

Some Physico-chemical Properties and Organic Acid Profiles of Herby Cheeses

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Summary

The aim of this study was to determine the effects of different herbs on the physicochemical properties and organic acid profiles of Herby cheeses. Five batches of cheese were manufactured: CC, cheese without herb; CH, cheese containing Helis (*Ferule* sp.); CK, cheese containing Kekik (*Thymus* sp.); CS, cheese containing Sirmo (*Allium* sp.) and CM, cheese containing Mendo (*Anhriscus* sp.). Analyses were performed for total solids, ash, fat, salt and protein contents on the first day of production while titratable acidity, pH and organic acid profiles were carried out on days 1, 7 and 14, 21 and 28 of storage. The results indicated that the herbs influenced significantly ($P<0.01$) all parameters investigated of the Herby cheeses during storage. Lactic, acetic and butyric acid contents increased during storage, while orotic, citric, pyruvic and propionic acid values decreased in all samples including control.

Keywords: Herby cheese, Organic acid, Storage period

Otlu Peynirlerin Bazı Fizikokimyasal Özellikleri ve Organik Asit Profilleri

Özet

Bu çalışmanın amacı, Otlu peynirin fizikokimyasal özellikleri ve organik asit profili üzerine farklı otların meydana getirdiği etkiyi saptamaktır. Peynir, beş grup halinde üretilmiştir: (Otsuz üretilen peynir; CH, Helis içeren peynir (*Ferule* sp.); CK, Kekik içeren peynir (*Thymus* sp.); CS, Sirmo içeren peynir (*Allium* sp.) ve CM, Mendo içeren peynir (*Anhriscus* sp.)). Örneklerin toplam kuru madde, kül, yağ ve protein içerikleri depolamanın 1. gününde belirlenmiş, titrasyon asitliği, pH ve organik asit profili ise depolamanın 1, 7, 14, 21 ve 28. günlerinde gerçekleştirilmiştir. Elde edilen sonuçlar, kullanılan otların depolama süresince Otlu peynirin tüm parametreleri üzerinde önemli ölçüde ($P<0.01$) etkili olduğunu göstermiştir. Örneklerin laktik, asetik ve bütirik asit içerikleri depolama süresince artarken orotik, sitrik, pürivik ve propiyonik asit değerleri ise kontrol dahil tüm örneklerde düşüş göstermiştir.

Anahtar sözcükler: Otlu peynir, Organik asit, Depolama

INTRODUCTION

Herby cheese, a traditional Turkish cheese type, is generally produced by adding some aromatic herbs, which are well known for many years in Turkey¹. Recent years, it has been not only produced in small-scale businesses with traditional methods but also in well-equipped factories. Even though more than 20 kinds of herbs have been used for the production, the most used kinds are Sirmo (*Allium* sp.), Kekik (*Thymus* sp.), Helis (*Ferule* sp.), Mendo (*Anhriscus* sp.), Cünk (*Rannunculus* sp.), Dereotu (*Anethum* sp.), Nane (*Mentha* sp.)². The herbs have usually the flavors characteristics in the spices. They

are harvested in their vegetative period of the springtime and usually added into the cheeses with two ways. In the first way, the herbs are used freshly after washed and sliced. The second way is to make pickle. For later way, after harvesting, herbs are washed and sliced, then put into the brine with 16% salt. After about 20 days they are ready to add into the cheese. They can also be stored for long time and can be used whenever needed³. Some authors⁴⁻⁶ reported that the herbs have the flavoring and preservative functions during storage. Numerous researches have already been carried out



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on Herby white pickled cheese⁵⁻⁹ and Herby cacik^{10,11}, but there is no any study about the overall composition and the changes of organic acids during the storage time of Herby cheese. The aim of this study was to determine the compositional properties and the changes of organic acids of Herby cheese during storage period.

