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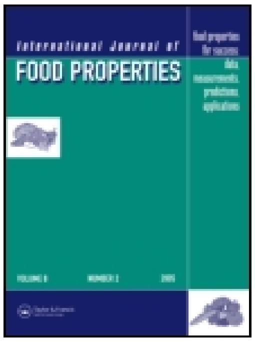

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DETERMINATION OF BIOGENIC AMINES IN HERBY CHEESE

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Herby cheese (Otlu peynir) is widely produced and consumed in eastern parts of Turkey, and is generally made from sheep milk. The biogenic amines and organic acids content, microbiological, and chemical properties of Herby cheese were determined on 30 samples obtained from retail markets in East Anatolia Region, Turkey. The most important biogenic amines were tyramine (range 18.0–1125.5 mg kg^{-1}), followed by cadaverine varied from not detected to 1844.5 mg kg^{-1}. Histamine content generally was found higher than 100 mg kg^{-1}. The concentration of amines in some cheeses was much higher than the toxic dose limits. Generally, total amine content was found high in samples that had high nitrogen fraction and organic acids value. Levels of biogenic amines were significantly correlated with the organic acids of Herby cheese. The basic organic acid was found the lactic acid ranging from 585.4 to 26480.3 mg kg^{-1}, followed by butyric acid varying between 314.8 and 7329.6 mg kg^{-1} values. Lipolysis and nitrogen fraction were determined high in ripened Herby cheese samples. Coliform counts of samples showed lower than 100 cfu g^{-1} in the samples. Significant ($p < 0.01$) positive correlation was observed between acetic acid and phenylethylamine, putrescine, cadaverin, histamine, and tyramine.

Keywords: ***Herby cheese, Biogenic amines, Organic acids, Nitrogen fraction.***

INTRODUCTION

Herby cheese, which has a semi hard texture and a salty taste, is produced in small family businesses for their needs and for commercial purposes. In addition, it is produced in well equipped factories. It is made from raw sheep milk in the Eastern and Southeastern parts of Turkey. If sheep milk is not available, a mixture of sheep and cow or sheep and goat milk can be used for cheese making. Herby cheese, named "Otlu peynir" in Turkish, is being home made in villages and some small manufactures for many years. Most people consume it as a part of almost every meal.[1–3]

To make herby cheese, sheep milk is first filtered immediately after milking and then coagulated with calf rennet at the milking temperature. After cutting the coagulum, whey is removed, and previously prepared herbs are added into the curd. About 25 kinds of herbs can be used to make herby cheese, e.g., *Allium* spp., *Thymus* spp., *Ferula* spp., *Anthriscus nemorosa,* etc. From these herbs, single or mixture of some herbs can be added. The rate of

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the addition of herbs changes between 0.5 and 2 kg per curd obtained from 100 L of milk. After the addition of herbs, the cheese is pressed for about 3 h to remove the remaining whey. The pressed cheese is then cut into blocks. Cheese blocks are ripened in brine or they are dry salted and placed in plastic containers. Then whey cheese or "cacik" (a quark-like product) is put between cheese blocks so that air is not allowed inside the container. All containers filled with cheese are turned upside down and kept in a cool place or placed in the soil to permit the moisture to come out. In this position, the bottom of the container is always left open to enable the moisture loss. This cheese is ripened for about 3 months to get the desired taste and flavour.[1,4,5]

Cheeses represent an ideal environment for the formation of proteolytic products, namely, free amino acids and biogenic amines, directly influenced by bacterial activity, pH, salt concentration and indirectly, by water availability, storage temperature, and ripening time. Biogenic amines, which are low molecular weight organic bases, can be formed and degraded as a result of normal metabolic activity in microorganisms.[6,7]

Many factors contribute to the presence and accumulation of biogenic amines, such as availability of free amino acids, pH level, water activity, and temperature,[8,9] and, primarily, the presence of micro-organisms possessing amino acid decarboxylase activity.[10] Non-starter lactic acid bacteria and *Enterobacteriaceae* have been reported to have a major role on the production of biogenic amines in cheese.[7,11] The decarboxylation of some amino acids leads to the formation of biogenic amines. Many bacterial species may contribute to the formation of biogenic amines in cheese. Biogenic amines at high concentrations have been found in fermented foods as a result of a contaminating microflora exhibiting amino acid decarboxylase activity. Since biogenic amines, in particular histamine and tyramine, have been reported to affect the well-being of susceptible consumers, their occurrence in food is not desirable.[12]

Microorganisms that can be present in milk and cheese, such as *Pseudomonas, Enterobacteriaceae* and *Micrococcoceae*, have some active decarboxylases.[10] LAB are less active in the decarboxylation of amino acids, but in the light of the high populations reached in cheese for long ripening periods, their contribution should not be disregarded. Non-starter lactic acid bacteria (e.g., *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc,* and *Streptococcus*) and some Gram-negative bacteria (e.g., *Pseudomonadaceae* and *Enterobacteriaceae*) have been reported to be capable of amine production.[10,13] Biogenic amine formation can be present in relatively high concentrations in cheese.

