GIDA (2015) 40 (1): 1-8 Araştırma/*Research*
doi: 10.15237/gida.GD14049

EFFECTS OF DIFFERENT HERBS ON BIOGENIC AMINE CONTENTS AND SOME CHARACTERISTICS OF HERBY CHEESE

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Geliş tarihi / *Received*: 01.07.2014
Düzeltilerek Geliş tarihi / *Received in revised form*:14.07.2014
Kabul tarihi / *Accepted*: 21.10.2014

Abstract

In this study, the effects of different herbs (*Allium* sp., *Ferula* sp. and *Anthriscus* sp.) on some properties of Herby cheese, during 90 days, were studied. Herby cheese samples were analyzed in terms of selected organic acids (formic, lactic, acetic, citric, and butyric) and biogenic amines (phenylethylamine, histamine, tyramine, cadaverine, putrescine, and tryptamine). The dominant organic acid and biogenic amine found in herb added cheeses were lactic acid and phenylethylamine, respectively. Heliz and Sirmo added cheeses contained higher phenylethylamine than those of the Mendo added and control group cheeses. The highest mean value of lactic acid was found in Heliz added cheeses. Differences among the fatty acids of all samples were not significant ($P<0.05$) statistically. Total counts of mesophilic aerobic bacteria, lactic acid bacteria, and coliform bacteria decreased during ripening in all cheese samples.

Keywords: Herby cheese, organic acids, biogenic amines, microbiological characteristics, chemical characteristics

FARKLI OTLARIN OTLU PEYNİRİN BİYOJEN AMİN İÇERİĞİ ve BAZI ÖZELLİKLERİ ÜZERİNE ETKİSİ

Özet

Bu çalışmada 90 günlük depolama süresi boyunca farklı otların (*Allium* sp., *Ferula* sp. and *Anthriscus* sp.) Otlu peynirin bazı özellikleri üzerine etkisi incelenmiştir. Otlu peynir örneklerinin organik asit (formik, laktik, asetik, sitrik, ve bütiric) ve biyojen amin (feniletilamin, histamin, tiyramin, kadaverin, putresin, ve triptamin) içerikleri belirlenmiştir. Otlu peynirlerde en yüksek miktardaki organik asidin laktik asit ve biyojen aminin ise feniletilamin olduğu bulunmuştur. Otlu peynirlerin kontrol grubu örneklere göre daha yüksek miktarda feniletilamin ve histamin içerdiği tespit edilmiştir. Peynir örnekleri arasında yağ asitleri açısından istatistiksel olarak önemli bir fark bulunmamıştır. Toplam mezofililik aerob bakteri, laktik asit bakterisi ve koliform bakteri sayılarının depolama süresi boyunca tüm peynir örneklerinde azaldığı saptanmıştır.

Anahtar kelimeler: Otlu peynir, organik asit, biyojen amin, mikrobiyel özellikler, kimyasal özellikler

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INTRODUCTION

Recently specific attention has been given to the adaptation of traditional cheeses to industrial practices. Turkey has a very long tradition of producing a large variety of cheeses. Herby cheese is one of the popular products traditionally produced from raw milk in Eastern Turkey. This cheese variety has a semi hard texture and a salty taste and is produced in small family or artisan workplaces and generally made from raw sheep's milk. Furthermore mixtures of sheep's, cow's or goat's milk are used in the manufacture of Herby cheese. This cheese variety is ripened for about 3 months in order to get the desired taste and flavor. Almost 25 kinds of herbs are used for the production of Herby cheese. However most preferred herbs are "sirmo" (*Allium* sp.), "heliz" (*Ferula* sp.) and "mendo" (*Anthriscus* sp.) (1-3). This dairy product, is generally produced in homes under poor hygienic conditions and marketed in open markets.

In cheese, toxic compounds such as biogenic amines are produced during ripening. This compounds originate in foods from decarboxylation of specific amino acids (4, 5). Levels of biogenic amines vary according to ripening period and microflora. As raw milk and herbs are used in the production of traditional Herby cheese, the process should be investigated in terms of biogenic amines and microbial characteristics. Sağun et al. (6) noticed that the level of histamine in Herby cheese was 21.9 mg/kg on the first day of ripening, then gradually increased, and reached 46.2 mg/kg at day 90. Andiç et al. (1) analyzed some biogenic amines in Herby cheese, and tyramine was found to be dominant biogenic amine with levels ranging from 18 to 1125.5 mg/kg. Durlu-Özkaya (7) detected the average amounts of putrescine, cadaverine, histamine, tyramine, and spermidine in 9 Herby cheese samples as 24.3, 8.0, 17.4, 182.4, and 115.7 mg/kg, respectively.