MATERIAL and METHODS

Materials

The milk used in this study was obtained from the pilot milk-processing plant of the Agricultural Collage of Atatürk University. Jars with 100 g capacity for packaged of Herby cheeses were purchased from local markets of Erzurum, Turkey. Sirmo (*Allium* sp.), Mendo (*Anhriscus* sp.), Helis (*Ferule* sp.) and Kekik (*Thymus* sp.) purchased from a cheese shopping center of Van City. While Sirmo and Mendo herbs were obtained in pickled form, Kekik and Helis were taken dry form from local markets in Van, Turkey.

Methods

To produce Herby cheese, a total amount of 75 kg whole-fat milk was used. Firstly, milk was left over a day for turn to sour at room temperature. Afterwards sour milk was heated to boiling point (90-95°C, 30 min). Then it was gently and continuously stirred and avoided from the boiling rapidly. Milk coagulation occurred gradually at 85-90°C then curd was cooled to 25-30°C. Then curds were collected in handle colander and transferred to cloth bag. The mouth of the cloth bag was tied and the bag was hung up to drain excess water. After the whey removed, cheeses were divided into 5 equal parts (CC, cheese without herb; CH, cheese containing Helis (*Ferule* sp.); CK, cheese containing Kekik (*Thymus* sp.); CS, cheese containing Sirmo (*Allium* sp.) and CM, cheese containing Mendo (*Anhriscus* sp.) and each herb was added to the curds at a ratio of 2% except control group and salted with high microbiologically and chemically qualified salt. For analyses, control and herby cheeses groups were filled into the sterile jars of approximately 200 g capacity and stored at 4±1°C for 28 days. The analyses were performed after 1, 7, 14, 21 and 28 days of storage. Two experiments were carried out, and results were given as the means of these experiments.

Physico-chemical Analysis

Dry matter and ash contents of cheeses were determined by the gravimetric method and total nitrogen by Kjeldahl method as described by IDF^{12,13}. Fat content was analyzed by the Gerber method as described by Kurt *et al.*¹⁴. For pH measurement, 10 g cheese sample was weighted and diluted with 20 mL of distilled water and was measured using a digital pH meter (WTW 340-1)¹⁵. Titratable acidity was determined as lactic acid percentage by titrating with 0.1 N NaOH. For salt analysis, 5 g sample was weighted and diluted with distilled water at 80°C and filtered from filter paper. Then 25 mL filtrate

was titrated with 0.1 N AgNO₃ using potassium chromate as indicator¹⁴.

Organic Acid Concentrations

The quantification of organic acids of Herby cheeses were analyzed by high-performance liquid chromatography (Agilent HPLC 1100 series G 1322 A, Germany) according to the modified methods described by Fernandez-Garcia and McGregor¹⁶ and Akalin *et al.*¹⁷. For the extraction of organic acids, 4 g of Herby cheese sample was diluted with 25 mL 0.001 N H₂SO₄ and centrifuged at the 5.000xg for 10 min. The supernatant was filtered through Whatman No.1 filter paper and through a 0.45 µm membrane filter (PALL, USA), and 2 mL aliquots were stored in HPLC vials at -20°C until HPLC analysis. The degassed mobile phase of 0.001 N H₂SO₄ was used at a flow rate of 0.6 mL/min. The wavelength of detection was optimized at 210 nm for quantification of organic acids. Duplicate injections (about 10 µl) were performed for all samples. The standard solutions of orotic, pyruvic, citric, lactic, acetic, butyric and hippuyric acids were prepared in distilled water to establish the elution times and calibration curves.

Statistical Analysis

The study was designed according to randomized complete block design by 5 (herbs) x 5 (storage period) factorial experiments. All statistical analyses were performed with SPSS 13.0 for Windows¹⁸. Differences between means were compared by Duncan's multiple range tests (P<0.05, P<0.01). The data analyzed are presented as the mean ± standard deviation (mean ± SD).

RESULTS

General Composition

The mean values of total solids, ash, fat, salt and protein contents of Herby cheeses are showed in [Table 1](#). When considering the general composition of the experimental cheeses, the obtained results were as expected.

