

QUALITY CHARACTERISTICS OF TRADITIONAL SEPET CHEESE

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**by
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ABSTRACT

QUALITY CHARACTERISTICS OF TRADITIONAL SEPET CHEESE

Investigation and registration of traditional foods contributes to the improvement of economy and the continuation of important elements of a nation's culinary heritage, culture. Various types of traditional cheese are present in different shape, color, taste in the world. Sepet cheese is one of the traditional cheeses produced in the Aegean region.

In this study, chemical, physical, microbiological, organoleptic, and aroma characteristics of traditional sepet cheeses were investigated. The changes in the quality characteristics were examined during production and ripening periods. The natural lactic acid bacteria flora of sepet cheeses during production and ripening was identified with phenotypic methods.

As a result of chemical analysis, average chemical characteristics of traditional sepet cheeses were found as 54.33 ± 5.17 total solid content, 0.82 ± 0.05 water activity, 25.11 ± 2.86 fat content, 5.58 ± 0.43 pH, 28.99 ± 2.12 protein content. According to microbiological analysis of sepet cheeses, average total aerobic, lactococci, lactobacilli, enterococci, psychrotrophic bacteria, *Staphylococcus aureus*, yeast, mold, coliform bacteria counts were 7.64 ± 1.18 , 7.38 ± 1.10 , 7.38 ± 0.99 , 6.99 ± 0.99 , 5.37 ± 1.15 , 1.25 ± 1.72 , 3.22 ± 0.25 , 0.95 ± 0.96 , 2.72 ± 1.82 log cfu/g, respectively. During descriptive profile analysis, traditional sepet cheeses were described with free fatty acid, cooked, creamy, whey, animal like, sulfurous aromatic descriptives with high salty basic taste. Free fatty acids were found to be the most abundant volatile compounds of sepet cheeses and had the highest aroma intensities in volatile fraction. According to phenotypic identification, isolates were closely related to *Lactococcus lactis* subsp. *lactis*, *Lactobacillus casei* spp. *ramnosus*, *L. plantarum*, heterofermentative *Lactobacillus* spp., *Streptococcus thermophilus*, *Leuconostoc* spp., *Enterococcus durans* and *E. faecium*.

ÖZET

GELENEKSEL SEPET PEYNİRİNİN KALİTE KARAKTERİSTİKLERİ

Geleneksel gıdaların araştırılması ve tanımlanması, kültürel mirasın devamlılığına ve ekonomik gelişime katkı sağlar. Dünyada farklı renk, görünüş ve tada sahip birçok geleneksel peynir çeşidi vardır. Sepet peyniri, Ege bölgesinde üretilen geleneksel peynirlerden biridir.

Bu çalışmada, geleneksel sepet peynirlerinin kimyasal, fiziksel, mikrobiyolojik, duyuşsal ve aroma karakteristikleri araştırılmıştır. Peynir yapımı ve olgunlaşması sırasında kalite karakteristiklerinde meydana gelen değişimler incelenmiştir. Bu aşamalarda sepet peynirlerindeki doğal laktik asit bakterileri izole edilmiş ve fenotipik metodlarla tanımlanmıştır.

Araştırma sonucunda, ortalama kimyasal değerler; 54.33 %±5.17 kuru madde, 0.82±0.05 su aktivitesi, 25.11 %±2.86 yağ, 5.58±0.43 pH, 28.99 % ±2.12 protein olarak belirlenmiştir. Yapılan mikrobiyolojik analizler sonucunda, sepet peynirlerinin ortalama toplam aerobik mikroorganizma, laktokok, laktobasil, enterokok, psikrotrofik mikroorganizma, *Staphylococcus aureus*, maya, küf, koliform mikroorganizma sayımları sırasıyla 7.64±1.18 , 7.38±1.10, 7.38±0.99, 6.99±0.99, 5.37±1.15, 1.25±1.72, 3.22±1.25, 0.95±0.961, 2.72±1.82 log cfu/g olarak bulunmuştur. Lezzet profil analizinde, eğitimli panelistler sepet peynirlerini yüksek miktarda tuzlu temel tada sahip ransit, pişmiş, kremamsı, hayvanımsı ve sülfürümsü aromatik terimleriyle tanımlamışlardır. Uçucu madde analizi sonucunda, serbest yağ asitleri en yüksek oranda bulunmuştur. Serbest yağ asitlerinin, sepet peynirlerinin aromasını önemli derecede etkilediği belirlenmiştir. Fenotipik tanımlama sonuçlarına göre, izolatlar *Lactococcus lactis* subsp *lactis*, *L. plantarum*, *Lactobacillus casei* spp. *ramnosus*, heterofermantatif *Lactobacillus* spp., *Streptococcus thermophilus*, *Leuconostoc* spp., *Enterococcus durans* and *E. faecium* olarak tanımlanmıştır.

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CHAPTER 1

INTRODUCTION

Traditional foods include foods that have been consumed locally or regionally for many generations (European Commission 2007, EuroFIR 2009). The methods for preparation of these local foods have been passed down from generation to generation (European Commission 2007). Investigation and registration of traditional foods are important for the continuation of important elements of a nation's culinary heritage and culture. Unfortunately some traditional foods are threatened with extinction. Therefore, there is a real need to study traditional foods to preserve important elements of culture (EuroFIR 2009).

The traditional dairy products, especially cheese, have an important place in rural-region food culture (Dost, et al. 2004). Many cheeses are part of the country's culture. Many European cheeses are trademark protected and origin protected against imitations. These systems aim to provide a simple system for the protection of food names on a geographical or traditional basis. Well known origin protected cheeses are Parmigiano-Reggiano from Italy and Roquefort from France (Blom, et al. 2002). These systems also contribute to promotion and protection of regional food products.

Turkey has a high diversity of culture. Therefore, it has wide variety of traditional foods. In Turkey, approximately 50 types of cheeses are produced in rural region. Some of these cheeses are home-made and factory-made. However, some types are still produced in only limited areas and most of them are forgotten depending on the changes of socioeconomic status (Dost, et al. 2004). Investigation of production methods and their characteristics give chance to produce traditional cheeses in large scale and then export throughout the continent and protect diversity of tradition (Tan and Ertürk 2002).

Objective of this study is to investigate the chemical, physical, microbiological, organoleptic, and aroma characteristics of traditional sepet cheeses which is important for economical aspects and continuation of cultural heritage.

CHAPTER 2

LITERATURE REVIEW

2.1. Milk

Milk is a fluid secreted by the female of all mammals and important for the function of meeting the complete nutritional requirements of the neonate of the species. It must supply energy, amino acids, vitamins, and minerals. Therefore, milk supplements good quality protein, calcium and vitamins, particularly vitamin A, riboflavin, niacin and folic acid, so it helps to balance human diet. Therefore, milk is an ideal food for both infants and adults (Tamime 2006). Moreover, many physiological functions are performed by milk constituents, milk contains several bio-protective molecule that ensure health security to humans including antimicrobial substances (immunoglobulins, lactoperoxidase, and lactotransferrin), enzymes and enzyme inhibitors, vitamin-binding carrier proteins (Fox, et al. 2000).

The utilisation of milk from different species depends on geographical conditions. There are more than 4,000 species (Fox, et al. 2000). The cow has long been the principal dairying species in many regions of the world. Buffaloes contribute significantly to milk production in the Indian subcontinent and Egypt. Sheep and goat are important in the Mediterranean regions, parts of the Middle East and some regions of Africa. The camel is an important source of milk in desert regions of North and East Africa, and the Middle East (Tamime 2006).

The composition of milk from milk producing species differs from each other. In addition to the species, geographical location and requirement for the neonates affect the milk composition (Tamime 2006). Also, genetic constitution of the individual species, age of the species, stage of lactation, number and time of milkings, and certain

disease conditions, seasons, motion influence the composition of milk (Üçüncü 2005). Table 1 shows the composition of milk from different species.

Sheep milk is rich in nutrients, having 18 percent total solids. These high-fat, high-protein milks make sheep milk good source for cheese and other manufactured dairy products (Üçüncü 2005).

Goat milk resembles with cow milk on the basis of nutrient composition. However, it differs in several characteristics. All the beta-carotene is converted to vitamin A in goat milk, so it is white. The fat globules are smaller and therefore remain suspended, but the fat content is about the same with cow milk. Goat milk curd forms into small, light flakes and is more easily digested, much like the curd formed from human milk. It is often suitable for people who are allergic to the proteins in cow milk and for some patients suffer from stomach ulcers (Üçüncü 2005).

