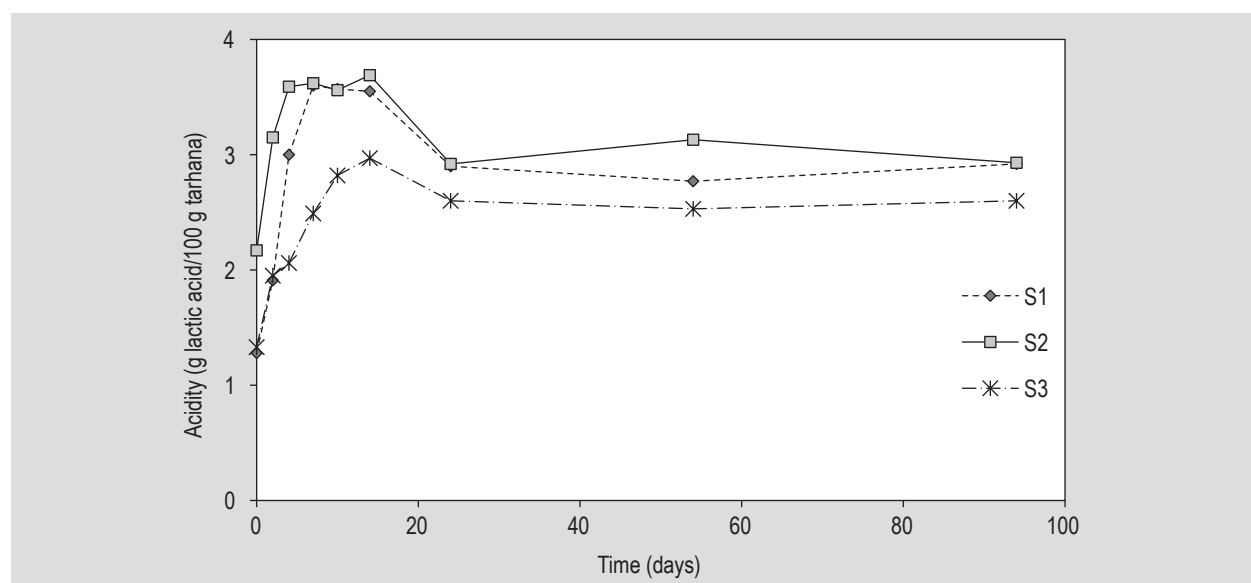
Table 2. pH, acidity (g lactic acid/100 g tarhana on dry base) and lactic acid bacteria (LAB) counts (log cfu/g tarhana on dry base) of tarhana samples during fermentation and storage.

Sample code	Type of analysis	Day of analysis								
		0	2	4	7	10	14	24	54	94
S1	pH	4.81 (0.2)	4.21 (0)	3.81 (0.3)	3.71 (0.3)	3.63 (0.6)	3.62 (0)	3.78 (0)	3.81 (0.3)	3.80 (0)
	Acidity	1.28 (0.8)	1.91 (5.2)	3.00 (2.3)	3.60 (2.2)	3.55 (0.8)	3.55 (0.8)	2.90 (2.4)	2.77 (0.7)	2.92 (2.1)
	LAB	4.28 (0.7)	7.94 (0.6)	9.08 (0)	8.84 (0.7)	8.41 (0.2)	8.55 (1.1)	2.30 (0)	2.04 (2.9)	2.02 (1.5)
S2	pH	4.10 (0)	4.05 (0.2)	4.02 (0)	4.07 (0.2)	4.04 (0)	4.09 (0)	4.32 (0)	4.34 (0)	4.32 (0)
	Acidity	2.17 (4.1)	3.15 (1.6)	3.59 (0.6)	3.62 (1.9)	3.56 (0.6)	3.69 (1.1)	2.92 (1.4)	3.13 (0.3)	2.93 (1.7)
	LAB	6.04 (0.3)	7.03 (0.1)	7.19 (0.1)	7.48 (0.1)	7.37 (0.7)	7.66 (0.4)	4.79 (0.4)	2.48 (2.7)	2.04 (2.9)
S3	pH	4.62 (0)	4.21 (0.2)	4.10 (0)	4.07 (0.2)	3.82 (0.3)	3.76 (0)	3.87 (0)	3.89 (0)	3.86 (0)
	Acidity	1.33 (1.5)	1.95 (2.6)	2.06 (2.4)	2.49 (0.8)	2.82 (1.1)	2.97 (0.7)	2.60 (1.2)	2.53 (0.4)	2.60 (1.5)
	LAB	5.65 (0.2)	6.75 (0.6)	7.23 (0.7)	8.04 (0)	8.08 (0)	8.33 (0.1)	3.23 (1.2)	2.30 (2.6)	2.11 (4.7)