The highest mean value of titratable acidity (0.28±0.06%) was found in CS and the lowest mean value (0.25±0.03%) in CK ([Table 2](#)) and the differences among the samples were determined statistically significant (P<0.01).

During storage period, titratable acidity values of Herby cheeses were lower than that of the control at the beginning of storage period. After 7 days, a higher increase was observed in the herby samples than the control in terms of titratable acidity. Additionally, titratable acidity contents of all samples including control reached the highest value on 14th day of storage in all groups. Then, a decrease was observed in varying rates between 14th and 21th days of storage. At the end of storage, a rise was again determined ([Fig. 1](#)).

The highest mean value of pH (5.82±0.07) were found in CC and CK and the lowest mean value was (5.79±0.07) in

Table 1. Some physicochemical properties of herby cheeses (mean±SD)**Tablo 1.** Otlu peynirlerin bazı fizikokimyasal özellikleri (ortalama±SD)

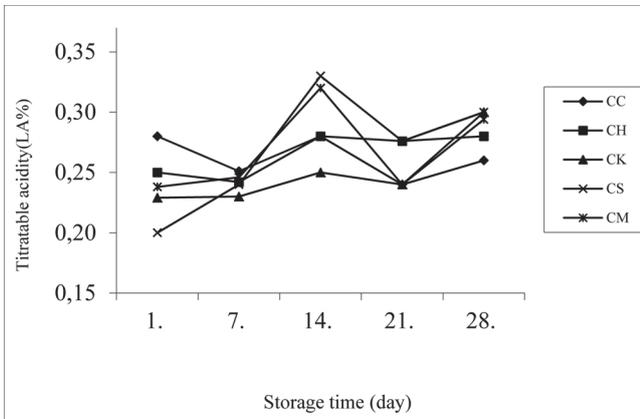
Group	Total Solids (%)	Ash (%)	Fat (%)	Salt (%)	Protein (%)
CC (Control)	58.15±0.28	5.25±0.57	21±1.15	3.63±0.14	35.48±3.52
CH	57.76±0.63	4.92±0.17	21.50±0.41	2.98±0.22	29.16±1.01
CK	58.10±0.43	4.42±0.44	21.75±1.26	2.75±0.35	29.53±0.48
CS	55.03±0.46	3.61±0.10	21.50±0.58	1.81±0.22	27.10±0.45
CM	53.12±1.92	3.87±0.29	21.00±1.15	2.57±0.19	21.72±0.77

CC: Cheese with no added herb (Control), CH: Cheese with Helis (*Ferule sp.*), CK: Cheese with Kekik (*Thymus sp.*), CS: Cheese with Sirmo (*Allium sp.*), CM: Cheese with Mendo (*Anhriscus sp.*)

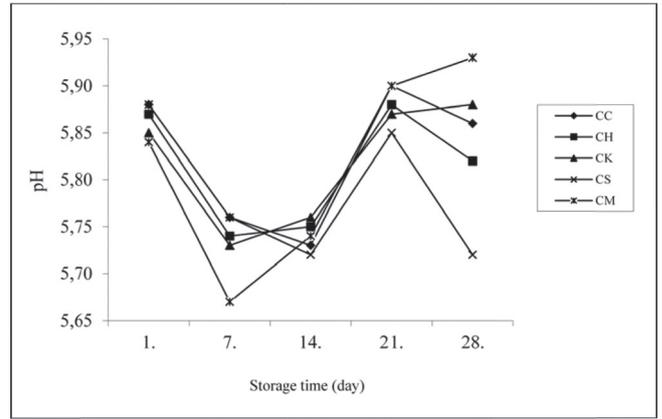
Table 2. Titratable acidity and pH values of herby cheeses (mean±SD)**Tablo 2.** Otlu peynirlerin titrasyon asitliği ve pH değerleri (ortalama±SD)