Table 1. Chemical composition (g 100g⁻¹) of milk from different species.
(Source: Hurley 2009)

Species	Fat	Protein	Lactose	Minerals	Total solids
Antelope	1.3	6.9	4.0	1.3	25.2
Bison	1.7	4.8	5.7	0.96	13.2
Buffalo	10.4	5.9	4.3	0.8	21.5
Camel	4.9	3.7	5.1	0.7	14.4
Cow (Holstein)	3.5	3.1	4.9	0.7	12.2
Cow (Guernsey)	5.0	3.8	4.9	0.7	14.4
Cow (Jersey)	5.5	3.9	4.9	0.7	15.0
Dolphin	14.1	10.4	4.9	–	30.4
Goat	3.5	3.1	4.6	0.79	12.0
Donkey	1.2	1.7	6.9	0.45	10.2
Horse	1.6	2.7	6.1	0.51	11.0
Human	4.5	1.1	6.8	0.2	12.6
Pig	8.2	5.8	4.8	0.63	19.9
Reindeer	22.5	10.3	2.5	1.4	36.7
Seal	53.2	11.2	2.6	0.7	67.7
Sheep	5.3	5.5	4.6	0.9	16.3
Whale	34.8	13.6	1.8	1.6	51.2

2.2. Dairy Products

Milk is a nutritious food for human, but milk is also a suitable media for microorganisms and can spoil easily. Therefore, in order to increase its resistance and to obtain different nutritious dairy products, milk is processed into different products such as butter, cheese, dried milks, ice cream, and condensed milk.

Milk, cheese, and yogurt provide the following beneficial nutrients in varying quantities. They have calcium, phosphorous, magnesium, protein, vitamin B₁₂, vitamin A, zinc, riboflavin, folate, iodine, vitamin C and have health benefits such as healthy bones and teeth, energy release, healthy muscle function, growth and repair, production of healthy cells, good eyesight and immune function, good immune system, healthy skin, production of healthy cells, formation of healthy connective tissues, and regulation of the body's rate of metabolism, respectively (The Dairy Council 2009).

2.2.1. Production and Consumption of Dairy Products

In Turkey, milk processing plant has equipped with high technology in the last years. Also, there is an increase in the number of foreign direct investments and joint venture cooperation in dairy processing sector. Therefore, the number of processing plants, and the amount of milk produced and processed have increased during the last decade (Sarışağılı 2009).

In Turkey the raw milk is processed into pasteurized milk, butter, cheese, yogurt, ice cream, milk powder, etc. Table 2 shows the amount of dairy products processed in Turkey.

Gönç et al. (1993) declared that 40 % of the produced milk was consumed as raw milk, 50 % was processed in dairy farms and 10 % was processed in modern factory in Turkey. In developed countries only 0.5-0.6 % of produced milk is consumed as raw milk (Tan and Ertürk 2002).

Table 2. Turkey's milk supply and processed dairy products in 2004-2008 (Tonnes)
(Source: Sarısaçlı 2009).

	2004	2005	2006	2007	2008
Milk production	11,438,141	11,686,319	11,903,957	12,087,531	12,217,108
Dairy Products Import (*)	202	160	52	62	78
Total Supply	12,428,515	12,904,092	13,171,649	13,325,698	13,389,767
Fluid Milk	1,467,197	1,489,500	1,509,449	1,524,543	1,539,789
Cheese Production	6,427,236	6,519,205	6,740,434	6,862,745	6,953,125
Yoghurt Production	2,266,335	2,241,597	2,253,464	2,271,663	2,288,948
Butter Production	1,119,954	1,277,294	1,318,493	1,345,124	1,348,118
Dry Milk Production	85,254	83,596	82,118	83,456	87,128
Ice Cream Production	72,165	75,128	77,148	78,358	79,158

(*)Milk equivalent

In Turkey, 40 % of 11 million tonnes milk is used for cheese production (Tan and Ertürk 2002). World production of cheese is approximately 35 % of total milk production. Europe, with a production of roughly 8×10^6 tonnes per annum, has the largest production amount in the world. Table 3 shows the amount of cheese production in the world. Reasons of increasing consumption of cheese are the health benefits, convenience and flexibility in use, and the great diversity of flavors and textures. Therefore, cheese is the principal growth product within the dairy sector. Moreover, the most rapid growth in cheese consumption has occurred in its use as an ingredient (Fox, et al. 2000).

The most important traditional export commodity among Turkish dairy products is cheese. Total cheese export of Turkey was over 76 million US Dollars in 2008. Ice cream is the second important milk product exported from Turkey with 21 million US Dollars export in 2008. Whey powder, milk, cream and yogurt exports of Turkish dairy industry is another important dairy products for Turkish dairy industry. In 2008 total milk and cream exports reached to 17 million US Dollars and yogurt exports was 8,505 million US Dollars, in the same year (Sarısaçlı 2009).

Table 3. World production of cheese, 1994.
(Source: Fox, et al. 2000)

<i>Country</i>	<i>Cheese Production (1,000 Tonnes)</i>	<i>Country</i>	<i>Cheese Production (1,000 Tonnes)</i>	<i>Country</i>	<i>Cheese Production (1,000 Tonnes)</i>
World	15,084	Colombia	51	Bulgaria	72
		Ecuador	7	Croatia	21
Africa	511	Peru	6	Czech Republic	139
Algeria	1	Uruguay	23	Denmark	291
Angola	1	Venezuela	70	Estonia	18
Botswana	2			Finland	89
Egypt	349	Asia	1,018	France	1,605
Ethiopia	3	Afghanistan	16	Germany	1,569
Mauritania	2	Armenia	9	Greece	216
Morocco	7	Azerbaijan	7	Hungary	88
Niger	14	Bangladesh	1	Iceland	3
Nigeria	7	China	206	Ireland	91
South Africa	37	Cyprus	5	Italy	1,017
Sudan	76	Georgia	3	Latvia	11
Tanzania	2	Iran	197	Lithuania	27
Tunisia	6	Iraq	17	Macedonia, FYR of	1
Zambia	1	Israel	92	Moldova Republic	3
Zimbabwe	2	Japan	114	Netherlands	688
		Jordan	4	Norway	89
North and Central America	4,130	Kazakhstan	34	Poland	397
Canada	323	Kyrgyzstan	5	Portugal	65
Costa Rica	6	Lebanon	15	Romania	42
Cuba	15	Mongolia	1	Russian Federation	477
Dominican Republic	3	Myanmar	29	Slovakia	44
El Salvador	3	Syria	86	Slovenia	16
Guatemala	11	Tajikistan	1	Spain	163
Honduras	8	Turkey	139	Sweden	128
Mexico	123	Turkmenistan	13	Switzerland	133
Nicaragua	6	Uzbekistan	14	United Kingdom	385
Panama	7	Yemen	10	Ukraine	71
United States	3,627			Yugoslavia, FR	14
		Europe	8,201		
South America	677	Albania	1	Oceania	544
Argentina	405	Austria	102	Australia	270
Bolivia	7	Belarus	39	New Zealand	274
Brasil	60	Belgium- Luxembourg	75		
Chile	51	Bosnia- Herzgovina	14		

2.3. Cheese

Cheese is a popular manufactured food product. Cheese making started out as an accidental curdling of milk. It is commonly believed that cheese evolved in the Fertile Crescent between the Tigris and Euphrates rivers, in Iraq, some 8,000 years ago, during Agricultural Revolution (Fox, et al. 2000). It is stated that the Turks knew how to make cheese before they migrated to Anatolia. Uighur Turks (750 A. D.) used words meaning cheese for the first time. In the “Divani Lugat-it-Turk”, udhitma was used which means fresh cheese (derived from udhit; meaning to make cheese, to settle, to solidify). As for “peynir or penir”, this word is encountered for the first time in Mamluk culture and Turks learned this word from Persian at the time of the migration of the Turks from Middle Asia to Anatolia. The principal evidence for this is that in Dede Korkut’s tales, the main foodstuff of the soldiers of Atilla was cheese. Also, various cheeses are mentioned in Yusuf Has Hacib’s work “Kutadgu Bilig” and in the folk poet Karacaođlan’s poems the “peynir” used for cheese (Kamber 2008A).

Cheese is a nutrient-dense food. Cheese provides a high concentration of nutrients relative to its energy content. The nutritional composition of cheese depends on the type of milk used and the manufacturing and ripening procedures (Üçüncü 2004). When milk is made into cheese, casein and fat are concentrated, because they are retained in the curd during manufacture. Other milk components are mainly removed along with whey. Therefore, cheese contains relatively small amounts of the watersoluble constituents (whey proteins, lactose, and water-soluble vitamins), which partition mainly into the whey. None of the milk components is fully retained in cheese and new substances may be added, notably salt (Walstra, et al. 2006 , Fox, et al. 2000). Table 4 shows .Percentage of nutrients present in the curd and the whey during cheddar cheese manufacturing.

Fat has important functions in cheese. It affects cheese firmness, adhesiveness, mouth-feel, and flavor. The concentration of protein in cheese varies can be 3% to 40%, depending on the variety (Üçüncü 2004, Fox, et al. 2000).

Table 4. Partition of nutrients in milk in making cheddar cheese
(Source: Walstra, et al. 2006).