For many different foods, the formation of biogenic amines may be a problem, since these compounds cause food poisoning. In general, the illness is a mild one and the symptoms usually disappear within a few hours but serious cases have also been reported. But, these compounds can represent a serious health hazard for humans when present in food in significant amounts, or ingested in the presence of potentiating factors, such as amine oxidase-inhibiting drugs, alcohol, and gastrointestinal diseases.[7,8] The presence of low levels of biogenic amines in cheeses is not considered a serious risk. However, if normal routes of catabolism of amines are inhibited, or the amount consumed thereof is large, various physiological effects may result, such as hypertensive crises, migraine headache and even death from cerebral hemorrhage in patients.[12]

The exact toxic threshold of biogenic amines is difficult to determine due to its dependence on the efficiency of detoxification mechanisms of different individuals. However, according to Nout,[13] Halasz et al.,[7] and Karovičová and Kohajdová,[14] 10 mg of histamine in 100 g of sample can cause histamine poisoning; 10–80 mg of tyramine can cause "cheese reaction" (6 mg if patient is receiving MAOI (mono amine oxidase inhibitor)); and 3 mg of phenylethylamine can cause migraine headache. The level of 1000 mg/kg (amine/food) is considered dangerous for health.[15]

Organic acids are the major products of carbohydrate catabolism by lactic acid bacteria (LAB). Their ability to produce acidic substances with the concomitant pH reduction is the major factor in milk fermentations. The resulting acidity is essential for the coagulation process and detrimental for the development of spoilage and pathogenic micro-organisms. Thus, it greatly improves the hygienic quality of dairy products although this acidic environment can also affect the lactic flora itself. Traditionally, milk fermentation and cheese making have been valuable processes to extend storage stability of an important foodstuff likely to undergo rapid spoilage. The capacity of LAB to effectively inhibit other bacteria depends not only on their ability to lower the pH, but also on the nature of the organic acids they produce. The undissociated fraction of these acids is the main inhibitory form. In dairy products, organic acids resulting from the hydrolysis of fatty acids, normal bovine metabolism processes, or direct addition as acidulants are also present. In particular, organic acids contribute to the flavour of most aged cheeses.[16]

Little publications are available on the biogenic amines levels in herby cheeses. This is one of the first studies that focus on biogenic amines and organic acids in Herby cheese. The aim of this study was to determine biogenic amines, organic acids and the characteristics of ripened Herby cheeses obtained randomly from East Anatolia Region markets.

MATERIALS AND METHODS

Sampling

Cheese samples were purchased at retail stores in East Anatolia Region, Turkey between March and May 2008. These cheese samples were maturated nearly 12 months. The selection of cheese samples represented the different types commercially available and cheese production in the East of Turkey. Cheeses were analyzed for biogenic amines contents (cadaverine, putrescine, tyramine, tryptamine, histamine, and phenylethylamine), organic acid contents (citric, formic, acetic, propionic, lactic, and butyric acid), lactic acid bacteria, coliforms counts, pH, total nitrogen (TN), water soluble-nitrogen (WSN), trichloro acetic acid-soluble nitrogen (TCA-SN), and phosphotungstic acid soluble nitrogen (PTA-SN), dry matter, fat, salt, protein, and acid value degrees (ADV) and ripening index. The samples were carried to the laboratory and kept in a refrigerator. Samples were taken for microbial and chemical analyses. Samples for biogenic amines determination were stored at −20°C until analyzed. The cheeses were grated, homogenized thoroughly and analyzed immediately. Each analysis was performed in duplicate.

Chemicals

Six aqueous standard solutions containing cadaverine dihydrochloride, putrescine dihydrochloride, tyramine hydrochloride, tryptamine hydrochloride, phenylethylamine hydrochloride, histamine dihydrochloride, 1.7-diaminoheptane (as internal standard) were purchased from Sigma (St. Louis, MO, USA). Citric, formic, acetic, propionic, lactic and butyric acids were purchased from Sigma (St. Louis, MO, USA), and gradient HPLC-grade

H_2SO_4 from Merck (Darmstadt, Germany). Milli-Q water (Bedford, MA, USA) was used to prepare buffers, stock solutions of each standard compound and the samples.

Biogenic Amine Analysis

Biogenic amine contents of the samples were determined according to the method of Eerola et al.[17] Biogenic amines were extracted from 2.0 g samples with 0.4 M perchloric acid and detected as their dansyl derivatives by HPLC. The gradient-elution system was 0.1 M ammonium acetate as solvent A and acetonitrile as solvent B. The graduate-elution program was started at 50% solvent B and ended at 90% solvent B in 25 min. The system was equilibrated for 10 min before next analysis. The flow rate 1.0 mL/min and column temperature was 40°C. A 20 ll sample was injected onto the column. Peaks were detected at 254 nm using the HPLC system with a column Spherisorb ODS2 150A, 150 × 4.60 mm (waters) and a gradient pump, which includes an Agilent HPLC (1100 series G 1322 A, Germany), vacuum degasser, DAD detector, and a computer including Agilent package program. The quantitative determinations were carried out by internal standard (1.7-diaminoheptane) method, using peak heights. Biogenic amine contents were expressed as mg kg^{-1}.