Group	Titratable Acidity (LA%)	pH
CC (Control)	0.27±0.02 ^{b*}	5.82±0.07 ^a
CH	0.26±0.02 ^{bc}	5.81±0.06 ^a
CK	0.25±0.03 ^c	5.82±0.07 ^a
CS	0.28±0.06 ^a	5.79±0.07 ^b
CM	0.26±0.04 ^{bc}	5.81±0.11 ^a

CC: Cheese with no added herb (Control), CH: Cheese with Helis (*Ferule sp.*), CK: Cheese with Kekik (*Thymus sp.*), CS: Cheese with Sirmo (*Allium sp.*), CM: Cheese with Mendo (*Anhriscus sp.*), * Different letters in the same row represent significant differences at P<0.01

**Fig 1.** Titratable acidity values of the herby cheeses during storage

Şekil 1. Otlu peynirlere ait titrasyon asitliği değerlerinin depolama süresince değişimi

**Fig 2.** pH values of the herby cheeses during storage

Şekil 2. Otlu peynirlere ait pH değerlerinin depolama süresince değişimi

CS. There were no significant ($P>0.05$) differences between the groups except cheese with Sirmo (*Allium sp.*) in terms of pH value (Table 2). The highest value was observed in the CM while the lowest value was found in the CS at the end of storage (Fig. 2).

Organic Acid Concentrations

In this research, seven different organic acids were determined in the cheese samples during storage period.

The highest mean value of orotic acid (1.93 ± 0.097) was found in CC and the lowest mean value (0.77 ± 0.32) was in CM. Differences among the samples were significant ($P<0.01$) statistically (Table 3).

Table 3. Organic acid profiles of Herby cheeses (mean±SD)**Tablo 3.** Otlu peynirlerin organik asit düzeyleri (ortalama±SD)

Group	Orotic Acid	Citric Acid	Pyruvic Acid	Lactic Acid	Acetic Acid	Propionic Acid	Butyric Acid
CC (Control)	1.93±0.097 ^{ab*}	77.49±5.23 ^a	10.45±0.52 ^a	91.54±23.58 ^c	15.08±4.76 ^d	47.05±25.00 ^d	30.65±4.15 ^b
CH	1.25±0.32 ^a	74.29±11.30 ^a	9.18±0.99 ^b	145.76±33.86 ^b	115.13±57.67 ^a	87.94±15.95 ^a	30.15±5.85 ^b
CK	1.07±0.19 ^{ab}	68.07±18.39 ^b	8.06±1.68 ^c	227.27±152.13 ^a	65.55±74.24 ^b	77.17±27.88 ^b	56.05±25.77 ^a
CS	1.03±0.09 ^b	55.68±17.40 ^b	9.44±0.85 ^b	196.12±130.72 ^a	41.55±19.77 ^c	17.58±11.09 ^e	45.19±22.59 ^a
CM	0.77±0.32 ^c	50.55±17.56 ^c	8.55±1.35 ^b	142.36±30.99 ^b	18.92±8.76 ^d	60.22±29.13 ^c	58.88±36.47 ^a

CC: Cheese with no added herb (Control), CH: Cheese with Helis (*Ferule sp.*), CK: Cheese with Kekik (*Thymus sp.*), CS: Cheese with Sirmo (*Allium sp.*), CM: Cheese with Mendo (*Anhriscus sp.*), * Different letters in the same row represent significant differences at P<0.01

Changes of orotic acid concentrations in samples during storage are shown in Fig. 3. In this experiment, the orotic acid value decreased after 14th day of storage until not detectable level. This could be attributed to the storage time.

The mean values of citric acid in the cheese samples changed between 77.49 ± 5.23 and 50.55 ± 17.56 $\mu\text{g/g}$. When the herby samples compared to the control, differences between the experiments were statistically significant ($P < 0.01$) except for CH sample in terms of citric acid level (Table 3). As seen from Fig. 4, citric acid contents in all samples decreased after 14th day of storage.

In groups, the mean concentrations of pyruvic acid changed between 10.45 ± 0.52 and 8.06 ± 1.68 $\mu\text{g/g}$. Differences between the control and herby samples were found to be statistically significant ($P < 0.01$) (Table 3). On the other hand, pyruvic acid values generally showed an irregular change during storage period (Fig. 5).