Nutrient	Percentage in Curd^a	Percentage in Whey^a
Water	6	94
Total Solids	48	52
Casein	96	4
Soluble Proteins	4	96
Fat	94	6
Lactose	6	94
Calcium	62	38
Vitamin A	94	6
Thiamin	15	85
Riboflavin	26	74
Vitamin C	6	84

^a Percentage of total in original milk at point of separation of curd and whey.

Casein is the main protein in cheese, although the water-soluble milk proteins lactalbumin and β -lactoglobulin may also be present, depending on the amount of whey entrapped in the cheese. Casein is slightly deficient in sulfur-containing amino acids, so the biological value of cheese protein is slightly less than that of total milk protein (Üçüncü 2004, Fox, et al. 2000). Cheese contains trace amounts of carbohydrate, primarily lactose. The residual lactose in cheese curd is, normally, fermented to lactic acid by starter bacteria during manufacture and ripening. Cheese is also a suitable nutrient for patients who have diabetes or lactose malabsorption, because of the low lactose ratio it contains. Since most of the milk fat is retained in the cheese curd, the fat-soluble vitamins in milk also partition into the curd. Most of the vitamin A in milk fat (80-85%) is present in cheese fat. Conversely, most of the watersoluble vitamins in milk partition into the whey during curd manufacture. The 10- 20 % of vitamin B₁, 20-30 % of vitamin B₂ and biotine, 30-60 % of folic acid and vitamin B₁₂ in milk can partition in cheese. Significant quantities of vitamin B₁₂ are produced in Swiss cheeses by propionic acid bacteria. Cheese is also an important source of calcium, phosphorus, and magnesium. The amount of salt added during the manufacture of different cheese varies significantly, resulting in large differences in the concentration of sodium in cheese (Üçüncü 2004, Fox, et al. 2000).

2.4. Cheese Production

Cheese making is a very old method for the preservation of milk. When fresh milk become sour, the casein aggregates and a gel is formed. Whey separation generally occurs when the gelled or clotted milk is kept for some time. These steps are the origin of cheese making. However, for centuries, milk has also been clotted by the addition of specific agents, especially rennet, an extract of calf stomach, and starter cultures (Walstra, et al. 2006).

There are numerous cheese varieties that have several different processes. Variations at one or more steps during production of cheese cause different textures and flavors, desired characteristics (Gunasekaran and Ak 2003). However, clotting, syneresis, shaping and ripening are essential steps for nearly all cheese varieties. Figure 1 summarizes the general steps of cheese production.

2.4.1. Clotting of Milk

In cheese making, milk should have good physical, chemical, sensorial, and microbiological characteristics. Milk should be free of chemical taints and free fatty acids, which cause off-flavors in cheese, and antibiotics, which inhibit the growth of bacterial cultures. Milk should also have high casein content (Üçüncü 2005, Fox, et al. 2000).

The essential steps of cheese making involve coagulation of the casein component of the milk to form a gel. Coagulation can be achieved by the action of a proteolytic enzyme, lowering the pH below the isoelectric point of protein (~ 4.6), heating to about 90°C at a pH of about 5.2 (Gunasekaran and Ak 2003).

In the acid coagulated cheese making, acid formed from lactose by lactic acid bacteria and/or by the addition of food-grade acid (e.g., lactic or citric) or acidogen, such as gluconic acid- δ -lactone (which hydrolyzes to gluconic acid) (Walstra, et al. 2006, Fox, et al. 2000).

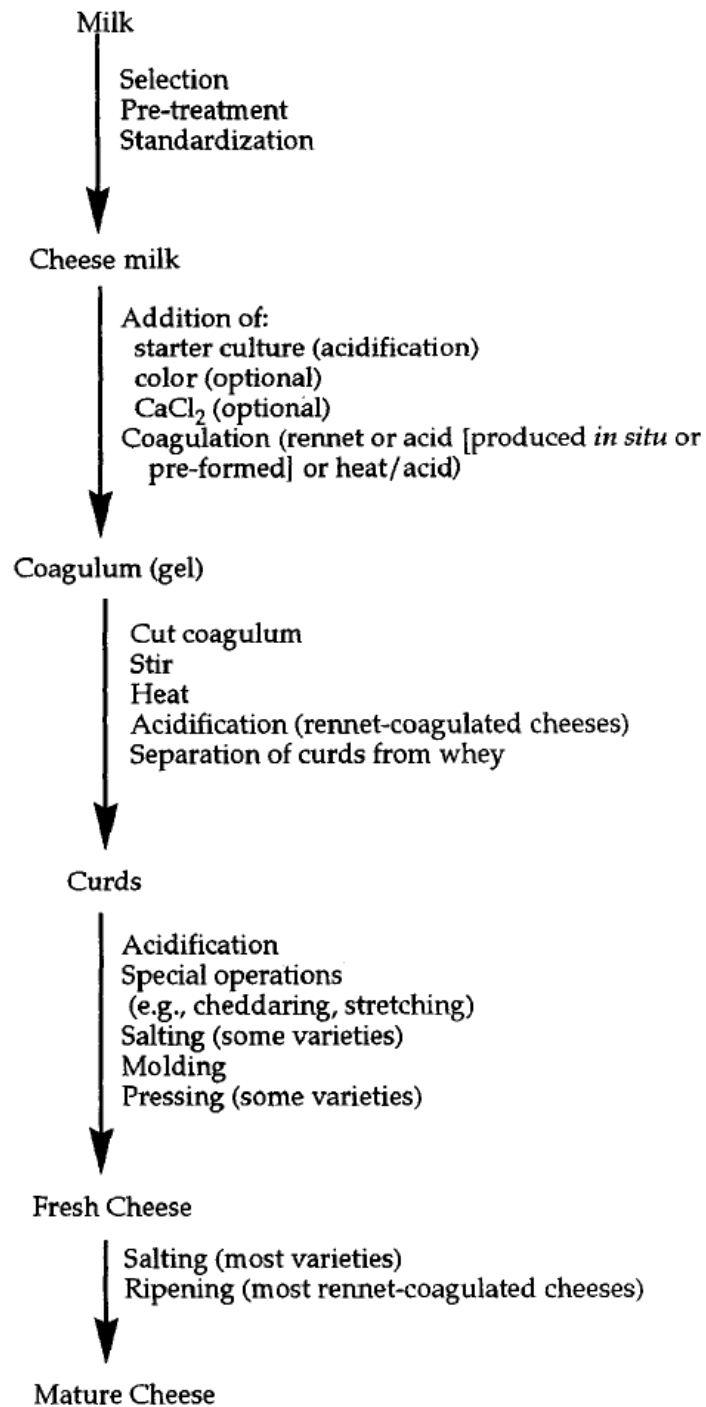


Figure 1. General protocol for cheese manufacture.
(Source: Fox, et al. 2000)

The acid-heat coagulated cheeses have a minor importance in all cheese varieties. They are usually produced from whey or a blend of whey. Whey contains lactose, vitamins, proteins and minerals along with traces of fat. Whey proteins primarily consist of α -lactalbumin and β -lactoglobulin. Depending on the method of manufacture, whey may also contain glycomacropeptides. Therefore, acid-heat coagulated cheeses cause recovery of the nutritionally valuable whey proteins (Fox, et al. 2000).

Acid milk gel formation occurs based on two principles of physicochemical changes. Firstly, colloidal calcium phosphate become soluble at 20-30°C and pH 5.2-5.3. As a result of solubilization, a tendency toward disaggregation of the casein micelles into a more disordered system occur. Secondly, tendency for the casein micelles to aggregate into a more ordered system occur. Aggregation is the result of the reduction of the negative surface charge on the casein micelles due to the production of lactic acid. Casein hydration also decrease in the pH range 5.3-4.6. Moreover, the ionic strength of the milk serum increase due to the increased concentrations of calcium and phosphate ions and this increase has a shrinking effect on the casein micelles. This physicochemical and microstructural changes are summarized in Figure 2 (Fox, et al. 2000).

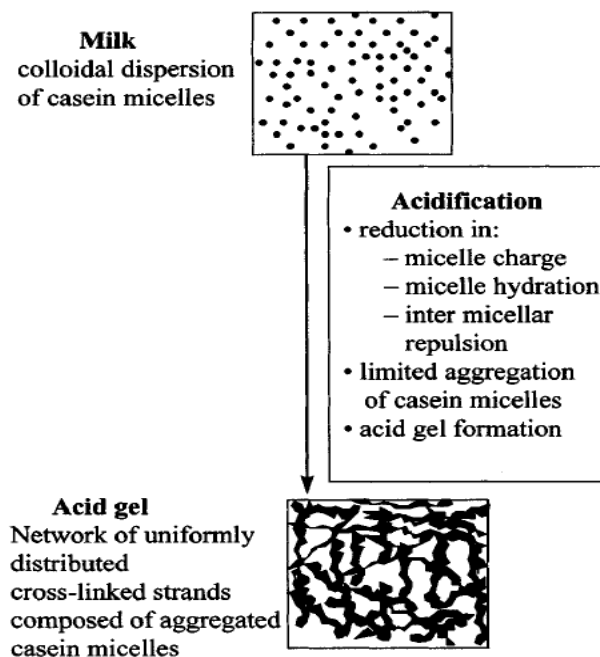


Figure 2. Schematic representation of the conversion of milk to a gel by acidification using a starter culture (Source: Fox, et al. 2000).