Organic Acid Analysis

Organic acid contents of the samples were determined according to the method of Bevilacqua and Califano.[18] Samples for organic acid determination were stored at −20°C until used for analysis. About 100 g of a representative cheese sample was ground (A-10 Analytical Mill, Tekmar, USA) and homogenized (Heidolp Silent Cruster M, Schwabach, Germany) from each cheese sample. Fifty milliliters of 0.009 N H2SO4 (mobile phase) was added to a 7 g of ground cheese and extracted for 1 h with agitation on a shaker (Heidolph Unimax 1010, Schwabach, Germany) and centrifuged (Hettich Zentrifugen Universal 32 R,Tuttlingen, Germany) 7000 g for 5 min, by modifying the method used by Bevilacqua and Califano.[18] The supernatant was filtered once through filter paper and twice through a 0.45 μm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA); 10 μL was injected. The HPLC system with a column (Aminex HPX - 87 H, 300 mm × 7.8 mm) was used (Bio-Rad Laboratories, Richmond, CA, USA) and a gradient pump, which includes an Agilent HPLC (1100 series G 1322 A, Santa Clara, CA, USA), vacuum degasser, DAD detector, and a computer including Agilent software package. The UV detector was set at 214 and 280 nm. Operating conditions were: mobile phase, 0.009 N H_2SO_4, filtered through 0.45 μm twice membrane filters. The quantitative determinations were external standard method, using peak area. Organic acid contents were expressed as mg kg^{-1}. Duplicate analyses were performed on all samples.

Microbiological Analysis

For microbiological analysis, a 10 g sample was prepared by homogenizing with 90 ml physiological saline water (0.85 NaCl%) in a tube and shaken for 1 min. Further decimal dilutions were prepared from this homogenized mixture. The following incubation conditions for microbiological analysis were used: De Man Ragosa Sharpe Agar (Merck) for 3 days at 37°C for lactic acid bacteria (LAB), Violet Red Bile Agar (Merck) for 2 days

37°C for coliform. Bacteria counts were expressed as colony-forming units per gram of sample (log cfu g^{-1}).

Physical and Chemical Analysis

pH value was measured by homogenizing 10 g of cheese in 100 mL of distilled water using a pH meter (Hanna Instrument pH 211; Microprocessor pH meter, Germany) equipped with an electrode (HI 1131, Germany). The cheese samples were analysed for total nitrogen using micro Kjeldahl digestion and distillation units (Nüve, Ankara, Turkey) (AOAC).[19] Dry matter content was determined by weight difference using a drying oven (Nüve, Ankara, Turkey) according to the methods described by Case et al.,[20] WSN, TCA-SN and PTA-SN compounds of cheeses were determined as described by Butikofer et al.[21] Salt content was determined by the Mohr method. The fat content of the cheese was measured by the Gerber method described by Case et al.[20] Lipolysis was done using the BDI method and measured as Acid Degree Value (ADV). For this test, 10 g of finely ground sample was placed in a lipolysis butyrometer. Twenty mL of BDI reagent (30 g triton X-100 70 g sodium tetra phosphate in 1 L distilled water) was added and the butyrometers were placed in a boiling water bath for 20 min to extract the fat. The mixture was centrifuged for 1 min and sufficient aqueous methanol was added to bring the fat into the neck of butyrometer and centrifuged for another minute. Then, the fraction of liquid fat was transferred into a 50 mL glass and was weighed. Five mL fat solvent (4:1 petroleum ether and n-propanal) was added to the flaks. This was titrated with 0.01 N tetra n-butyl ammonium hydroxide and total free fatty was calculated. All measurements were duplicated.

Statistical Analysis

SPSS for Windows statistical software[22] was used for all statistical analyses in this study. All data are presented as mean ± standard error of means.

RESULTS AND DISCUSSIONS

Biogenic Amine Analysis

The aromatic biogenic amines (TRP, HIS, PA, and TYR) and diamines (PUT and CAD) of the 30 cheese samples analyzed are summarized in Table 1. As seen from the table, there are significant differences in the contents of biogenic amines in the samples. In our study, it was found that biogenic amines were high in some Herby cheese. Biogenic amines were detected higher than 1000 mg kg^{-1} in 10 of 30 samples (Table 1). For this reason, amine contents in ripened cheeses were, in general, much higher than those in unripened cheeses, from 10 to 2000 times higher. Moreover, a wide variation was observed among BA contents in the ripened cheeses, which could be related to the intensity of the ripening process, as several authors were reported.[23,24] The proteolysis occurring during cheese manufacture may increase the release of amino acids from casein. Those free amino acids can be decarboxylated by bacterial enzymes, giving rise to the accumulation of BAs. Therefore, prolonged ripening seems to increase BA production.[25–27]

Tyramine levels were found to be higher than the other amine levels in Herby cheeses (Table 1). Tyramine was the main aromatic biogenic amine in Herby cheese (Table 1).

Table 1 The results of biogenic amines of herby cheese samples (mg kg^{-1}).