The mean lactic acid values of cheeses ranged from

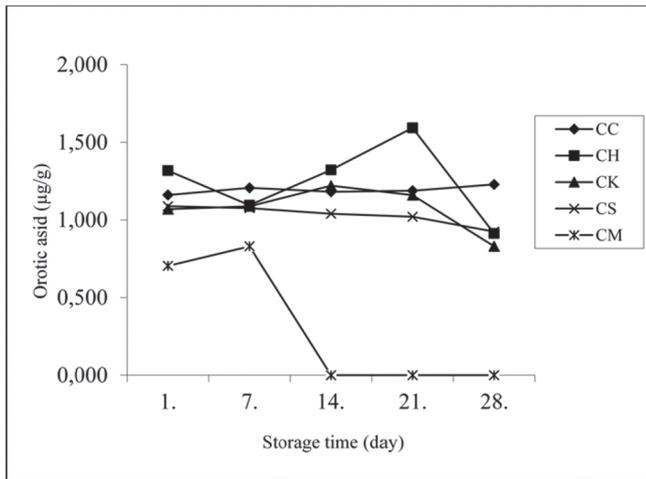


Fig 3. Orotic acid levels of the herby cheeses during storage

Şekil 3. Otlu peynirlere ait orotik asit düzeylerinin depolama süresince değişimi

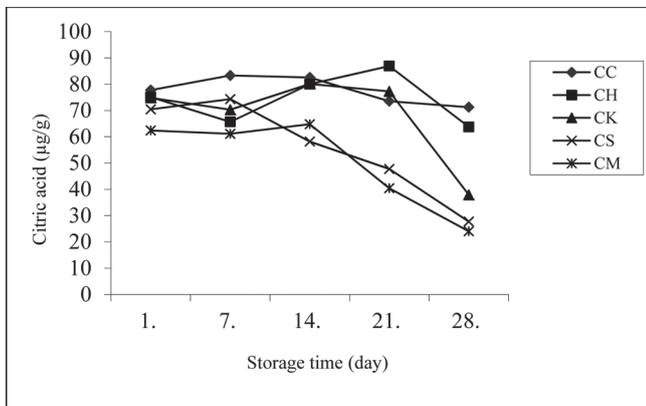


Fig 4. Citric acid levels of the herby cheeses during storage

Şekil 4. Otlu peynirlere ait sitrik asit düzeylerinin depolama süresince değişimi

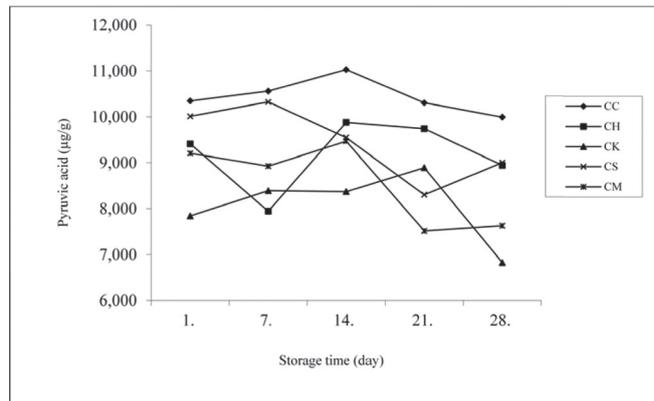


Fig 5. Pyruvic acid levels of the cheeses during storage

Şekil 5. Otlu peynirlere ait pirüvik asit düzeylerinin depolama süresince değişimi