The vast majority of cheese varieties are produced by rennet coagulation. The rennet (proteinases)-induced coagulation of milk include two stage process. The primary phase involves the specific enzymatic hydrolysis of κ -casein to produce para-casein micelles. Secondary stage involves aggregation of the rennet-altered (para-casein) micelle into a threedimensional gel network coagulum in the presence of Ca^{2+} at temperatures above 20°C (Fox, et al. 2000). Figure 3 summarizes the rennet coagulation of milk.

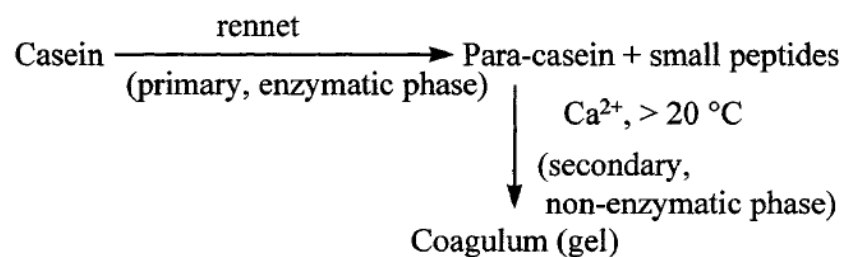


Figure 3. Summary of the rennet coagulation of milk
(Source: Fox, et al. 2000).

κ -casein is the only protein hydrolyzed during the rennet coagulation of milk and it is hydrolyzed specifically at the peptide bond between Phenylalanine 105-Methionine 106. As a result, κ -casein is converted into para- κ -casein and glycomacropeptide. Glycomacropeptide is hydrophilic so remain as whey. There are about 10 forms of κ -casein that differ in sugar content; hence, 10 caseinomacropeptide are produced. All the caseinomacropeptides are soluble in 2 % trichloroacetic acid (TCA) but only the glycosylated forms are soluble at higher concentrations of TCA. Therefore, TCA-soluble nitrogen can be used to monitor the primary phase of rennet coagulation (Üçüncü 2004, Fox, et al. 2000).

In the secondary or clotting phase, when approximately 85% of the total κ -casein has been hydrolyzed, the stability of the micelles is reduced. As a result, micelles collide and remain in contact and build into a three-dimensional network, referred to as a coagulum or gel (Fox, et al. 2000). There are two hypotheses about how the micelles aggregate. One is that hydrophobic bonding occurs between the para- κ -casein and the other is that calcium and calcium phosphate bonding occurs in α – and β – caseins. Gel formation in the secondary phase is dependent on the milk's temperature and calcium

content. The coagulation rate is also dependent on the concentration and activity of the enzyme solution. Increases in both of these factors shorten coagulation time and increase firmness (Gunasekaran and Ak 2003).

2.4.2. Removal of Whey

The gel is subjected to syneresis. The objective of syneresis is to remove whey from the gel and effectively concentrate the casein and fat. This process enables the cheese makers to control the moisture content of the cheese. Therefore, it gives the manufacturer opportunity to control the activity of microorganisms and enzymes in the cheese, and hence the biochemistry of ripening and the stability and quality of the finished cheese. Cheeses which have higher moisture content, will mature faster, but it will be less stable. High-moisture cheeses have a much greater tendency to develop off-flavors than low-moisture varieties (Fox, et al. 2000). Because of removing lactose and other solubles from the curd, syneresis adjusts the pH of the cheese independently (Gunasekaran and Ak 2003).

Syneresis is generally intensified by cutting and stirring the gel. After this process whey and curd mixture are obtained. The curd is approximately 10 to 30% of the volume of milk (Walstra, et al. 2006). In some kind of cheese productions, dehydration is done in the cheese vat by fine cutting the gel, extensive cooking of the curds whey mixture at approximately 40 – 50°C and vigorous agitation during cooking. For the softer varieties, the gel may be scooped directly into the molds without cutting or cooking, and syneresis occurs in the molds while the pH of curd decreases. Moreover, curds for some varieties (e.g., Cheddar, white cheese and Swiss) are subjected to pressure in the molds to aid whey removal (Üçüncü 2004, Fox, et al. 2000). Washing by adding hot water to the curd – whey mixture also enhances the syneresis (Gunasekaran and Ak 2003). Salting also causes the loss of moisture from the curd. Approximately 2 kg water are lost per kg of salt absorbed in cheese (Fox, et al. 2000).

2.4.3. Shaping and Salting

When the curd is separated from the whey, the curd particles are shaped into some form and salted (Gunasekaran and Ak 2003). Salting does not apply to some fresh-type cheeses such as quarg (Walstra, et al. 2006).

The concentration and distribution of salt in cheese have some effects on cheese quality. Firstly, salt inhibits or retards the growth of microorganisms, including pathogenic microorganisms. Also, salting restrains the activity of various enzymes in cheese. Salting affects the syneresis of cheese curd, resulting in whey expulsion but it is not enough for controlling the moisture content of cheese curd (Fox, et al. 2000). As a result, salt affects durability, safety, flavor, and consistency of cheese (Walstra, et al. 2006).

Cheese is salted by one of four methods;

1. In dry salting method, dry salt and curd are mixed prior to molding and pressing.
2. Dry salt or a salt slurry is spreaded on the surface of the formed cheese.
3. The formed cheese is immersed in a concentrated NaCl brine.
4. A combination of two of these methods can be used. For example, curd may be partially dry-salted before stretching and molding, then placed into brine (Fox, et al. 2000).

2.4.4. Ripening

Cheese is a biochemically dynamic product and undergoes significant changes during ripening. The major biochemical changes involved during cheese ripening are proteolysis, lipolysis, metabolism of residual lactose, lactate and citrate, and the formation of volatile compounds (Beuvier and Buchin 2004). Ripening gives to different cheeses characteristic flavors, textures, and appearances (Gunasekaran and Ak 2003). Fresh cheeses constitute a major proportion of the cheese consumed in some countries. Most of these cheeses are produced by acid coagulation. However, most

rennet-coagulated cheeses are ripened for a period ranging from about 3 weeks to more than 2 years (Fox, et al. 2000). Ripening is the main factor determining on the typical flavor and texture of a given cheese variety. To achieve this, cheese is kept for a variable time under suitable conditions. The storage conditions vary widely with the type of cheese involved (Walstra, et al. 2006).

Action of microorganisms present within the curd and on its surface affect the ripening. Ripening is also influenced by residual enzymes present in the cheese curd. Proteins, carbohydrates and fat are metabolised by both microbial activities (starter and non-starter microorganisms), and by the action of indigenous milk enzymes and residual coagulant (Beuvier and Buchin 2004). Scott et al. (1998) declared the primary factors in ripening;

1. Storage temperature and humidity.
2. Chemical composition of the curd (fat content, level of amino acids, fatty acids, and other by-products of enzymatic action).
3. Residual microflora of the curd (Gunasekaran and Ak 2003).

2.4.5. Additional Process Steps

Nowadays, some additional process steps are commonly applied in cheese making. Their main objectives are to decrease variations in the conditions occurring during the manufacturing process and in the properties of the cheese.

2.4.5.1. Standardization of Cheese Milk

Milk for cheese is subjected to a number of pretreatments, with various objectives. For some cheeses, the milk is standardized to give a fat content that is particular to a given cheese. Different cheese varieties have a certain fat in dry-matter content, in effect, a certain fat-protein ratio, and this content has legal status in the "Standards of Identity" for many cheese varieties (Hutkins 2006, Gunasekaran and Ak

2003). Depending on the ratio required, it can be modified by removing some fat by natural creaming, adding skim milk, adding cream or adding milk powder, evaporated milk, or ultrafiltration retentate (Fox, et al. 2000).

For inhibit production variation, calcium and starter culture can be added to milk. Calcium is important in the coagulation of milk by rennet and hence it is common practice to add CaCl_2 (e.g., 0.01%) to cheese milk. Starter culture can be added to adjust the pH. The pH of milk is a critical factor in cheese making. Addition of 1.5-2% starter culture reduces the pH of the milk immediately by about 0.1 unit (Üçüncü 2004, Fox, et al. 2000).

2.4.5.2. Pasteurization of Cheese Milk

Heat treatment destroys microorganisms and enzymes that can cause damage during ripening. Also, pasteurization kill pathogens that can survive for some time, especially in soft-type cheeses. To avoid recontamination after pasteurization, strict hygienic measures have to be taken (Walstra, et al. 2006).