It was reported in the studies which were carried out to determine the microbiological characteristics of Herby cheese that *S. aureus* and *E. coli* were present in fairly high numbers, with average of 6.10 and 3.68 log CFU/g, respectively in 50 unripened Herby cheese (8). Sancak (9) stated that enterococci count was $6.3x10^4$ log CFU/g.

Many studies were carried out to determine amine contents of different types of cheese, but there are only few studies focusing on the effect of herb addition on biogenic amine accumulations in Herby cheese. The aim of this study was to determine the effect of different herbs on the biogenic amines and other characteristics (organic acids, and some microbiological, chemical, and biochemical properties) of Herby cheese during 90 days of storage.

MATERIAL and METHODS

Manufacture of the Herby Cheese

The pickled forms of the most preferred herbs are "sirmo" (wild garlic) (*Allium* sp.), "heliz" (*Ferula* sp.), and "mendo" (*Anthriscus* sp.); they were separately used for cheese production. The raw milk mixture [cow (50%) and sheep (50%)] was heated to 32°C and coagulated with microbial rennet (Mayasan Company , Istanbul, Turkey) for 45 min. After coagulation, the curd was cut, partly drained, and divided into 4 groups. (1 control group, 2 mendo-added groups, 3 heliz-added groups, and 4 sirmo-added groups). Then the pickled herbs were added to the cheese curds and mixed. Levels of the herbs in pickled form were 1% of milk weight used. The control cheese contained no herb. After that, the curds were drained and pressed for 3 h. After draining, cheeses were cut into blocks of 7x7x5 cm. These were placed into plastic boxes and filled with brine containing 14% NaCl. Samples were taken on the 30^{th}, 60^{th}, and 90^{th} days of the ripening period for chemical and microbiological analyses. The experiment was repeated twice and the analyses were done in duplicate.

Chemical Analysis

Titratable acidity, dry matter, fat, total nitrogen, and salt contents of the cheese samples were determined according to the methods described by AOAC (10). Water-soluble nitrogen (WSN), trichloroacetic acid-soluble nitrogen (TCA-SN), and phosphotungstic acid-soluble nitrogen (PTA-SN) ratios were determined according to the methods given by Bütikofer et al. (11). Lipolysis measurement was done by using BDI method and recorded as acid degree value (ADV) (12). pH values of the cheese samples were measured by using a HANNA pH meter (HANNA Instruments, Cluj-Napoca, Romania).

Analysis of Biogenic Amines

Six aqueous standard solutions containing cadaverine dihydrochloride, putrescine dihydrochloride, tyramine hydrochloride, tryptamine hydrochloride, phenylethylamine hydrochloride, histamine dihydrochloride, and 1,7-diaminoheptane (as internal standard)

from Sigma (St. Louis, MO) were derivatized as described for the cheese samples. Biogenic amine contents of the samples were determined according to the method of Eerola et al. (13). 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 gradient-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 the next analysis. The flow rate was 1.0 mL/min. and the column temperature was 40 °C. A 20-µL sample was injected onto the column. Peaks were controlled at 254 nm using the HPLC system with a column Spherisorb ODS2 150A, 150 x 4.60 mm (Waters, Milford, MA, USA) and a gradient pump, which included an Agilent HPLC (1100 series, G1311A quaternary pump, G1315A diode array detector, G1313A auto sampler, and G1322A vacuum degasser; Agilent, Palo Alto, CA), and a computer including the Agilent package program. The quantitative determinations were carried out with internal standard (1.7-diaminoheptane) method, by using peak heights. Biogenic amine contents were expressed as mg/kg.

Analysis of Organic Acids

Extraction and determination of organic acids were performed according to the modified method of Bevilacqua and Califano (14). In order to do this, approximately 100 g of a representative cheese sample was ground (A-10 analytical mill, Tekmar, Cincinnati, OH, USA) and homogenized (Heidolph Silent Cruster M, Schwabach, Germany). Fifty mL of 0.009 N H_2SO_4 (mobile phase) was added to 7 g of ground cheese and extracted for 1 h by mixing in a shaker (Heidolph Unimax 1010, Schwabach, Germany) and centrifuged at 7000 x g for 5 min. (Hettich Zentrifugen Universal 32 R, Tuttlingen, Germany). The supernatant was filtered once through coarse filter paper and twice through a 0.45-µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, Billerica, MA, USA) and 10 µL was injected into an HPLC system (1100 series G 1322 A, Santa Clara, CA, USA) equipped with an Aminex HPX-87 H column (300 mm x 7.8 mm). The UV detector was set at 214 and 280 nm. The mobile phase was 0.009 N H_2SO_4. The quantitative determinations were based on external standard method, using peak areas. Organic acid contents were expressed as mg/kg.