The numbers in parentheses are the coefficient of variation values.

the drying procedure the pH value of the samples slightly increased and finally had a value of 3.80 on the 94th day, at the end of the storage period. Nearly the same trend was observed in the S3 samples. The S2 samples exhibited nearly a constant pH of about 4 during the fermentation period. The pH value of the samples increased after the drying procedure and showed a final value of 4.32 on the 94th day. The observation of the acidity values during the processing gave a clearer picture of fermentation. Figure 1 demonstrates that the acidity of all samples increased significantly during the fermentation period. Following the drying procedure acidities showed a decrease and became

nearly constant during the storage period. According to the Pearson correlation test significant correlations were observed among the three samples in terms of changes in acidity with time ($P < 0.05$). These results were in accordance with the findings of Yücel and Üren (2008), in their study it was reported that acidity changes during fermentation of pickled cabbage had the same trends. At the same time, it was concluded that fermentation of tarhana samples began in the early stages of the fermentation period and continued until the 14th day. According to the paired t-test results, acidity values of the S3 samples were significantly smaller than those of the S1 and S2 samples ($P < 0.05$).

**Figure 1.** Changes in acidity of tarhana samples during fermentation and storage. S1, S2, S3 are different formulations for production of tarhana.

The changes in LAB counts during fermentation and storage periods are presented in Figure 2. As in the case of acidities, LAB counts of the three samples increased significantly during the fermentation period, but following the drying procedure showed a decrease and became nearly constant during the storage period. These results were in accordance with the findings of Sengun *et al.* (2009) which reported that LAB and total viable microorganism in tarhana increased during the fermentation and then decreased to the initial number and that the highest LAB population was 10^5 - 10^7 cfu/g during the fermentation and 10^3 - 10^4 cfu/g in dried tarhana. A significant correlation was observed between LAB count changes and acidity changes for the S2 samples ($P<0.05$). In addition, significant correlations were observed among the three samples in terms of changes in LAB counts with time ($P<0.05$). According to the results of the paired t-test, LAB counts of the S1 samples were significantly greater than those of the S2 and S3 samples between the 2nd and the 14th day ($P<0.05$).

The types and concentrations of amines detected in tarhana samples from different formulations are shown in Table 3. Because of interfering compounds agmatine determination couldn't be realised. Detection limits of the biogenic amines for the applied method were reported by Özdestan and Üren (2009) as 0.5 mg/kg or less, except for tyramine that had a limit of detection value of 2.5 mg/kg. The changes in putrescine and tyramine concentrations during fermentation and storage periods are demonstrated in Figures 3 and 4, respectively. Figure 3 shows that the putrescine content of the S1 samples increased significantly up to 633 mg/kg during the first 10 days of fermentation. Then it decreased to 194 mg/kg on the 24th day and became nearly constant during the storage period. According to

the Pearson correlation test, a significant correlation was observed between putrescine changes and acidity changes during the fermentation and storage periods for the S1 samples ($P<0.05$). At the same time, the putrescine content of the S1 samples that had the highest LAB counts was greater than those for the other two samples. In addition, a significant correlation was observed between putrescine and acidity changes during the fermentation and storage periods for the S3 samples ($P<0.05$).

The changes in tyramine concentration during fermentation and storage are shown in Figure 4. The tyramine changes in the S1 samples had a similar trend as putrescine and there was a correlation between putrescine and tyramine curves for the S1 samples ($P<0.05$). There was also a correlation between tyramine and acidity changes for the S1 samples ($P<0.05$). At the same time, tyramine content of the S1 samples with the highest LAB counts was greater than those for the other two samples.