There are alternatives to heat treatments for reducing the number of microorganisms in milk. These are:

1. treatment with H_2O_2
2. activation of the lactoperoxidase- H_2O_2 - thiocyanate system
3. bactofugation
4. microfiltration (Fox, et al. 2000).

2.4.5.3. Regulation of Cheese Color

Cheese color can be different according to milk species. Carotenoids in bovine milk can be bleached by treatment with H_2O_2 or benzoyl peroxide or masked by chlorophyll or titanium oxide (TiO_2), although such practices are not permitted in all

countries. If highly colored cheese, butter are preferred by consumers, intense colors may be obtained by adding carotenoids (synthetic or natural extracts) (Fox, et al. 2000).

2.5. Rennet

Several proteinases will coagulate milk under suitable conditions. Milk clotting enzymes can be animal origin, plant origin or microbial origin (Üçüncü 2004).

Rennet that is an animal derivative is extracted from the fourth stomach of a calf or young goat. Rennet contains an enzyme called rennin which has the property of clotting milk to form a solid curd (Carroll 1994). Animal rennets are prepared by extracting the dried (usually) or salted gastric tissue (referred to as *veils*) with 10% NaCl and 1% boric acid. For this extraction process, pH is adjusted to 4.7 with HCl solution. This process lasts about 6-7 days at 17-20°C. Pepsin is also animal origin proteinases. However, it has high proteolytic activity which is not wanted by cheese producers. Pepsin increases in stomach of animal after consumption of solid food by calf or young goats (Üçüncü 2005).

There are a number of plants that have coagulating properties. Papain from *Carica papaya*, bromelain from *Ananas sativa* and ricin from *Ricinus communis* have high proteolytic effect (Üçüncü 2005). In ancient Rome, cheesemakers used an extract of fig tree bark. The flower of the thistle plant called *Cynara cardunaculus* is used to make Sera de Estrella cheese (Üçüncü 2005, Carroll 1994).

Microbial origin rennets can be bacterial origin or fungal origin. *Bacillus subtilis*, *B. polymyxa*, *B. cereus* can be used by bacterial rennet producers. *Mucor pusillus*, *M. Miehei*, *Endothia parasitica* are used to produce fungal origin rennets (Üçüncü 2005). The early fungal rennets were considerably different from chymosin or pepsins, but the present products have been modified to make them having similar properties to that of chymosin. Microbial rennets are relatively cheap. Therefore, rennets have attracted the attention of recombinant technology. The gene for prochymosin has been cloned in *Escherichia coli*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* var. *lactis*, *Aspergillus nidulans*, *A. niger*, and *Trichoderma reesei*. As a result of recombination, enzymatic properties of the recombinant enzymes

are indiscernible from those of calf chymosin. The cheese making properties of recombinant chymosins have been assessed on many cheese varieties, mostly with very satisfactory results (Fox, et al. 2000).

2.6. Texture of Cheese

Texture perceptions result from a complex arrangement of sensory inputs. These sensory inputs are related both prior to and during food consumption (Fox, et al. 2000). The texture of cheese is the macroscopic expression of the structure of the cheese matrix. The texture is affected during two stages of cheese processing: production and ripening (Beuvier and Buchin 2004). Figure 4 illustrates the main changes occurring during production and ripening of cheese in a schematic way.

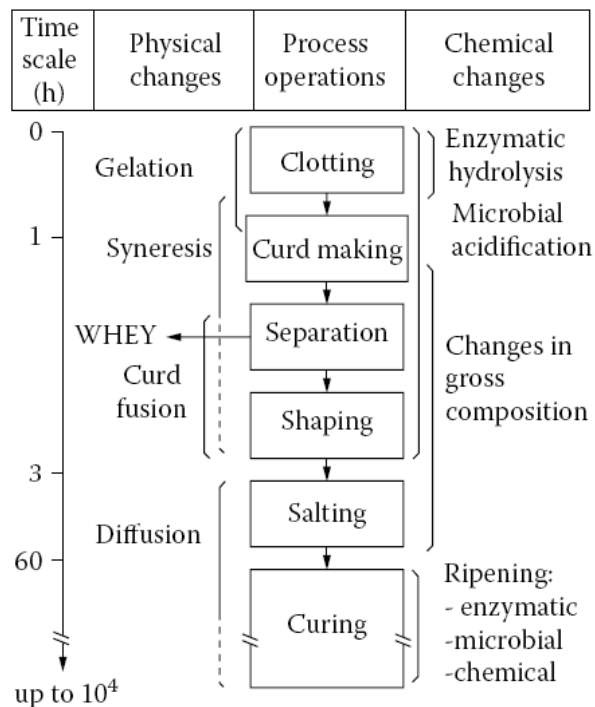


Figure 4. Schematic of the most essential physical and (bio)chemical changes occurring during the transformation of milk into cheese (Source: Walstra, et al. 2006).

During manufacture, the cheese matrix is formed. It begins with coagulation. Coagulation is the step during which milk undergoes a profound physical and rheological change. The structure affects the rheological properties (Everard, et al. 2007, Gunasekaran and Ak 2003). In this step, caseins organise themselves into a network, entrapping fat globules, water and gas bubbles. This network structure is critically affected by composition of milk and technological conditions of coagulation (Fox, et al. 2000, Gunasekaran and Ak 2003). High concentration of rennet in making cheese results in high aggregation rate and a coarse casein network that is responsible for the firm gel and cheese (Gunasekaran and Ak 2003). Jack and Peterson (1995) also declared that higher curd scalding temperature leaves the curd springy, and the resulting cheese becomes rubbery (Gunasekaran and Ak 2003). Moisture content, acidity, and pH of curd affect the texture of final cheese. Everard et al. (2007A) reported that increasing the emulsifying salt in processed cheese caused increasing pH and increased firmness. Hydration and interactions of caseins are affected by acidification that influences the extent of mineralisation of the caseins (Beuving and Buchin 2004). The hydrophobic interactions are important for a stable casein matrix structure. This structure are weakened by adsorption of water by proteins to solvate the ionic charges. Moisture in processed cheese reduced the firmness of cheese (Everard, et al. 2007B). Higher-moisture content cheeses, at same pH and salt content, are less firm than lower moisture content cheeses (Gunasekaran and Ak 2003). Salting influences cheese proteins. Therefore, salting affects cheese texture due to protein solubility, and probably protein conformation (Fox, et al. 2000). Olson (1982) found that Mozzarella cheese with a higher salt content (1.78 vs. 1.06 %) is less stringy (Gunasekaran and Ak 2003). Bryant et al. (1995) claimed that the microstructure of lower-fat Cheddar cheese has a more compact protein matrix with less open spaces than the microstructure of a regular-fat cheese (Gunasekaran and Ak 2003). Gwartney et al. (2006) also concluded that reduced fat cheeses were more waxy, fracturable, chewy, hard, and springy and less sticky, cohesive, meltable, and smooth than full-fat cheeses. In study of Emmons et al. (1980), Olson and Johnson (1990), and Bryant et al. (1995), reduced-fat cheeses tended to be more elastic and more adhesive (Gunasekaran and Ak 2003). Firmness increases with casein content and decreases with fat content. the cohesiveness of cheese also decreases with fat content. Moreover, reduced-fat content results in springier texture because of fewer fat globules with more casein being deformed per unit volume (Gunasekaran and Ak 2003).

During ripening, changes occur in the matrix because matrix is influenced by the loss of water and proteolysis (Beuviel and Buchin 2004). Generally, the duration of ripening is inversely related to the moisture content of the cheese and affect the texture (Fox, et al. 2000).

The most notable change with age is the decrease in fracture strain and springiness and increase in creaminess with the effects of proteolytic breakdown of the protein matrix (Gunasekaran and Ak 2003). Everard et al. (2007B) also found that mouthcoating was the only textural parameter to be significantly affected by aging from 2 to 4 weeks after manufacture.

Lawrence et al. (1987) list the following three factors during production periods as having an effect on ripening: pH at which whey is drained from the curd, salt in moisture ratio, pH of cheese after salting. The pH at which whey is drained from the curd determines the proportions of chymosin and plasmin in the cheese. Salt-in-moisture ratio affect the activity of residual rennet and plasmin in cheese. These effects influence the texture of cheese during ripening. The temperature and relative humidity conditions of ripening conditions also affect the texture development (Gunasekaran and Ak 2003). Increasing the ripening temperature from 0 to 15°C results in a significant decrease in the mean concentration of intact casein, a decrease in the level of expressible serum. These affect the texture of cheeses (Gunasekaran and Ak 2003).