Analysis of Fatty Acid

Extraction of total lipids was carried out according to Folch et al. (15). After removing the solvent phase with a rotary evaporator at 40 °C, 0.2 g of fat was transferred into a tube, dissolved in 2 mL hexane, and then fatty acid methyl esters (FAMEs) were prepared by using 0.2 mL 1 N methanolic KOH. Analysis of FAMEs was made on an Agilent 6890 GC equipped with a 5973 mass-selective detector (Santa Clara, CA, USA) and fitted with a fused silica capillary column (DB-23; 60 m x 0.25 mm; film thickness 0.25 µm; JandW Scientific Co, Folsom, CA, USA). Helium was used as the carrier gas. Inlet temperature was 250 °C. The initial oven temperature was 60 °C and increased to 120 °C at 10 °C/min. Then oven temperature was increased to 200 °C at 14 °C/min and the final temperature maintained for 45 min. One µL and a 1:5 split ratio were used for injection. Supelco FAME 37 component FAME mix (Supelco, Bellefonte, PA, USA) was used as the standard. Chromatograms were evaluated by using MS software (Chem Station, A.10.02, Agilent) and MS (NIST) database. Percentages of FAMEs were quantified according to their relative area.

Microbiological Analysis

The number of viable total mesophilic-aerobic bacteria (TMAB), lactic acid bacteria (LAB), and coliform counts in the samples were enumerated by using dilution plating technique. For microbiological analysis 10 g of sample was prepared by homogenizing with 90 mL of physiological saline solution (0.85% NaCl) in a sterile polyethylene bag by using a Stomacher 400 (Seward Laboratory, London, UK) for 1 min. Serial dilutions of homogenized samples were also made in physiological saline solution (0.85% NaCl) and drop-plated onto appropriate media. Samples were spread-plated on duplicate on plate count agar (PCA) (Oxoid, Basingstoke, Hampshire, UK) for TMAB; on acidified (pH 5.4) de Man, Rogosa, Sharpe agar (MRS) (Merck, Darmstadt, Germany) for LAB; and on violet red bile agar (VRBA) (Merck) for coliform group bacteria. PCA plates were incubated at 37 °C for 48 h MRS plates were incubated at 37 °C for 72 h and VRBA plates were incubated at 37 °C for 48 h. Then, viable counts of microorganisms were determined. Log_{10} transformations were applied on microbiological data.

Statistical Analysis

Statistical analysis was performed by using SPSS software version 13.0 (16). Variance analysis and Tukey's multiple comparison tests were used in order to determine the differences between samples.

RESULTS AND DISCUSSION

Chemical properties of experimental cheese samples are given in Table 1. The use of herb did not significantly affect (P>0.05) the dry matter, fat, salt, and total nitrogen levels of the cheese samples. In the previous studies on the Herby cheese, the dry matter, salt and total nitrogen amounts were reported (17, 9, 18, 19) as 47.67, 6.39, 3.75%; 58.14, 7.21, 3.99%; 47.23, 6.45, 3.75%; and 43.05, 6.63, 2.03%, respectively. The amount of fat in Herby cheeses noted by Sancak (9) and Sönmezsoy (19) were higher (23.38% and 24.03%, respectively) than our findings.

The WSN, TCA-SN, and PTA-SN contents of the samples increased significantly (P<0.05) during the ripening period. Similar findings were reported by Javidipour and Tunçtürk (20), Özer et al. (21), Chander et al. (22), and Fulco et al. (23) for different types of cheese. Ekici et al. (2) reported that WSN:TN ratio of Herby cheese supplemented with *Allium* sp. increased during ripening period and reached 34.07±2.143% at the end of ripening.

Allium sp. (sirmo)-incorporated samples had significantly (P<0.05) higher WSN, TCA-SN, and P-TA-SN values than the other groups at the end of the ripening period.