No	TRP	PA	PUT	CAD	HIS	TYR	Total
1	165.4	ND	72.3	71.7	213.3	286.3	809.0
2	123.1	ND	80.2	68.0	286.9	541.0	1099.2
3	73.5	ND	121.6	68.4	159.9	227.6	651.0
4	92.7	71.8	106.5	39.9	169.8	360.3	841.0
5	85.8	ND	0.87	4.7	95.8	61.6	248.7
6	135.2	69.1	13.5	28.9	248.6	417.5	912.8
7	113.3	ND	320.0	83.4	157.4	317.6	991.7
8	99.0	78.8	377.3	602.0	168.8	418.6	1744.5
9	65.7	ND	32.3	63.2	27.4	122.6	311.2
10	70.9	23.8	179.1	249.5	126.2	271.9	921.4
11	72.5	49.4	57.8	22.4	114.3	309.7	626.1
12	ND	ND	ND	ND	5.9	93.6	99.5
13	100.3	44.2	168.9	194.0	155.9	381.1	1143.9
14	93.9	47.3	362.7	388.7	256.8	540.7	1690.1
15	54.1	17.3	122.8	115.1	102.4	233.0	644.7
16	167.4	ND	45.1	97.5	65.3	104.2	479.5
17	93.7	19.9	114.4	69.9	166.2	214.3	678.4
18	78.2	100.0	662.1	1453.9	676.7	1097.9	4068.8
19	143.9	32.5	119.2	154.6	145.7	276.5	872.4
20	172.6	41.8	470.6	514.2	279.2	483.0	1961.4
21	91.0	36.8	15.0	72.5	286.9	452.7	954.9
22	124.7	99.8	847.0	1844.5	681.5	1125.5	4723.0
23	60.3	ND	ND	27.2	ND	18.0	105.5
24	122.3	36.2	40.8	13.7	112.4	267.3	592.7
25	142.1	12.1	30.4	41.7	129.9	187.9	544.1
26	92.6	39.8	454.4	663.8	255.5	544.4	2050.5
27	127.8	66.6	366.3	874.9	248.4	492.4	2176.4
28	107.1	18.2	90.6	102.6	159.2	217.7	695.4
29	115.2	82.5	283.3	492.9	327.5	532.5	1833.9
30	114.6	19.6	220.3	229.9	113.3	214.2	911.9
Min	ND	ND	ND	ND	ND	18.0	
Max	172.6	100.0	847.0	1844.5	681.5	1125.5	
Std. error of mean	103.2 ± 6.7	33.6 ± 5.8	192.5 ± 38.2	288.4 ± 79.4	197.9 ± 28.3	360.3 ± 46.3	

TRP: tryptamine; PA: phenylethylamine; PUT: putrescine; CAD: cadaverine; HIS: histamine; TYR: tyramine; ND: not detected; Min: minimum value; and Max: maximum value.

The level of tyramine ranged from 18.0 to 1125.5 mg kg^{-1}; it was detected in two samples at a level of higher than the 1000 mg kg^{-1} and in others in the range 18.0–541.0 mg kg^{-1}. Durlu-Ozkaya et al.[27] investigated some biogenic amines in tulum cheese, and tyramine was found to be the dominant biogenic amine in the stored samples. The level of tyramine ranged from 109.6 to 1575.5 mg kg^{-1}. The presence of high quantities of these amines in Herby cheese should be considered as a consequence of a poor hygienic milk quality. Several authors have showed that tyramine is the most important biogenic amine in cheese.[7,10] In this study, it was found almost similar results with these authors. In most cases consumption of food containing biogenic amines does not lead to illness, because amine-destroying enzymes in the digestive tract prevent the uptake of these potentially hazardous compounds into the blood stream. The tyramine levels detected in this work are less then 541.0 mg kg^{-1}, only two samples did include high tyramine, which is the upper limit that can be consumed without noticeable effects.[28]

Tryptamine and phenylethylamine were detected sporadically, at lower amounts compared to histamine, putrescine, cadaverine, and tyramine. Tryptamine was found in low concentrations. It was detected in 29 of 30 samples and its levels in the range of not detected to172.6 mg kg^{-1} (Table 1). Only one sample did not contain detectable amounts of tryptamine. Similar results were observed by Chang et al.[11] The toxic threshold of tryptamine is not known[28]. Phenylethylamine was found in 19 of the 30 samples and levels of almost half of cheese samples were found to be higher than 100 mg kg^{-1}. The prevalence of this amine was high, however the levels detected were low, below its toxic threshold (30 mg kg^{-1}).

Maximum level of putrescine was 847.0 mg kg^{-1}, while it was 1844.5 mg kg^{-1} for cadaverine (Table 1). Biogenic amines were found the relatively high levels found in some samples, a wide variability was observed in the distribution of BAs; in CAD, for example, the levels ranged from non detected to 1844.5 mg kg^{-1} of sample. This variability was within the same type cheeses, which could be attributed to differences in the manufacturing process: type of milk (sheep or cow), heat treatment of milk (such as pasteurization), ripening time, microflora, cheese mass. Several authors carried out studies on ripened cheeses and they reported similar trends in amine distribution to those reported here.[26,27] Cadaverine and putrescine were present at levels up to 1110.0, and 173.7 mg kg^{-1} in Brazilian cheese, respectively.[29] These results are quite higher than that found in this study.