Table 3 shows that tyramine, putrescine and cadaverine were the major biogenic amines. Spermidine, spermine and histamine were found irregularly and at very low concentrations. Methylamine, tryptamine and 2-phenylethylamine were not detected in any sample. Total amounts of biogenic amines were 595.3 mg/kg, 233.8 mg/kg and 191.5 mg/kg on the 94th day for the S1, S2 and S3 samples, respectively. The concentrations of the biogenic amines were below the maximum permissible limits. It was seen explicitly that during the fermentation period acidity, LAB counts and biogenic amine contents increased as a result of activities of microorganisms. Subsequently a decrease was observed. Although acidity and LAB counts remained nearly constant during the storage period,

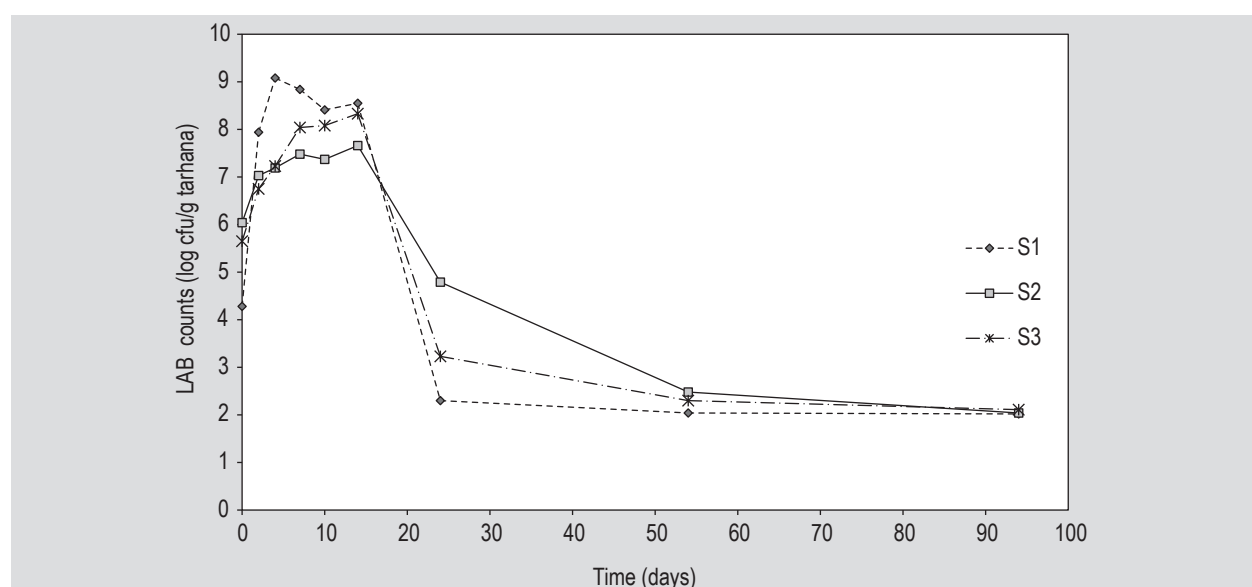


Figure 2. Changes in lactic acid bacteria (LAB) counts of tarhana samples during fermentation and storage. S1, S2, S3 are different formulations for production of tarhana.

Table 3. Biogenic amine contents/(mg/kg on dry base) of tarhana samples during fermentation and storage.