ADVs of all groups significantly increased (P<0.05) during ripening. *Allium* sp. (sirmo) added samples had significantly (P<0.05) higher ADVs than the other treatments. ADVs of herb-added cheeses were significantly higher than that of the control (P<0.01). Differences between the samples in the study could be resulted from microflora of herbs besides microflora of raw milk.

Titratable acidity of all herb-added cheeses was significantly higher (P<0.05) than the titratable acidity of the control sample. Lactic acid bacteria from raw milk and herbs led to the formation of high acidity in Herby cheese. Similar findings were reported for 90 day-old Herby cheese by Ekici et al. (2) and for Turkish white cheese by Öner et al. (24). As expected, increases in the acidity during ripening correlated with the pH values of the cheese samples very well.

Biogenic amines determined in ripened samples after 90 days of storage were phenylethylamine, histamine, and tyramine. Cadaverine, putrescine, and tryptamine were not found in the samples during ripening period. Phenylethylamine was the major biogenic amine in all ripened cheeses, however, it was not found in the fresh samples (except in *A llium* sp.-added treatment) Phenylethylamine was also found in *Anthriscus* sp. (mendo)-added treatment on the 30th day of ripening. Phenylethylamine content significantly increased (P<0.05) starting from the 60th day until the end of ripening period in all treatments (Table 2). Phenylethylamine level was reported to be 3.77 mg/kg in Feta cheese by Valsamaki et al. (25); 0.74 mg/kg in white cheese by Öner et al. (24); and 27.34 mg/kg in goat cheese by Novella-Rodr guez et al. (26).

Histamine, which was not found in the fresh control, *Anthriscus* sp. (mendo)-added, and *Ferula* sp. (heliz)-added samples was determined on the 60th and the 90th days of the ripening period. Histamine content of *Allium* sp. (sirmo)-incorporated sample significantly increased up to the 30th day of storage (P<0.05) and it then slightly increased up to the end of the ripening period. Histamine is accumulated in cheese during ripening as a result of the activity of lactic acid bacteria in milk and other sources. Ekici et al. (2) reported that addition of herbs caused an increase in histamine accumulation in cheese.

Tyramine was the most abundant biogenic amine in fresh samples. Tyramine significantly increased (P<0.05) at the late stages of ripening period. Valsamaki et al. (25) indicated that tyramine and putrescine were the major amines in ripened Feta cheese (120 days) and the concentrations of tyramine and histamine were 204 and 76.4 mg/kg, respectively, on the 90th day of storage. Histamine in herb-added cheese was reported to be between 0.00 and 52.5 mg/kg (7). Sancak et al. (27) found that histamine amount in 47 Herby cheeses ranged between 25.62 and 957.62 mg/kg. Andiç et al. (1) stated that phenylethylamine, histamine, and tyramine amounts of Herby cheese ranged between 0 and 100 mg/kg; 0 and 681.5 mg/kg; 18.0 and 1125.5 mg/kg respectively. Nout (28) pointed out that maximum allowable levels in foods should be in the range of 50-100 mg/kg for histamine and 100-800 mg/kg for tyramine. Concentrations of histamine and tyramine found in our study were lower than these levels.

Cheese samples were analyzed for formic, butyric, citric, lactic, and acetic acids. Formic and butyric acids were not found in the samples. Overall, organic acid contents of herb-added cheese samples had a tendency to increase during ripening period (Table 3). Cheese samples with *Allium* sp. (sirmo) had the highest citric acid content at the end of 90 days. Manolaki et al. (29) reported that lactic, citric, and acetic acids