Primary proteolysis that begins with coagulation is the result of internal hydrolysis of casein molecules, by the coagulant or indigenous enzymes of milk, such as plasmin. Peptide α_{s1} -I that result of primary proteolysis causes the initial softening of the cheese (Gunasekaran and Ak 2003). In Grappin and Beuviel's study (1997) supposed that removal of the native microflora from raw milk may alter the texture of subsequent cheeses by two major mechanisms. The heat treatment of the milk used to destroy the microflora may alter the structure of the casein matrix by denaturation of whey proteins or the loss of water, or modify the proteolysis patterns by denaturation, activation or modified retention of enzymes. On the other hand, the elimination of most of the indigenous microflora, either by heating or microfiltration, may modify the biochemical changes in cheeses, in particular proteolysis (Beuviel and Buchin 2004).

Secondary proteolysis occurs essentially during ripening, by the action of peptidases of microorganisms. Textural change during secondary proteolysis is based on the rate of proteolysis and increase in pH. In this phase, each peptide is cleaved and two new ionic groups are generated. Increasing the solvation of the protein chains result in

reducing the amount of free water in the matrix. As a result of ripening, the protein matrix becomes less cohesive and hardens (Fox, et al. 2000, Gunasekaran and Ak 2003). Olson (1982) expressed that the reason of “curdy” texture in Mozzarella cheese is insufficient proteolysis due to high salt content (Gunasekaran and Ak 2003).

Cheese texture is a sensory property, so it is ultimately expressed in sensory terms or descriptors. However, instrumental methods are easier and quickly carried out, standardize, and reproduce and require the involvement of fewer trained people. Comparative studies on the evaluation of texture by compression and sensory methods have been undertaken by Green et al. (1985), Jack et al. (1993), Lee et al. (1978). Good correlations have been reported, in general, for hardness, chewiness, adhesiveness, and springiness. Rheological texture assessment of cheese can be used to replace or supplement sensory analysis for quality control purposes (Everard, et al. 2007A, Fox, et al. 2000).

2.7. Flavor of Cheese

One of the quality characteristics of cheese is its flavor (Fox, et al. 2000). Solvent extraction, vacuum distillation can be used to characterize the aroma of cheese. The typical odor of a food product results from a component balance in a complex mixture of volatile compounds present in the headspace around the cheese (Lecanu, et al. 2002). Therefore, static and dynamic headspace techniques, as well as headspace solid-phase microextraction can be used for characterization of aroma components. Solid-phase microextraction (SPME) is a new technique developed by Arthur and Pawliszyn (Jaillais, et al. 1999). SPME that involves concentration of volatile components by adsorption on a fiber, is a simple and sensitive, solvent free technique (Lecanu, et al. 2002, Jaillais, et al. 1999).

Formation of flavor compounds depends on moisture, salt content, pH of cheese and ripening conditions, such as storage temperature, air humidity in the ripening room and duration of the ripening period (Forde and Fitzgerald 2000). Based on sensory evaluation and chemical analysis of cheeses, various groups of volatile compounds have been identified as being responsible for the final taste and aroma of cheese (Ayad

2004). These volatile compounds which are hydrocarbons, alcohols, aldehydes, ketones, esters, free fatty acids, as well as nitrogen- and sulfur-containing compounds are the results of protein and lipid digestion, amino acid and fatty acid degradation (Forde and Fitzgerald 2000). Cheese flavor is mainly derived from the breakdown of milk proteins, fats and lactose by enzyme activities. Enzymes can be either indigenous to milk (plasmin and lipase) or added to the milk (chymosin or rennet substitute and pregastric esterase), or secreted by microorganisms (especially molds and other microorganisms in the surface smear), or released from microbial cells following cell death and lysis. A few volatile compounds are produced by chemical reactions, for example, via the Maillard and Strecker reactions between amino acids and various carbonyls, especially dicarbonyls, e.g., diacetyl, glyoxal, or methyl glyoxal (Fox, et al. 2000, Forde and Fitzgerald 2000).

Lactose ratio affects the flavor of cheese. Lactic acid and acetic acid which are the result of glycolysis, cause decrease in the pH of cheese and can cause unpleasant flavor. Therefore, flavor of the low-lactose cheeses are described as clean and mild. Flavor development was substantially faster in the high lactose cheese than in the washed-curd cheeses. Acetate may contribute to the cheese flavor. Acetate occurs from the oxidation of lactate depends on the nonstarter lactic acid bacteria population and the availability of O₂. Acetate may also be produced by starter bacteria from lactose or citrate or from amino acids by starter bacteria and lactobacilli. High concentration of acetate may cause off-flavors. Strains of *Lactococcus lactis* subsp. *lactis* and *Leuconostoc* spp. metabolize citrate to diacetyl in the presence of a fermentable sugar during production and early ripening. Therefore, diacetyl is a very significant aroma compound for unripened cheeses (Fox, et al. 2000).

Lipids are important for cheese flavor. The first reason is that lipids function as solvents for aroma compounds which are produced from lipids, proteins and lactose. However, lipids may also absorb from the environment compounds that cause off-flavors. The second reason is being the source of short chain fatty acids that have strong and characteristic flavors. Fatty acids are produced by lipolysis and a lower proportion of free fatty acids originate from the degradation of lactose and amino acids (Fox, et al. 2000, Curionia and Bossetb 2002). These reactions depend on the action of the indigenous lipases of milk or the action of microbial lipases. In Molimard and Spinnler's study (1996), it is stated that the shorter chain fatty acids can also be derived from ketones, esters and aldehydes by oxidation. In general, long-chain fatty acids (>12

carbon atoms) have less characteristic flavor than short and moderate-chain, even numbered fatty acids (C₄–C₁₂). For example, ethanoic (acetic) acid and propanoic (propionic) acid have a typical vinegar odor which have importance for flavor of many cheeses (Curionia and Bossetb 2002). Acetic acid may be the result of the catabolism of lactose, citrate, amino acids, or the propionic acid fermentation. Moreover, propionic acid, butyric acid, hexanoic acid and branched chain volatile fatty acids are important for some kind of cheese flavor. Propionic acid is the end product of lactose fermentation by propionibacteria. Butyric acid can originate from the catabolism of triglycerides and from lactate fermentation by clostridia. Hexanoic acid is liberated by lipolysis of triglycerides. Branched-chain volatile fatty acids which are 2-methyl propanoic (isobutyric), 2-methyl butanoic, 3-methyl butanoic (isovaleric) acids result from catabolism of the amino acids valine, isoleucine and leucine, respectively (Beuvier and Buchin 2004).

In some cheeses which have high redox potential, unsaturated aldehydes that are strongly flavored and cause a flavor defect, may be originated by the oxidation of polyunsaturated fatty acids (Fox, et al. 2000).

Fatty acids are also precursors of methyl ketones, alcohols, lactones and esters which have important influences on cheese flavor (Beuvier and Buchin 2004). For example, metabolism of fatty acids in cheese by *Penicillium* spp. involves release of fatty acids by the lipolytic systems, oxidation of β -ketoacids, decarboxylation to methyl ketone with one less carbon atom and reduction of methyl ketones to the corresponding secondary alcohol under aerobic conditions (Fox, et al. 2000). Therefore, their levels depend on the balance between production and degradation, which depends on the degree of maturity of the cheese (Beuvier and Buchin 2004).

Ketones especially methyl ketones have typical odors and important for aroma of surface-mould ripened and blueveined cheeses (Curionia and Bossetb 2002). The concentration of methyl ketones is related to lipolysis. In surface-ripened cheese, these compounds is originated from the enzymatic activity of *Penicillium roqueforti*, *P. camemberti* or *Geotrichum candidum* on keto acids. Keto acids naturally present at low concentrations in milk fat (Fox, et al. 2000; Curionia and Bossetb 2002).

Lactones possess a strong aroma and they have an important role in cheese flavor (Fox, et al. 2000). Lactones are produced from keto acids released by lipolysis, followed by reduction to hydroxyacids and spontaneous cyclisation of the hydroxy-

acids (Fox, et al. 2000; Beuvier and Buchin 2004). In the study of Urbach (1997), occurrence of lactones in cheese were linked to feeding (Beuvier and Buchin 2004).

Proteolysis are important for cheese flavor. Small peptides and amino acids which are the result of proteolysis contribute directly to cheese flavor. The presence of amino acids in cheeses clearly depends on aminopeptidase activity which is related to lactococcal cell lysis. Then, amino acids may be catabolized to aromatic compounds which are amines, acids, carbonyls, and sulfur-containing compounds etc. and major contributors to cheese flavor (Fox, et al. 2000).

Indole and skatole are nitrogen containing compounds which seems to contribute significantly to the aroma profile of some cheeses. Indole is resulted from a degradation product of tryptophan by yeasts, micrococci and *Brevibacterium linens*. In Gallois's study (1984), it was found that pyrazines which gives raw potato like aroma defect is produced from l-valine degradation by *Pseudomonas taetrolens* (Curionia and Bossetb 2002).