Sample code	Biogenic amines	Day of analysis								
		0	2	4	7	10	14	24	54	94
S1	Methylamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Putrescine	7.1 (25)	5.5 (36)	240 (7)	498 (4)	633 (9)	446 (5)	194 (4)	260 (3)	267 (9)
	Cadaverine	4.8 (19)	5.0 (22)	nd	nd	nd	5.1 (10)	7.3 (60)	nd	25.3 (31)
	Tryptamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	2-phenylethylamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Spermidine	7.8 (20)	4.3 (26)	nd	nd	nd	nd	nd	nd	nd
	Spermine	5.3 (19)	nd	nd	nd	nd	nd	4.4 (9)	nd	nd
	Histamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Tyramine	33 (31)	73 (30)	198 (23)	322 (17)	240 (34)	176 (31)	168 (29)	309 (39)	303 (2)
S2	Methylamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Putrescine	17.4 (22)	12.1 (6)	19.7 (23)	14.1 (16)	18.5 (9)	22.2 (10)	6.7 (12)	8.5 (24)	16.5 (73)
	Cadaverine	6.3 (53)	14.7 (1)	nd	nd	nd	nd	nd	nd	7.3 (19)
	Tryptamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	2-phenylethylamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Spermidine	21.2 (20)	12.2 (45)	13.8 (6)	10.0 (7)	9.8 (24)	8.4 (16)	8.8 (14)	nd	nd
	Spermine	12.5 (8)	6.3 (0)	11.1 (15)	11.1 (5)	14.2 (25)	10.5 (15)	10.8 (27)	nd	nd
	Histamine	3.2 (7)	nd	2.3 (9)	nd	8.0 (56)	4.8 (7)	4.9 (16)	nd	nd
	Tyramine	22 (16)	nd	82 (21)	nd	67 (16)	nd	nd	nd	210 (53)
S3	Methylamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Putrescine	7.2 (9)	10.3 (30)	7.2 (1)	11.0 (11)	16.7 (27)	17.6 (17)	13.1 (5)	15.2 (26)	14.5 (25)
	Cadaverine	nd	22.6 (85)	nd	3.3 (9)	3.4 (14)	nd	nd	7.8 (34)	125 (8)
	Tryptamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	2-phenylethylamine	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Spermidine	4.2 (28)	5.6 (24)	5.8 (6)	9.4 (3)	4.3 (4)	nd	6.7 (11)	nd	nd
	Spermine	2.9 (17)	3.2 (12)	4.3 (18)	10.2 (11)	5.4 (17)	9.7 (21)	7.4 (21)	nd	nd
	Histamine	nd	nd	nd	nd	nd	nd	4.6 (9)	nd	nd
	Tyramine	nd	128 (85)	nd	60 (51)	138 (35)	38 (11)	47 (7)	63 (28)	52 (48)

nd = not detected. The numbers in parentheses are coefficient of variation values.

concentrations of some biogenic amines increased on the 94th day; tyramine concentration in the S2 samples and cadaverine in the S3 samples. It may be concluded that during the fermentation and storage periods not only the LAB counts but also the composition of LAB changed. Sengun *et al.* (2009) identified LAB during the fermentation of tarhana and found that *Streptococcus* species were the prevailing bacteria in the early stages of processing, but *Lactobacillus* species were predominant finally.

4. Conclusions

Tarhana is one of the most important traditional fermented foods in Turkey, which is produced by LAB and yeast fermentation. In the present study, tarhana was used instead of bakers' yeast for the yeast fermentation. A relatively long fermentation period was preferred to improve the nutritional properties, aroma and flavour

of tarhana dough. Consequently, three different types of tarhana were produced by using different formulations. The acidity and LAB populations of all tarhana samples increased significantly during the fermentation period and then decreased by drying procedure and became nearly constant during the storage period. Tyramine, putrescine and cadaverine were the major biogenic amines and the changes in these biogenic amines had the same trend as the acidity and the LAB counts. There were significant correlations between acidity and tyramine, putrescine and cadaverine concentrations, and also between acidity and LAB counts. In general, tarhana samples acquired elevated levels of biogenic amines during the fermentation period. The concentrations of the biogenic amines decreased at the end of the fermentation possibly due to the activities of biogenic amine degrading bacteria. Lonvaud-Funel (2001) reported that some strains of bacteria were able to degrade biogenic amines, which were unable to decarboxylate amino