Table 1. Effects of Herbs on the Some Chemical Characteristics of Herby Cheese

		Storage Period (day)			
Item	Sample	0	30	60	90
Dry Matter (%)	1	43.30±0.16^{aA}	42.62±0.35^{aA}	42.40±0.38^{aA}	42.62±0.39^{aA}
	2	44.61±0.24^{aA}	42.22±1.95^{aA}	43.97±0.15^{aA}	43.09±0.73^{aA}
	3	43.45±0.23^{aA}	43.59±0.45^{aA}	42.54±0.80^{aA}	43.19±0.56^{aA}
	4	43.29±0.41^{aA}	44.08±0.29^{aA}	42.67±0.23^{aA}	43.34±0.01^{aA}
Fat (%)	1	19.75±0.35^{aA}	20.25±1.06^{aA}	21.00±0.71^{aA}	20.50±1.02^{aA}
	2	19.75±0.35^{aA}	19.75±0.35^{aA}	20.00±0.00^{aA}	20.50±2.12^{aA}
	3	20.00±0.26^{aA}	20.00±0.54^{aA}	20.50±0.71^{aA}	20.00±0.71^{aA}
	4	20.50±0.71^{aA}	19.75±0.35^{aA}	19.75±0.35^{aA}	20.00±1.41^{aA}
Salt (%)	1	5.03±0.16^{aA}	4.91±0.16^{aA}	4.97±0.91^{aA}	4.56±0.61^{aA}
	2	4.80±0.16^{abA}	4.68±0.16^{aA}	5.00±0.58^{aA}	4.62±0.66^{aA}
	3	4.66±0.46^{abA}	4.74±0.25^{aA}	4.74±0.41^{aA}	5.03±0.43^{aA}
	4	4.74±0.08^{abA}	4.80±0.16^{aA}	5.15±0.33^{aA}	4.97±0.58^{aA}
Total Nitrogen (%)	1	2.40±0.69^{aA}	2.30±0.09^{aA}	2.38±0.31^{aA}	2.29±0.16^{aA}
	2	2.60±0.14^{aA}	2.47±0.15^{aA}	2.50±0.16^{aA}	2.47±0.35^{aA}
	3	2.68±0.21^{aA}	2.52±0.29^{aA}	2.44±0.24^{aA}	2.67±0.21^{aA}
	4	2.56±0.31^{aA}	2.65±0.14^{aA}	2.56±0.11^{aA}	2.54±0.25^{aA}
Water-soluble N (%)	1	0.11±0.02^{aC}	0.15±0.01^{cB}	0.20±0.02^{bA}	0.22±0.01^{cA}
	2	0.12±0.02^{aC}	0.17±0.01^{bcB}	0.21±0.01^{bA}	0.22±0.01^{cA}
	3	0.12±0.02^{aC}	0.19±0.01^{abB}	0.21±0.02^{bB}	0.26±0.02^{bA}
	4	0.12±0.03^{aC}	0.22±0.02^{aB}	0.30±0.01^{aA}	0.32±0.02^{aA}
Trichloroacetic	1	0.04±0.02^{aC}	0.12±0.01^{bB}	0.17±0.01^{bA}	0.18±0.01^{cA}
acid-soluble N (%)	2	0.05±0.03^{aC}	0.14±0.01^{abB}	0.17±0.01^{bAB}	0.20±0.01^{bcA}
	3	0.05±0.03^{aC}	0.14±0.02^{abB}	0.20±0.02^{aAB}	0.22±0.01^{bA}
	4	0.05±0.03^{aC}	0.15±0.01^{aB}	0.23±0.01^{aA}	0.26±0.01^{aA}
Phosphotungstic	1	0.03±0.01^{aC}	0.04±0.01^{bC}	0.06±0.01^{aB}	0.07±0.01^{bcA}
acid-soluble N (%)	2	0.03±0.01^{aC}	0.04±0.01^{bC}	0.05±0.01^{bB}	0.06±0.01^{cA}
	3	0.02±0.01^{aD}	0.05±0.01^{aC}	0.06±0.01^{aB}	0.08±0.01^{abA}
	4	0.03±0.01^{aC}	0.04±0.01^{bC}	0.06±0.01^{aB}	0.09±0.01^{aA}
ADV (mEq/100 g of fat)	1	0.58±0.01^{bD}	0.79±0.01^{bC}	1.03±0.06^{cB}	1.33±0.08^{cA}
	2	0.60±0.03^{bD}	0.79±0.02^{bC}	1.30±0.09^{bB}	1.62±0.03^{bA}
	3	0.54±0.03^{bD}	0.80±0.01^{bC}	1.24±0.01^{bB}	1.72±0.04^{bA}
	4	0.81±0.02^{aC}	1.01±0.14^{aC}	1.73±0.09^{aB}	2.42±0.05^{aA}
pH	1	5.16±0.05^{aA}	4.85±0.05^{bB}	4.89±0.15^{aB}	4.95±0.01b^{AB}
	2	5.04±0.01^{bA}	4.84±0.01^{bB}	4.67±0.02^{bC}	4.70±0.01^{dC}
	3	5.03±0.01^{bA}	4.88±0.01^{bC}	4.97±0.03^{aB}	4.90±0.01^{cC}
	4	5.07±0.01^{bA}	5.01±0.01^{aB}	5.06±0.01^{aA}	4.98±0.01^{aB}
Titratable acidity (%)	1	0.69±0.01^{cD}	0.85±0.01^{cC}	0.92±0.04^{cB}	1.24±0.01^{bA}
	2	0.83±0.01^{aD}	1.08±0.01^{aC}	1.24±0.01^{aB}	1.35±0.02^{aA}
	3	0.82±0.01^{aC}	0.96±0.03^{bB}	1.01±0.02^{bB}	1.30±0.02^{abA}
	4	0.77±0.01^{bD}	0.86±0.01^{cC}	1.05±0.01^{bB}	1.34±0.05^{aA}