Esters play an important role in cheese flavour. They have sweet, fruity and floral aroma. Especially ethyl esters have important role in the formation of a fruity flavor in cheese (Curionia, et al. 2002, Beuvier, et al. 2004). Bosset and Liardon (1984) recorded that esterification reactions occur between short to medium chain fatty acids and primary-secondary alcohols (Curionia and Bossetb 2002).

Alcohols have effects on flavor of most cheeses. Molimard and Spinnler (1996) declared that many metabolic pathways are involved in the biosynthesis of the alcohols such as lactose metabolism, methyl ketone reduction, amino acid metabolism as well as degradation of linoleic and linolenic acids (Curionia and Bossetb 2002). The presence of the native microflora in milk affects the production of alcohols in cheeses (Beuvier and Buchin 2004).

Aldehydes are important aromatic compounds for cheese flavor. Aldehydes originate from amino acids by transamination or by Strecker degradation which includes nonenzymatic reactions during ripening. Aldehydes are transitory compounds in cheese because they are rapidly reduced to primary alcohols or oxidised to the corresponding acids (Curionia and Bossetb 2002).

Volatile sulphur compounds play an important role in the flavor of cheese. These are hydrogen sulfide (H_2S), dimethylsulfide $[(CH_3)_2-S]$, dimethyldisulfide ($CH_3-S-S-CH_3$), and methanethiol (CH_3SH) and these compounds are found in most cheeses. The sulphur compounds in cheese derive from the sulphur amino acids (Fox, et al. 2000,

Beuvier and Buchin 2004). These compounds have strong garlic and very ripe cheese odors. Moreover, Berger (1999) reviewed that they are probably involved in the final aroma of mould-surface ripened and soft smear cheeses (Curionia and Bossetb 2002).

In conclusion, the study of flavor is useful both to characterize the cheese and to define its quality, linking it with the area and the methodology of production (Ziino, et al. 2005).

2.8. Microbiology of Cheese

Microorganisms, including bacteria, yeast and moulds, are present in cheese throughout ripening and have effects on maturation process. They contribute either directly through their metabolic activity or indirectly through the release of enzymes into the cheese matrix through autolysis. However, other microorganisms such as foodborne pathogens, can be found in cheese and have a negative impact on cheese quality. Therefore, cheese making technology should include precautions to remove or prevent pathogens entry to cheese (Beresford and Williams 2004).

Milk is sterile in the udder of healthy animals. However, contamination occurs during milking and storage. Bramley and McKinnon (1990) reviewed that when milk cooled to 15-21°C is dominated by mesophilic microorganisms, particularly *Lactococcus* and *Enterobacter* species. Cooling milk to 4°C greatly retards the growth of most microorganisms, but psychrotrophic bacteria, such as *Pseudomonas*, *Flavobacterium* and *Acinetobacter* continue to grow slowly (Beresford and Williams 2004). Water activity, concentration of salt, oxidation-reduction potential, pH, NO₃, ripening temperature, and the presence or absence of bacteriocins are the factors for controlling the growth of microorganisms in cheese. Compounds produced during curd manufacture and ripening such as H₂O₂ and fatty acids, also inhibit microbial growth, but the concentrations of these compounds in cheese are not sufficiently high to have a significant effect on microbial growth (Fox, et al. 2000).

Recent studies have shown that artisanal cheeses have different and typical microbial population dynamics related to the local production technology and geographic area of origin (Vidojevic, et al. 2007). The microflora of cheese and milk

have effects on cheese quality. The microflora associated with cheese ripening is extremely diverse. The microflora during ripening are divided into two groups which are the starter lactic acid bacteria and the secondary microflora. The secondary microflora are involved in the ripening process. The secondary microflora includes nonstarter lactobacilli, *Pediococcus*, *Enterococcus*, and *Leuconostoc*, propionic acid bacteria, moulds, yeasts, etc. (Beresford and Williams 2004). Faced with change in milk production and cheese manufacture, there is a need for knowledge of the natural biodiversity of microorganisms, their role, and the need and the way of preserving it. Therefore, nowadays many researches have been done in order to demonstrate the specific characteristics of raw milk cheeses (Beuvier and Buchin 2004).

Activities of nonstarter lactic acid bacteria during ripening have impact on cheese quality. Crow et al. (2001) reviewed that mesophilic lactobacilli are important in the maturation of cheeses because they can catabolise citrate and could be involved in proteolysis and in other enzymatic processes during cheese ripening (Vidojevic, et al. 2007). *Leuconostoc* spp. also have the ability to co-metabolise sugars and citrate. In addition, *Enterococci* spp. metabolize citrate and can form acetaldehyde, acetoin and diacetyl. Ray (1995) also reported that pediococci has the ability of diacetyl production from glucose. Moreover, nonstarter lactic acid bacteria contribute to the protein breakdown. Lactic acid bacteria could involve in amino acid breakdown by using α -ketoglutarate dependent transaminase pathway. The resultant α -keto acids are subjected to further enzymatic or chemical reactions to hydroxy acids, aldehydes, alcohols and carboxylic acids which are important for cheese flavor (Beresford and Williams 2004). Lipase and esterase activities are found in nonstarter lactobacilli and have effect on cheese characteristics. Tsakalidou (1993) reported the beneficial effect of enterococci in cheese making as contribution to the hydrolysis of milk fat by esterases. The released fatty acids can be further converted into methyl ketones and thioesters which have been implicated as cheese flavour compound (Beresford and Williams 2004). Enterococci have ability to live at wide range of growth temperatures and have high tolerance to heat and salt. Therefore, enterococci are found at high numbers in many cheeses which are made around the Mediterranean. Many of these are artisanal raw milk cheeses (Fox, 2000).

Lactic acid bacteria can protect the cheese against spoilage bacteria by producing lactic acid, H₂O₂, antibiotics such as nisin. Giraffa (2003) reviewed that *Enterococcus faecium* is capable of producing a variety of bacteriocins, called

enterocins with activity against *Listeria monocytogenes*, this has an important impact on the safety of cheeses that are made from raw milk (Abdi, et al. 2006).

In conclusion, microbial diversity in raw milk and cheese have effects on quality characteristics of traditional cheeses. Therefore, characterization of the microbial communities in raw milk, cheese and follow the dynamics of the entire populations throughout the cheese making and ripening processes are important.

2.9. Classification of Cheeses

Olson (1995) reported that it is estimated that more than 2000 cheese varieties exist, and the list may grow due to scientific researches and investigations of traditional cheeses (Gunasekaran and Ak 2003). The diversity of cheeses prompted the need for classification to more effectively describe and compare cheeses from different regions. There are differences in the same kind of cheeses such as different shapes, and packages. Moreover, some different kind of cheeses resembles with each other. Therefore classification of cheeses is very complex (Üçüncü 2004).

Cheeses may be classified according to the country of origin, manufacturing process, or some end-use property. Other classifications of cheeses, e.g., according to milk source, overall appearance (color, size, shape), chemical analysis, etc., are also possible. Davis (1965) recognized the difficulty in classifying cheeses and attempted to group them based on the nature and extent of chemical breakdown during ripening or according to flavor. Such a classification is still not available (Gunasekaran and Ak 2003).

Fox (1993) grouped cheeses based on the method of milk coagulation:

- Cheeses manufactured by enzymatic coagulation of milk (Kashar, Cheddar, Edam, etc.)
- Cheeses manufactured by coagulation with lactic acid bacteria (Cottage, Quark, Quesco, etc.)
- Cheeses manufactured by coagulation with heat and acid combination (Ricotta, Sapsago, Ziger, etc.)
- Cheeses manufactured by concentration (Mysost)

Walter and Hargrove (1972), who classified cheeses on the basis of manufacturing technique, suggested that there are only 18 distinct types of natural cheeses:

1. Very hard (grating)
 - 1.1 Ripened by bacteria (e.g., Parmesan)
2. Hard
 - 2.1 Ripened by bacteria, without eyes (e.g., Cheddar)
 - 2.2 Ripened by bacteria, with eyes (e.g., Emmental)
3. Semi-soft
 - 3.1 Ripened principally by bacteria (e.g., Gouda)
 - 3.2 Ripened by bacteria and surface microorganisms (e.g., Limburger)
 - 3.3 Ripened principally by blue mold in the interior (e.g., Roquefort)
4. Soft
 - 4.1 Ripened (e.g., Brie)
 - 4.2 Unripened (e.g., Cottage) (Fox, et al. 2000).

Marketers of cheese often classify cheeses by country of origin, which is logical to create a merchandizing image, but this causes confusion and overlap of many cheese varieties. More systematic classifications use composition, firmness and maturation agents as criteria. Classification according to this approach is useful for regulatory purposes and for comparing physical properties of cheese types. However, categorization by composition obviously groups cheeses of greatly different flavor characteristics into a single class (Üçüncü 2004).