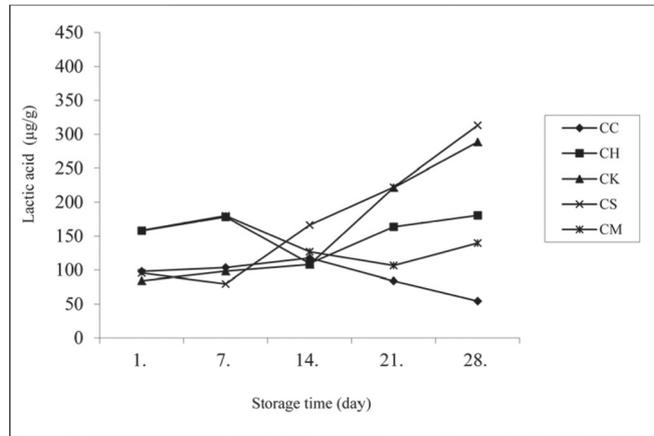


Fig 6. Lactic acid levels of the herby cheeses during storage

Şekil 6. Otlu peynirlere ait laktik asit düzeylerinin depolama süresince değişimi

227.27 \pm 152.13 to 91.54 \pm 23.58 $\mu\text{g/g}$. According to statistical evaluations, CM and CH cheeses were similar to each other, and CS and CK cheeses showed a similar trend with respect of statistical evaluations. In contrary, control cheese differed from the herby samples ($P < 0.01$) (Table 3). On the other hand, lactic acid contents of all samples increased during storage period except for control (Fig. 6).

The mean acetic acid values of the groups ranged from 15.08 ± 4.76 to 115.13 ± 57.67 $\mu\text{g/g}$. The levels of acetic acid were higher in the herby cheeses compared with the control sample and this was found to be significant statistically ($P < 0.01$) (Table 3). Acetic acid concentrations of cheeses were shown in Fig. 7.

The amounts of propionic acid in cheeses changed between 17.58 ± 11.09 and 87.94 ± 15.95 $\mu\text{g/g}$, and a variation was observed among the experiments in terms of propionic acid levels in herby samples and control, these differences were significant ($P < 0.01$) statistically (Table 3). Propionic acid contents of cheeses were shown in Fig. 8.

The lowest mean value (30.15 ± 5.85 $\mu\text{g/g}$) of butyric acid

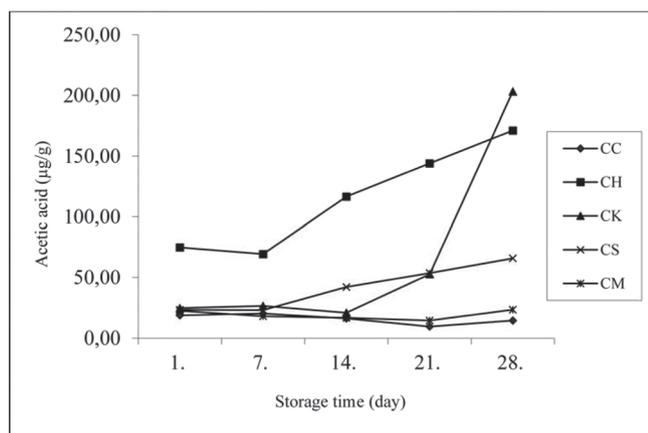


Fig 7. Acetic acid levels of the herby cheeses during storage

Şekil 7. Otlu peynirlere ait asetik asit düzeylerinin depolama süresince değişimi

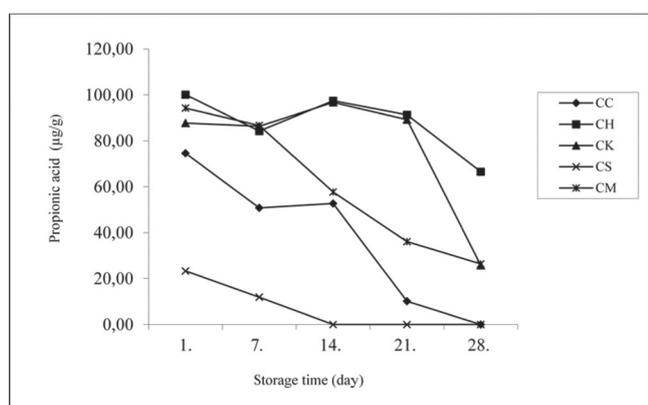


Fig 8. Propionic acid levels of the herby cheeses during storage

Şekil 8. Otlu peynirlere ait propiyonik asit düzeylerinin depolama süresince değişimi