^{a,b,c,d}Different lowercase letters within a column and item indicate significant differences between cheese samples (P<0.05).
^{A,B,C,D}Different uppercase letters within a row (sample) and item indicate significant differences between ripening periods (P<0.05).
1: control cheese; 2: mendo added cheese; 3: heliz added cheese; 4: sirmo added cheese.

are the main organic acids found in all Feta and Feta-type cheeses. Park and Lee (30) stated that the citric acids content of soft goat milk cheese aged at 4 °C for 4 weeks was 0.72 mg/kg. Kaminarides et al. (31) reported that lactic acid concentration reached 4.07 mg/kg in Halloumi cheese after 45 days of ripening. Bevilacqua and Califano (14) reported that lactic acid dominates organic acids in aged cheese and its concentration in different cheeses ranged from 1.94 to 17.4 mg/kg. Acetic acid was not found initially in cheese samples but was found on the 60th day and increased until the end of ripening in herb-added treatments. Interestingly, acetic acid was not found initially and throughout ripening period in the control group. An increase in acetic acid content during ripening of Feta cheese was reported by Manolaki et al. (29). Kaminarides et al. (31) noted acetic acid as the second most abundant organic acid in Halloumi cheese.

Fatty acid profile of fresh cheese samples is classified according to their chain lengths and saturation levels (Table 4). Addition of herbs did

Table 2. Effect of Herbs on the Biogenic Amines Contents of Herby Cheese

		Storage Period (day)			
Biogenic Amines (mg/kg)	Sample	0	30	60	90
Phenylethylamine	1	ND	ND	13.37±1.97^{aB}	83.65±5.63^{bA}
	2	ND	4.67±0.82^{aBC}	14.27±2.62^{aB}	94.53±7.18^{bA}
	3	ND	ND	7.68±1.18^{bB}	183.35±19.45^{aA}
	4	1.49±0.41^{B}	2.56±0.64^{bB}	14.85±1.64^{aB}	144.16±18.60^{aA}
Histamine	1	ND	ND	11.20±1.55^{aA}	11.34±3.04^{aA}
	2	ND	ND	9.97±2.62^{aA}	11.00±3.76^{aA}
	3	ND	ND	13.29±2.60^{aA}	14.53±3.76^{aA}
	4	1.25±0.21^{bB}	8.56±0.79^{aA}	11.11±1.60^{aA}	12.63±3.75^{aA}
Tyramine	1	7.89±1.89^{aB}	9.65±1.63^{aB}	14.58±2.28^{aB}	32.00±4.96^{aA}
	2	3.65±1.50^{bB}	5.27±1.20^{bcB}	8.74±1.17^{bB}	21.56±2.98^{bA}
	3	2.36±0.92^{bC}	3.68±1.12^{cC}	8.42±1.00^{bB}	14.52±2.62^{bA}
	4	4.56±0.90^{abC}	8.65±1.19^{abBC}	11.74±1.80^{ab}	16.84±2.76^{bAB}

^{a,b,c}Different lowercase letters within a column and biogenic amine indicate significant differences between cheese samples (P<0.05).
^{A,B,C}Different uppercase letters within a row (sample) and biogenic amine indicate significant differences between ripening periods (P<0.05).
1: control cheese; 2: mendo added cheese; 3: heliz added cheese; 4: sirmo added cheese; ND: not detected.

not affect the fatty acids profile of herb-added cheese during storage period. SFA and PUFA contents of Turkish white cheese reported by Javidipour and Tunçtürk (20) were 72.30 and 1.26%, respectively.

Generally, TMAB, LAB, and coliforms counts of samples significantly decreased (P<0.05) throughout ripening. TMAB and of different treatments showed comparable counts at the same ripening period. The LAB counts of herby samples were higher than that of control sample. It was reported that some herbs and spices influence the growth and activities of lactic acid bacteria at different levels (32). Coliform bacteria counts decreased to an undetectable level in *Anthriscus* sp. (mendo)-added samples after 60 days of ripening. At the end of ripening, lowest LAB count was determined in the control samples.