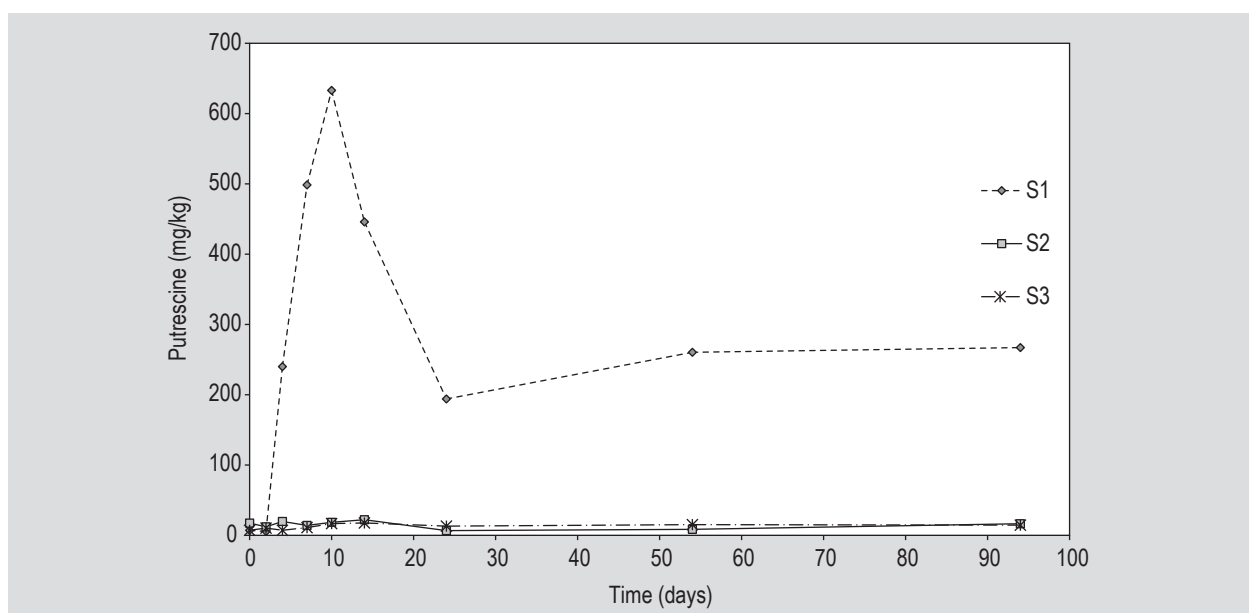


Figure 3. Putrescine contents of tarhana samples during fermentation and storage. S1, S2, S3 are different formulations for production of tarhana.

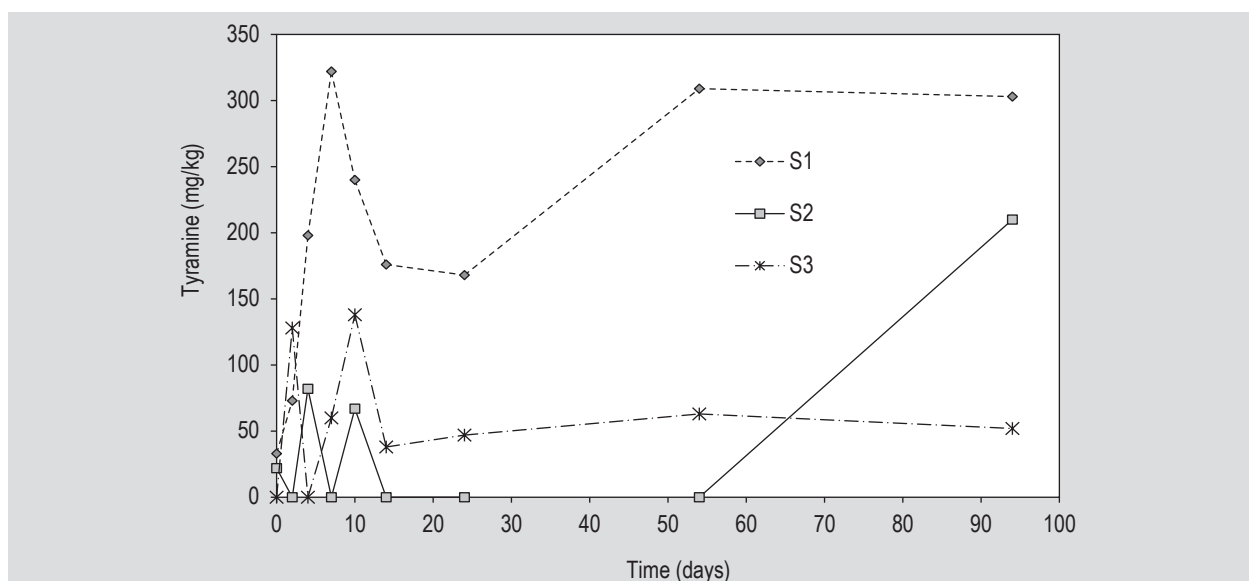


Figure 4. Tyramine contents of tarhana samples during fermentation and storage. S1, S2, S3 are different formulations for production of tarhana.

acids. On the other hand, contents of some biogenic amines increased at the end of the storage period because of the microorganisms cultivated during the storage period. Biogenic amine contents of tarhana samples were found to be different at the end of the storage period. These differences were normal as ingredients and original bacterial status might affect biogenic amine levels of the product. The concentrations of the biogenic amines in tarhana samples were below the maximum permissible limits.

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