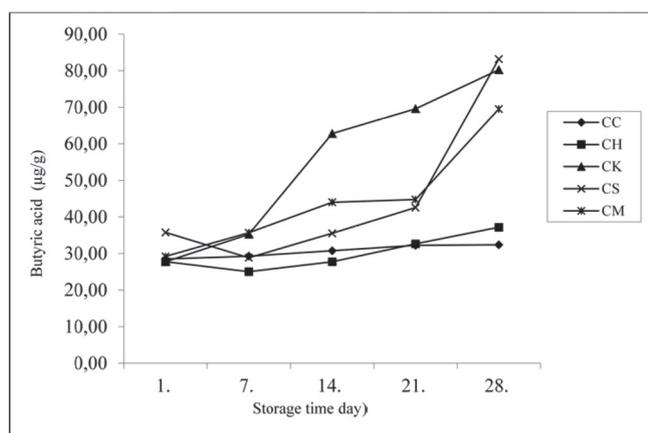


Fig 9. Butyric acid levels of the herby cheeses during storage

Şekil 9. Otlu peynirlere ait bütirik asit düzeylerinin depolama süresince değişimi

was found in cheese with helis (CH) and the highest mean value ($58.88 \pm 36.47 \mu\text{g/g}$) was found in cheese with mendo (CM). On the other hand, differences among the samples were found statistically significant ($P < 0.01$) (Table 3). Changes of

butyric acid concentrations in samples during storage were shown in Fig. 9.

DISCUSSION

Titrateable Acidity and pH

The titrateable acid values of cheese samples were affected significantly ($P < 0.01$). This could be attributed to the characteristics of herbs used. Zaika and Kissinger¹⁹ reported that some herbs and spices influence the growth and activities of lactic acid bacteria at different levels.

Differences between titrateable acidity values of the experimental cheeses during storage can be explained by the stimulating effects of the herbs on lactic acid bacteria. Bakirci²⁰ suggested that nitrogenous compounds and carbohydrates in herbs might serve as additional source of carbon and nitrogen for nonstarter lactic acid bacteria (NSLAB) and contribute to the activity of the cultures. A similar titrateable acidity pattern was also reported by Tarakci⁸ and Şenel²¹ for herby cheese.

The effects of herbs on pH values were similar to control (CC) during storage. This situation can be explained with alkaline compounds forming as a result of proteolytic degradation, yeast and moulds activities and compositional characteristics of herbs²²⁻²⁵.

Organic Acid Concentrations of Herby Cheeses

Organic acids are the major products of carbohydrate catabolism of lactic acid bacteria. They contribute to cheese quality, playing an integral role in flavor²⁶ and affect the flavors of most mature cheeses. They are formed as a result of hydrolysis of milk fat during lipolysis, bacterial growth, normal ruminant metabolic processes or addition of acidulants in cheese making^{16,27-30}. Quantitative determination of organic acids is an important tool for flavor and nutritional quality as well as being an indicator of bacterial activity in mature cheeses, as the total aroma intensity is correlated with organic acid levels in grading cheeses^{31,32}.

Differences among the samples were found statistically significant ($P < 0.01$). This might be explained by the properties of herbs used. The effects of herbs on the orotic acid were similar except CM.

Larson and Hegarty³³ indicated that the orotic acid levels in cheese products showed wide variations. The authors stated that washing of curd and fermentation degree effect the orotic acid level in cheese and suggested that the mature cheeses contain the lowest level of orotic acid. Also, Fernandez-Garcia and McGregor¹⁶; Tormo and Izco³⁰; Güzel-Seydim *et al.*³⁴; Kristo *et al.*³⁵ suggested that orotic acid concentrations of milk and milk products reduced to the levels of 45-48% during fermentation and storage.