Numbers of microorganisms such as coliforms, enterococci, and staphylococci found in cheese at relatively high levels were indicative of hygienic quality. These counts suggest that contamination was very high in raw milk (24). Ekici et al. (2) stated that TMAB counts in 90 days-old Herby and control cheese samples were 8.12 log CFU/g and 8.29 log CFU/g, respectively. Tekinşen and Özdemir (8) reported that *S. aureus* and *E. coli* were present in extremely high numbers in Herby cheeses, with average value of 6.10 and 3.68 log CFU/g, respectively.

CONCLUSION

Herbs used in the production of the cheeses led to increases in WSN, TCA-SN, PTA-SN, ADV, pH and titratable acidity values. Maximum values of

Table 3. Effect of Herbs on the Organic Acids Content of Herby Cheese

		Storage Period (day)			
Organic acids (mg/kg)	Sample	0	30	60	90
Citric acids	1	4560.00±1927.57^{aA}	5508.00±1571.19^{aA}	5360.00±1414.21^{abA}	6123.00±230.52^{bcA}
	2	5322.00±1738.06^{aA}	5074.00±1312.39^{aA}	3998.00±285.67^{bA}	4296.00±436.99^{cA}
	3	5240.00±1480.68^{aA}	4991.00±992.78^{aA}	7298.00±421.44^{aA}	7781.00±1166.73^{abA}
	4	5061.00±2542.76^{aB}	5185.00±1227.54^{aB}	7729.00±1035.20^{aAB}	9908.00±1288.35^{aA}
Lactic acids	1	9031.00±434.16^{aC}	20661.00±1797.46^{aB}	23854.00±1432.60^{abAB}	26443.00±2218.90^{bcA}
	2	9382.00±937.62^{aC}	20395.00±1975.66^{aB}	21894.00±1391.59^{bB}	27077.00±1508.97^{abA}
	3	10225.00±489.32^{aD}	21436.00±919.24^{aC}	27558.00±817.41^{aB}	31225.00±1735.24^{aA}
	4	10251.00±1578.26^{aB}	19981.00±1458.05^{aA}	20764.00±2604.98^{bA}	22229.00±438.41^{cA}
Acetic acids	1	ND	ND	ND	ND
	2	ND	ND	597.00±8.48^{cB}	756.00±100.41^{cA}
	3	ND	ND	1921.00±328.10^{aA}	2549.00±414.36^{bA}
	4	ND	ND	1291.00±272.94^{bB}	3597.00±520.43^{aA}

^{a,b,c}Different lowercase letters within a column and organic acid indicate significant differences between cheese samples (P<0.05).
^{A,B,C,D}Different uppercase letters within a row (sample) and organic acid indicate significant differences between ripening periods (P<0.05).
1: control cheese; 2: mendo added cheese; 3: heliz added cheese; 4: sirmo added cheese; ND: not detected.

Table 4. Effect of Herbs on the Fatty Acids of Herby Cheese

Fatty Acids (relative area)	Sample	Storage Period (day) 0	90
SCFA	1	5.06±1.29^{aA}	7.39±2.54^{aA}
	2	5.45±1.84^{aA}	6.70±0.56^{aA}
	3	6.50±2.33^{aA}	6.51±0.61^{aA}
	4	5.92±0.19^{aA}	7.00±0.15^{aA}
MCFA	1	19.24±0.48^{aA}	20.69±0.53^{aA}
	2	20.00±2.48^{aA}	18.10±0.82^{bA}
	3	19.21±0.21^{aA}	20.33±1.32^{abA}
	4	17.19±0.40^{aA}	20.62±1.20^{aA}
LCFA	1	75.70±1.77^{aA}	71.92±3.07^{aA}
	2	74.55±4.32^{aA}	75.20±1.38^{aA}
	3	74.28±2.12^{aA}	73.15±0.70^{aA}
	4	76.89±0.60^{aA}	72.38±0.66^{aA}
SFA	1	67.65±0.68^{aA}	69.44±1.41^{aA}
	2	68.05±2.07^{aA}	66.70±0.64^{aA}
	3	68.25±0.66^{aA}	68.94±0.89^{aA}
	4	66.10±0.54^{aA}	68.94±0.75^{aA}
MUFA	1	27.30±0.52^{aA}	25.92±1.29^{bA}
	2	27.05±1.80^{aA}	28.17±0.32^{aA}
	3	26.86±0.50^{aA}	26.28±0.36^{aA}
	4	28.61±0.38^{aA}	26.33±0.38^{aA}
PUFA	1	5.05±0.16^{aA}	4.63±0.11^{aA}
	2	4.89±0.26^{aA}	5.12±0.32^{aA}
	3	4.89±0.16^{aA}	4.78±0.15^{aA}
	4	5.29±0.16^{aA}	4.73±0.13^{aA}