Histamine is of interest not only because of its toxicological risk, but also as an indicator of food quality.[11] The minimal concentration of histamine in food that would elicit a toxic response has been estimated to be 100 mg of histamine in 1000 g of sample.[10,12] In this study, histamine content generally showed higher than 100 mg kg^{-1}. Histamine levels in various cheeses are known to be variable. In Herby cheese, the histamine content was reported between 0.0 and 52.5 mg kg^{-1}.[30] Sancak et al.[31] found that the histamine contents of ($n = 47$) Herby cheeses ranged between 25.62 and 957.62 mg kg^{-1} (with a mean of 211.82 mg kg^{-1}). These findings agree with those reported by authors. The usage of raw milk or because of post-contamination in Herby cheese may result in high levels of histamine formation. Generally the most abundant biogenic amines in cows and ewes milk cheese are tyramine and histamine, which occur in high levels in overripe cheeses.[15,32] Also putrescine and cadaverine have been detected in large quantities in overripe cheeses.[15] These findings agree with those reported by authors.

Differences in the micro environmental conditions throughout the cheese could explain the different accumulation of the amines related to microbial activity. It was known that initial *Enterobacteriaceae* species should be produce putrescine, cadaverine and considerable levels of histamine.[10,33] Therefore, the high these biogenic amines could be produced by these microorganism during the initial of ripening period and storage time. Among cheeses, there were great differences in biogenic amines. The prevailing amine was in all cases tyramine, followed by putrescine or cadaverine in the Herby cheese. Histamine was the following amine but in general showed higher values. Also, in this study, Herby ripened cheeses from raw milk had higher content both cadaverine and putrescine. Herby cheeses contained the highest histamine and tyramine content much higher than the accepted limit of histamine and putrescine are less than 100 mg kg^{-1} and between 100–800 mg kg^{-1}, respectively. Also, total amine content was higher than 1000 mg kg^{-1} in 10 of 30 samples. According to those values, the tested Herby cheeses are not acceptable. From this perspective, almost none of our samples exceed the acceptable level for good hygiene and manufacturing practices. Depending on the results, it can be concluded that the maturated Herby cheeses contained high biogenic amines content.

Organic Acid Analysis

Organic acid contents of the 30 cheese samples are shown in Table 2. As seen from the table, there are significant differences in the contents of organic acids in the samples. It was observed that levels of lactic acid were the highest among all organic acids tested, followed by acetic and butyric acids. Organic acids in cheese occur due to the hydrolysis of fatty acids, bacterial growth, normal bovine metabolic processes or direct addition of acidulants,[34–36] and the composition of organic acids may vary among different types of cheese. The organic acids produced contribute not only to the flavour and aroma of fermented dairy products but also to their preservation.

The primary purpose of a dairy starter culture is to produce lactic acid from lactose at a high rate in the early stages, where lactic acid acts to inhibit contaminants (i.e., coliforms).[35] As expected, the level of lactic acid was found much greater than citric, formic, butyric, propionic and acetic acids; the range for lactic acid was 585.4 to 26,480.3 mg kg^{-1}, which is less than the case for pickle white cheese (30,000 mg kg^{-1}).[35] Lactic acid dominates in aged cheese and its concentration in cheese ranged from 1940 to 17,400 mg kg^{-1}.[18,37] Lues et al.[38] reported the values of lactic acid in cheddar cheese at 23,413.9 mg kg^{-1}. The lactic acid content was similar to the results reported by authors. The percentage of the lactic acid produced is influenced by the age of the cheese. The production of lactic acid is essential for consistent ripening. Formation of lactic acid is essential for proper production, flavor development during normal ripening and good keeping quality.[39]

Acetic acid is the second most abundant acid detected in Herby cheese (Table 2). Acetic acid was detected in ranges from not detected to 10,669.5 mg kg^{-1} in Herby cheese. It is produced either by the metabolism of lactose by lactic acid bacteria or the metabolism of citric and lactic acid or the catabolism of amino acids.[40] Citric acid was detected in ranges from not detected to 2250.0 mg kg^{-1} in Herby cheese (Table 2). It is likely that most citric acid was lost in the whey because 94% of the citric acid is in the soluble phase of the milk. Nevertheless, the lack of citric acid in Herby cheese may also be due to the metabolism of citric acid being vulnerable to volatile flavor compounds (acetic acid, diacetyl, acetoin, and 2,3-butanediol) in some lactic acid bacteria.[40] The concentration of formic acid was found to be between not detected and 2400.2 mg kg^{-1} in Herby cheese, which was higher compared to pickle white cheese (1000 mg kg^{-1}).[35] It could be explained that the heterofermentative metabolism of lactose, by means of a flora different from the starter/secondary flora, may produce formic acid, acetic acid and ethanol.[41]

Hough et al.[16] stated that propionic acid was a good indicator of flavor development in cheese. Propionic and butyric acids were detected in ranges from not detected to 2243.8 and from 314.8 to 7329.6 mg kg^{-1} in Herby cheese, respectively (Table 2). There were differences in propionic and butyric acids among the Herby cheese samples. Propionic acid is formed from lactic acid by propionic bacteria. Butyric acid production may be originated not only from fats but also during proteolysis and subsequent deamination of caseins.[39]