Citric acid is the most abundant organic acid present in

raw milk and is available at the level of 0.2% concentration averagely^{30,36}. Lactic acid bacteria produce diacetyl from citric acid in milk by using the pyruvate that occurred as an intermediate product during fermentation^{37,38}. Citric acid is not the first energy source of bacteria, but can be metabolized very rapidly by *Lactococcus lactis* subsp. *diacetylactis* or *Leuconostoc* spp. in cheese³⁰. In all samples, citric acid concentrations decreased after 14th day of storage. This can be explained by the fermentation of citrate to some organic acids including acetic acid, propionic acid and volatile compounds^{39,40}. Similar results were reported by Ong and Shah⁴¹ for cheddar cheese.

Pyruvic acid occurs as an intermediate product of protein and carbohydrate metabolism. Firstly, pyruvic acid is formed from lactose by bacterial fermentation, then this product is converted to the lactic acid and other metabolites by an enzyme series^{34,36}. Pyruvic acid concentrations of samples showed an increases and decreases during storage. Because pyruvate is readily formed through the glycolytic pathway. Also, it acts as a substrate of several metabolic reactions such as the formation of formic acid, ethanol, diacetyl, acetoin and 2,3-butylene glycol⁴².

Formation of lactic acid is essential for flavor development and keeping quality of cheeses. Generally lactic acid concentrations of samples showed an increase during storage period except for control sample. Control cheese showed statistically important differences ($P < 0.01$) from herby cheese samples. This was probably due to the effects of herbs, which are known a stimulating effect towards the lactic acid production¹⁹. Lactic acid concentrations of samples increased except for control during storage and the highest value was observed in the cheese with sirmo (CS), the lowest value was found in control sample (CC) at end of the storage.

Acetic acid is one of the important flavor compounds in many cheeses²⁶. Acetic acid can be produced from citrate, lactose and amino acids⁴³. In general, it gives vinegar taste and aroma to the product. Therefore, the acceptability of the products shows a very rapid decline during storage⁴⁴. The acetic acid contents of CK, CH and CS samples increased during storage time, while CC and CM samples remained relatively constant during this period (Fig. 7). A similar result was reported by Bouzas *et al.*³⁹ for acetic acid content of Cheddar cheese.

Propionic acid bacteria are used in dairy industry during Emmental type cheese maturation to produce CO₂, volatile fatty acids and diacetyl that represent characteristic flavours of this cheese⁴⁵. In addition, it is well known that the propionic acid fermentation leads to characteristic eyes and nutty flavour to the cheese⁴⁶. Among samples, significant differences were observed in terms of propionic acid concentrations ($P < 0.01$). This was probably due to the inhibitory effect of herbs on propionic acid-producing bacteria⁴⁷. It was observed a decline in all samples in terms of propionic acid contents during storage, but the sharply decrease was seen in

the cheese containing sirmo (Fig. 8). This situation can be explained by the result of the lipolytic and proteolytic activities of non-starter bacteria³¹.

Butyric acid appears in cheeses as a result of, either lipolysis or deamination of amino acids^{36,37}. The butyric acid contents of herby samples were higher than that of control sample. This was presumably due to the stimulating effect of herbs used. In addition, the butyric acid contents of samples progressively increased during storage, but the increase in control and CH samples became a slower rate during the storage (Fig. 9).

This study suggested that the use of some herbs in herby cheese affected the physicochemical properties and organic acid composition of cheeses at different levels. It was seemed that titratable acidity values of Herby cheeses were higher than that of control depending on the herb types. Titratable acidity values of all cheeses increased until 14 day of storage, then an irregularly change was determined. PH values of the cheeses showed a similar trend in all experiments including control during storage. Orotic, citric, pyruvic and propionic acid values of herby samples were lower than that of control, and these values decreased after 14th day of storage in all cheeses. On the contrary, lactic, acetic and butyric acid values generally increased after 14th day of storage in herby cheeses, but decreased in control and some herby samples. It was concluded that further studies are essential to evaluate the effects of herbs on the properties of this type cheeses.

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