^{a}Different lowercase letters within a column and item indicate significant differences between cheese samples (P<0.05).
^{A}Different uppercase letters within a row (sample) and item indicate significant differences between ripening periods (P<0.05).
1: control cheese; 2: mendo added cheese; 3: heliz added cheese; 4: sirmo added cheese; SCFA: short chain fatty acids; MCFA: medium chain fatty acids; LCFA: long chain fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

these parameters were obtained in sirmo-added cheese. No significant difference in terms of histamine and tyramine contents of cheese samples was determined; only phenylethylamine contents of cheeses that were produced by using heliz and sirmo increased significantly at the end of the ripening period.

Citric, acetic, and lactic acid concentrations increased in all cheese samples during storage. However, organic acid variations were different among the samples. The highest values for citric and acetic acids were determined in sirmo-added samples; while highest lactic acid values were in heliz-added cheese samples. Total aerobic mesophilic bacteria, lactic acid bacteria, and coliform bacteria counts decreased significantly in all groups during storage. Coliform bacteria counts decreased to an undetectable level only in mendo-added cheese samples by the end of the storage period. Herb addition had no effect on fatty acids profiles of cheeses. However, some biogenic amines and organic acids levels and microbiological and ripening criteria of the cheeses were influenced by herbs.

Funding

This research has been funded by the Scientific Research Projects Foundation of Yüzüncü Yıl University (Project No. 2008-ZF-B071).

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Table 5. Effect of Herbs on the Microbiological Characteristics of Herby Cheese

Microorganisms	Sample	Storage Period (day) 0	30	60	90
Total aerobic mezofilic bacteria (log_{10} CFU/g)	1	8.52 ± 0.19^{aA}	7.13 ± 0.01^{cC}	7.75 ± 0.06 ^{aB}	7.40 ± 0.08^{aC}
	2	8.63 ± 0.03^{aA}	7.00 ± 0.01^{cC}	7.47 ± 0.24 ^{aB}	7.04 ± 0.19^{bC}
	3	8.37 ± 0.07^{aA}	7.36 ± 0.08^{bC}	7.02 ± 0.09^{bD}	7.58 ± 0.01^{aB}
	4	8.65 ± 0.25^{aA}	7.58 ± 0.07^{aB}	6.99 ± 0.12^{bC}	7.57 ± 0.02^{aB}
Lactic acid bacteria (log_{10} CFU/g)	1	8.41 ± 0.15^{bcA}	7.64 ± 0.15^{aB}	7.44 ± 0.14^{bB}	6.83 ± 0.18^{cC}
	2	8.65 ± 0.02^{abA}	7.69 ± 0.01^{aB}	8.92 ± 0.27^{aA}	7.94 ± 0.13^{aB}
	3	8.35 ± 0.10^{cA}	7.62 ± 0.02^{aBC}	7.78 ± 0.08^{bB}	7.54 ± 0.02^{bC}
	4	8.82 ± 0.03^{aA}	7.86 ± 0.21^{aB}	7.64 ± 0.06^{bB}	7.57 ± 0.01^{bB}
Coliform bacteria (log10 CFU/g)	1	3.66 ± 0.07^{abA}	3.28 ± 0.34^{aB}	2.96 ± 0.16^{aBC}	2.58 ± 0.16^{aC}
	2	3.58 ± 0.02^{bA}	3.05 ± 0.14^{aB}	ND	ND
	3	3.57 ± 0.02^{bA}	3.24 ± 0.05^{aB}	3.16 ± 0.02^{aB}	2.15 ± 0.21^{aC}
	4	3.81 ± 0.13^{aA}	3.50 ± 0.06^{aAB}	3.10 ± 0.14^{aB}	2.23 ± 0.34^{aC}

^{a,b,c} Different lowercase letters within a column and item indicate significant differences between cheese samples (P<0.05).
^{A,B,C,D} Different uppercase letters within a row (sample) and item indicate significant differences between ripening periods (P<0.05).
1: control cheese; 2: mendo added cheese; 3: heliz added cheese; 4: sirmo added cheese; ND: not detected.

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