In this study, generally, samples with low contents of total organic acid also showed lower concentrations of total biogenic amines (Samples 12 and 23). According to the results of this research, there were significant ($p < 0.01$) positive correlation ($r = 0.463$, 0.507, 0.505, 0.594 and 0.614) between acetic acid and phenylethylamine, putrescine, cadaverin, histamine and tyramine, respectively. Also, there was significant ($p < 0.01$) positive correlation between total organic acids and histamine ($r = 0.473$) and tyramine ($r = 0.452$). These correlations were an indication of the relationship between organic

Table 2 The results of organic acid of herby cheese samples (mg kg^{-1}).

No	Citric	Lactic	Formic	Acetic	Propionic	Butyric	Total
1	754.8	15048.9	412.4	1711.0	844.9	3673.0	22445.0
2	1350.1	17349.7	ND	1851.4	1100.4	6844.5	28496.1
3	670.4	26480.3	ND	2915.6	1091.7	4617.8	35775.8
4	650.4	14207.1	484.1	ND	1241.4	5363.2	21946.2
5	2250.0	6304.9	531.7	ND	ND	4325.1	13411.7
6	626.2	11391.6	1885.7	6529.0	993.0	4214.6	25640.1
7	513.8	14497.9	1135.1	4828.3	808.3	5173.0	26956.4
8	188.2	10652.2	1788.8	4422.8	885.7	4355.2	22292.9
9	1387.9	6771.8	727.9	2358.6	111.5	1324.2	12681.9
10	1230.4	11756.7	1753.9	2060.3	910.4	3359.1	21070.8
11	565.2	13966.8	1560.9	3113.9	525.7	6669.7	26402.2
12	797.5	585.4	2400.2	2745.1	155.0	314.8	6998.0
13	989.2	11697.1	1184.5	6160.2	748.7	1614.8	22394.5
14	1304.6	13374.5	1697.5	9041.9	786.6	2962.8	29167.9
15	1830.5	13792.5	1223.4	3009.4	1484.7	3192.2	24532.7
16	676.8	5510.0	246.6	5015.9	378.6	6445.3	18273.2
17	733.8	13367.7	300.1	1249.1	1502.2	5659.8	22812.7
18	ND	7559.9	647.8	10669.5	1504.7	2243.8	22625.7
19	758.0	9399.7	517.6	1822.4	319.6	4851.9	17669.2
20	924.7	10744.0	ND	2190.1	1412.3	5505.6	20776.7
21	675.5	14588.1	992.3	4828.3	ND	7289.3	28373.5
22	ND	14789.4	2018.4	7586.9	2243.8	5159.6	31798.1
23	356.1	5435.5	190.7	2229.1	ND	5429.1	13640.5
24	664.5	14629.7	ND	2357.5	868.4	4131.7	22651.8
25	807.9	14738.5	491.9	3755.4	1308.0	4459.4	25561.1
26	627.5	13626.7	ND	3356.9	ND	4388.5	21999.6
27	637.8	12857.3	84.8	ND	ND	5525.8	19105.7
28	1189.8	13278.4	425.4	4493.3	ND	6520.7	25907.6
29	738.9	16316.5	2178.4	4252.7	ND	4045.1	27531.6
30	631.8	15893.2	ND	3265.7	ND	7329.6	27120.3
Min	ND	585.4	ND	ND	ND	314.8	6998.0
Max	2250.0	26480.3	2400.2	10669.5	2243.8	7329.6	35775.8
Std. error of mean	817.74 ± 87.42	12353.73 ± 857.12	829.33 ± 138.92	3594.01 ± 458.68	707.52 ± 111.67	4566.30 ± 316.83	–

ND: not detected; Min: minimum value; and Max: maximum value.

acids and biogenic amines, which might be a valuable quality criterion of cheese. In contrast, generally, samples with high contents of total organic acids also had higher concentrations of all determined biogenic amines (Samples 14, 18 and 22). In conclusion, this is the one of the first result found the correlation between organic acids and biogenic amines content in cheese.

Microbiological Analysis

Microbiological counts and physicochemical results of samples are shown in Table 3. Lactic acid bacteria counts of samples varied from 2.00 to 5.47 log cfu g^{-1}. The lactic acid bacteria counts of almost half of cheese samples had lower than 4.00 log cfu g^{-1}. Yetismeyen[42] found that the lactic acid bacteria varied from <3.00 to 7.07 log cfu g^{-1} in herby cheese. Some lactic acid bacteria produce biogenic amines (tyramine, histamine, phenylethylamine and tryptamine) in cheese.[12,33] The primary purpose of a dairy starter culture is to produce lactic acid from lactose at a high rate in the early stages, where lactic acid acts to inhibit contaminants (i.e., coliforms).[35] *Enterobacteriaceae* and coliform counts are considered indicative of the microbiological quality of cheese.[43] Coliform counts of samples had lower than 100 cfu g^{-1} which shows us there is no contamination during the storage condition or coliforms may be disappeared during the ripening time, owing to the conditions of the medium (high concentration of salt, lactic acid, low pH and microorganism occurrence). In our study, some samples had higher concentrations of putrescine, cadaverine, histamine and tyramine (Samples 18 and 22). It was known that initial lactic acid bacteria species should be produce putrescine, cadaverine and considerable levels of histamine.[10,33] Therefore, the high biogenic amines could be produced by these microorganisms during the initial of ripening period and storage time.

Physical and Chemical Analysis

The pH value is an important factor influencing amino acid decarboxylase activity, which is stronger in an acidic environment, the optimum pH being between 4.0 and 5.5.[12] pH values of cheese samples varied from 4.03 to 6.09 (Table 3). In this study, generally, the pH value was found high in samples that had low biogenic amines levels (samples 5, 9, 12, 16, and 23). Yetismeyen[42] found that the pH values varied from 4.22 to 5.19 in herby cheese. Higher organic acids or lower pH in ripened herby cheeses is found to be specific result for this kind of cheese. The reason is that while cheese blocks are placed into the container, whey cheese is sometimes used to fill the empty places between cheese blocks. Since whey cheese is rich in lactose, this might cause higher organic acids or lower pH value, as seen in Table 2 and 3. Tarakci et al.,[3] reported that the pH of herby cheese ripened with whey cheese decreased during ripening.

Proteolysis is the most complex and, in most varieties, the most important of the primary biochemical events that occur in most cheeses during ripening.[44–46] High levels of WSN, TCA-SN and PTA-SN (indices of proteolysis) were found in ripened Herby cheeses (Table 3). WSN, TCA, PTA values were determined to be between 0.19–077%, 0.10–0.71% and 0.06–0.30%, respectively. When tasted, the cheese had a strong flavour since the protein degradation and fat hydrolysis occur under different environmental (such as storage temperature and humidity) and cheese making conditions (pH, salt, different herbs, etc.), and many kinds of microorganisms that hydrolyse proteins and lipids might be responsible since the cheeses are made using raw milk with different microflora. In

Table 3 Microbiologica and physicochemical properties of herby cheese samples.

No	LAB (CFU g^{-1})	Dry Matter (%)	pH	TN (%)	WSN (%)	TCA-SN (%)	PTA-SN (%)	Fat (%)	Salt (%)	Protein (%)	ADV (%)
1	4.19	44.16	4.28	2.48	0.40	0.34	0.15	18.50	10.53	15.83	3.55
2	2.00	54.30	4.50	4.09	0.42	0.41	0.15	17.25	9.88	26.12	10.00
3	2.00	56.10	4.35	2.83	0.28	0.25	0.11	22.50	12.34	18.08	5.83
4	4.31	50.20	4.16	3.00	0.34	0.32	0.15	17.75	9.24	19.16	5.45
5	2.84	53.00	5.23	2.81	0.47	0.30	0.16	21.50	10.64	17.93	1.96
6	3.75	53.94	4.67	3.12	0.30	0.28	0.15	25.00	4.73	19.94	6.29
7	2.17	60.90	4.11	3.84	0.36	0.32	0.15	30.25	6.61	24.55	2.83
8	4.96	52.18	4.57	3.44	0.35	0.35	0.14	24.25	5.85	22.00	4.63
9	3.76	60.96	5.27	3.06	0.47	0.24	0.12	27.25	10.64	19.52	10.49
10	2.00	55.03	4.57	3.18	0.36	0.32	0.12	18.75	11.46	20.34	3.98
11	4.05	54.95	4.03	3.55	0.33	0.31	0.14	26.50	8.24	22.68	2.69
12	5.09	46.58	6.09	2.36	0.19	0.10	0.06	21.25	9.76	15.06	1.61
13	4.83	57.21	4.78	3.44	0.36	0.32	0.12	27.00	8.24	21.95	3.76
14	3.81	63.71	4.66	4.28	0.43	0.38	0.15	27.00	7.83	27.35	7.11
15	3.47	60.83	4.28	2.84	0.42	0.36	0.13	30.00	11.99	18.17	4.11
16	5.11	50.85	5.27	2.93	0.32	0.20	0.10	17.75	11.58	18.74	8.66
17	3.46	59.19	4.32	2.93	0.33	0.26	0.11	27.00	9.71	18.74	4.45
18	2.77	54.22	5.12	3.37	0.69	0.64	0.30	30.50	6.66	21.55	10.07
19	5.47	41.85	5.13	1.90	0.42	0.30	0.11	18.00	9.36	12.14	13.32
20	4.74	57.79	4.76	3.94	0.53	0.50	0.19	21.75	6.61	25.14	1.81
21	2.30	50.52	4.73	3.40	0.52	0.40	0.15	17.25	9.12	21.70	5.15
22	4.51	63.47	5.11	3.93	0.77	0.71	0.29	27.50	7.13	25.13	11.73
23	5.00	53.99	5.58	3.40	0.35	0.16	0.08	21.00	13.80	21.75	6.50
24	4.63	50.80	4.13	3.14	0.32	0.28	0.12	23.50	8.01	20.07	4.35
25	3.57	44.19	4.10	2.39	0.36	0.28	0.12	18.25	9.71	15.29	3.59
26	5.04	54.25	4.62	3.13	0.44	0.40	0.16	27.50	8.71	19.99	5.18
27	5.25	54.52	4.32	3.20	0.36	0.32	0.15	29.75	5.03	20.42	5.29
28	5.06	58.61	4.67	3.15	0.33	0.25	0.11	27.00	9.18	20.12	4.51
29	4.44	49.93	4.51	3.21	0.44	0.36	0.14	23.25	9.06	20.51	4.93
30	3.92	62.02	4.19	4.01	0.23	0.20	0.10	27.25	8.71	25.61	4.16
Min	2.00	41.85	4.03	1.90	0.19	0.10	0.06	17.25	4.73	12.14	1.61
Max	5.47	64.71	6.09	4.28	0.77	0.71	0.30	30.50	13.80	27.35	13.32
Std. error of mean	3.95 ± 0.19	54.3 ± 1.03	4.6 ± 0.1	3.2 ± 0.09	0.4 ± 0.02	0.32 ± 0.02	0.14 ± 0.009	23.73 ± 0.8	9.01 ± 0.4	20.51 ± 0.6	5.59 ± 0.54

LAB: lactic acid bacteria; TN: total nitrogen; WSN: water soluble-nitrogen; TCA-SN: trichloro acetic acid-soluble nitrogen; PTA-SN: phosphotungstic acid soluble nitrogen; Min: minimum value; Max: maximum value; and ADV: Acid degree value.

our study, samples with high contents of WSN, TCA and PTA also had higher concentrations of putrescine, cadaverine, histamine and tyramine (Samples 18 and 22). According to the results of research, there were significant ($p < 0.01$) positive correlation ($r = 0.488$, 0.683, 0.748, 0.805 and 0.766) between WSN and phenylethylamine, putrescine, cadaverin, histamine and tyramine, respectivelty. Also, there was significant ($p < 0.01$) positive correlation between TCA, PTA and biogenic amines. These correlations were an indication of the relationship between nitrogen fractions and biogenic amines. In contrast, samples with low contents of WSN, TCA and PTA also had lower concentrations of all determined biogenic amines (Samples 12 and 23).

The salt content of the samples was found between 4.73 and 13.80%, also the calculated average salt content in dry matter changed from 8.77 to 25.57% for all cheese samples. Factors such as available substrate pH, salt concentration, temperature,[13,29] and the presence of pyridoxal phosphate[13] influence the build up of biogenic amines in cheese. Generally, the salt value was high in samples that had low biogenic amines levels (samples 3, 9, 15, 16, and 23). The effects of salt include control of microbial growth and activity, control of various enzyme activities in cheese, reduction of cheese moisture content, and physical changes in cheese proteins that can influence cheese texture, flavour development and formation of biogenic amines from free amino acids.[47] The fat content of samples was found between 17.25 and 30.50%. It can be concluded that the salt contents influence the build up of biogenic amines in Herby cheese samples.

Higher lipolysis values were found for ripened Herby cheese (Table 3). There were dissimilarities in values of ADV among the Herby cheese samples (Table 3). In this study, acid degree value (ADV) of samples varied from 1.61-13.32%. These values are higher than the results reported by Yetişmeyen[42] on commercial Herby cheese. Since Herby cheese is normally made of raw sheep milk, the indigenous milk lipase remains in milk and cheese, and results in higher lipolytic activity. In addition, other factors such as high number of lipase-producing microorganisms (such as moulds, some psychrotrophs and lactic acid bacteria that have little activity) might be another reason.[3–5,48] Tarakcı et al.[49] reported values of ADV at 2.60 for 90-days old Herby cheese (used pasteurised milk). Also, values of ADV for ripened Herby cheese were reported by Tarakcı et al.[3] at 3.92. Herby cheese is produced from raw and unpasteurised materials (both milk and herbs), and found that the cheese produced from raw materials had higher ADV values than that produced from pasteurised cheese. Therefore, values found in this study were higher than to those reported by authors. The ADV values in this study indicate that the levels of free fatty acids have been elevated and are responsible for the characteristic Herby cheese flavor.

CONCLUSIONS

Tyramine and cadaverine are the most important biogenic amines for herby cheese. However, there were significant variations in the biogenic amines contents of herby cheese. In this study, samples with high contents of WSN, TCA, and PTA also showed higher concentrations of putrescine, cadaverine, histamine and tyramine (Samples 18 and 22). Variation in the biogenic amines levels of herby cheese could be due to the hygienic quality of raw material, manufacturing techniques, the specific flora, ripening period and the type of culture. The determination of organic acids in Herby cheese showed that lactic acid was the main acid in all kinds of Herby cheese. The organic acids are important flavor substances of most aged cheese types. Use of good hygienic conditions during the manufacture of Herby cheese are necessary to prevent high amine formation, together with good quality raw material with low total counts of contaminant microorganisms but also free of amine-producing microorganisms. Efforts should be made to understand biogenic amines formation in Herby cheese in order to optimize technology and secure low amines levels.

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