Classification according to firmness;

- Very hard cheese (Water in fat free substances < 51%)
- Hard cheese (Water in fat free substances 49-56%)
- Semi-hard cheese (Water in fat free substances 54-63%)
- Semi-soft cheese (Water in fat free substances 61-69%)
- Soft cheese (Water in fat free substances > 67%)

The term, water in fat free substance, related to a ratio of water content to the protein (caseins) content and the structural matrix of cheeses. Firmness of cheeses is closely related to that ratio but is also influenced by the percentage of fat in dry matter of cheese which is a ratio of the fat content to total of fat, protein, and mineral contents. Classification according to fat content in dry matter;

- High fat cheeses (fat content in dry matter > 60 %)
- Whole fat cheese (fat content in dry matter 45-60 %)
- Fat cheese (fat content in dry matter 25-45 %)
- Low fat cheese (fat content in dry matter 10-25 %)
- Non-fat cheese (fat content in dry matter < 10 %) (Üçüncü 2004).

International Dairy Federation cheese catalog list includes more than 500 cheeses from 29 countries and classification according to type of milk (animal species), texture, country of origin, physical characteristics of rind, body, and weight, fat content in dry matter, water & water in fat-free cheese (IDF General Secretariat 1981).

2.10. Traditional Cheeses in Turkey

Turkey has a lot of traditional foods because of its history, different cultures and climates. Cheese is an important traditional food in Turkish cuisine. There are many traditional cheese types produced and consumed locally in Turkey (Turkoglu, et al. 2003).

The first information about varieties of cheese is found in the Ottaman era. In a times of Beyazit II, taze lor, taze dil cheese, taze cayir cheese, madurnu cheese, sumu cheese, karaman cheese, sofa cheese, esme cheese, midili cheese, white cheese, cimi tulum cheese, ızmır tulum cheese, rumeli tulum cheese, taze kaskaval cheese and balkan kaskaval cheese are mentioned that are brought to Istanbul. Evliya Çelebi's travel book also mentioned approximately 400 types of cheeses were present in Istanbul such as Kaskaval Cheese, Kesme Cheese, and Teleme Cheese. White cheese, kashar, tulum, lor and çökelek are the main cheeses produced in all regions of Turkey. Cow's milk, ewe's milk and goat's milk are used in the production of these cheeses. Also, in the production of some traditional cheeses only ewe's and goat's milk can be used. Some examples of ewe milk cheeses are ayas ovma, abaza, mihaliç, koponesti, çimi, Urfa white, Antep sikma, divle, cabalti çökelegi, Erzincan tulum, tomas, and kazikli. Some examples of goat milk cheeses are Balıkesir and Karaburun goat's sepet cheese, Ezine goat's cheese, Gönen yörük cheese, Malatya goat's salamura cheese, Isparta sütçüler çayır cheese,

Hatay yayladağ goat's cheese, Isparta Eğridir goat's cheese, Konya teneke salamura goat's cheese (çepni), and Denizli white goat's cheeses (Kamber 2008A).

Koponesti cheese, tire çamur cheese, İzmir teneke tulum, kırktokmak cheese, kirlihanım cheese, kuru çökelek cheese, posa cheese, sepet cheese, Afyon tulum, karaburun lor goat's cheese, and kuru ezme cheese are the examples of traditional cheeses in Aegean region. In Aegean Region, ewe's and goat's milk are used in the majority of cheeses (Çetinkaya 2005; Kamber 2008B).

2.10.1. Sepet Cheese

Sepet Cheese is a traditional cheese of Aegean region in Turkey. Local people in the region learned sepet cheese making from immigrants. Baskets are used in the shaping of cheese. Therefore, this traditional cheese is named as sepet cheese. The baskets are made from stalks which are collected near river and moist areas.

Sepet cheese is produced in Ayvalık, Dikili, Burhaniye, Foça, Çeşme, Urla, Karaburun, Ödemiş and Söke which are the towns of Aegean region. In the production of sepet cheese usually woolly goat milk is used. In Ödemiş and Söke, a blend of cow's and ewe's milk can be used. In Ayvalık and Polonezköy, cow milk is usually used in the production of sepet cheese. According to producers, 1 kg sepet cheese can be produced from 9 - 10 l cow's milk or 6 - 7 l goat's milk or 4,5 l ewe's milk (Büke 1981, Kınık, et al. 1999).

Figure 5 summarizes the production steps of Sepet cheese. In the production of sepet cheese, milk is heated to 30-40°C and inoculated with rennet. After inoculation, heat is turned off. Coagulation occurs during 2 hours. When the curd become firm, curds are broken down until no large lumps remain. This process helps the syneresis. The curds separate from the whey and settle at the bottom of the container. The deposited curds are placed in to baskets. 0.5-1.0 kg cheeses mostly placed into baskets. Without any weight being placed upon it, the cheese in the basket is left to strain by itself and during this period, as it strains, the soft cheese assumes the shape of the basket. The cheese is turned upside-down to enable the top surface to take on the shape of the basket too. The cheese is then removed from the basket. The upper and lower

surfaces are sprinkled with fine salt, the cheese is returned to the basket and the straining process continues (Büke 1981, Kınık, et al. 1999). Approximately 18 hours later, when the cheese can support itself as a single block, it is taken out of the basket and placed in brine (%18-24 NaCl). The sepet cheese in brine can be stored in a cool place for between 6 months and 1 year. Even if the cheeses are removed from the brine, they can be kept in air without spoiling for a long period (Kamber 2008B).

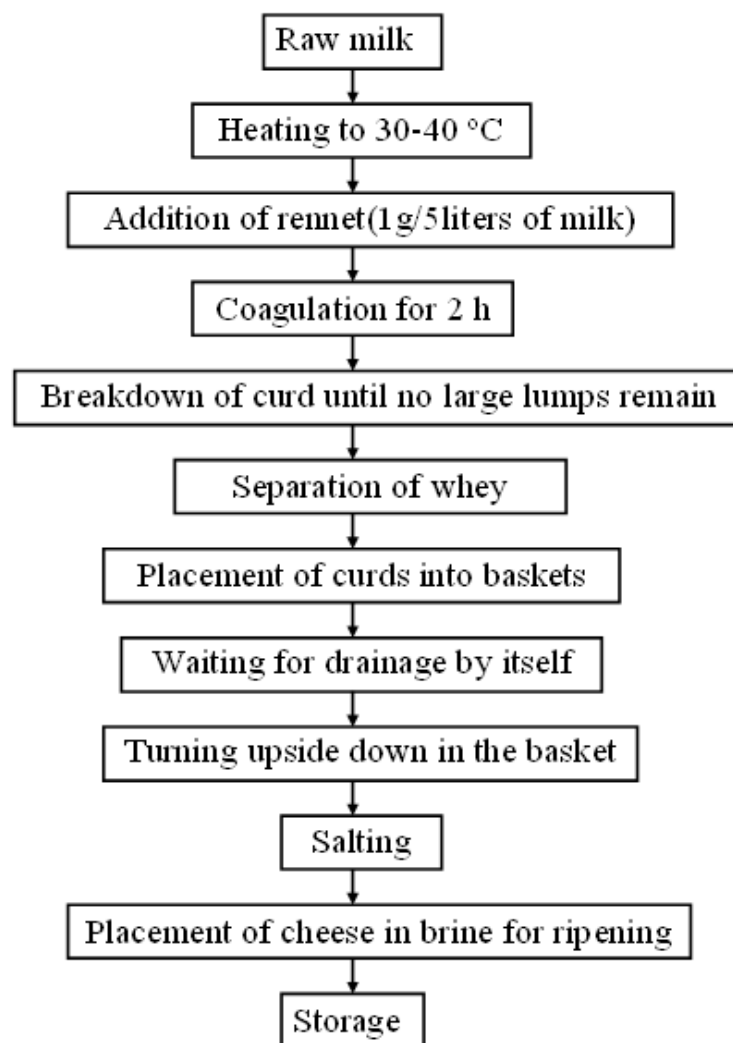


Figure 5. Production of Sepet Cheese

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals

The chemicals used in the study are listed in Appendix A.

3.1.2. Samples

In the first part of the study, the quality characteristics of thirty sepet cheeses were investigated. The cheese samples were collected from Çeşme, Karaburun, Zeytineli, Gülbahçe, Ovacık, Barbaros, Üçkuyular, Kemalpaşa, Turgutlu, Ayvalık, Balıkesir, Polonezköy. Sample types and their locations are given in Table 5. In the second part of the study, two sepet cheeses were produced in Germiyan and Zeytineli towns and they were analysed during production and ripening. Goat milk was used in the production of these sepet cheeses. In Zeytineli, rennet was used as coagulant agent which was produced from the fourth stomach of young goat. In Germiyan, industrial enzyme (mayasan) was used in the production. The cheeses were ripened under refrigeration conditions for 6 months in brine. Milk, curd, cheeses at 1st day, cheese at 1st, 2nd, 3rd, 4th and 6th months were analysed. Samples were stored under refrigeration conditions for physical and chemical analysis and analysed in one week. Samples also packaged under vacuum and stored at -20°C for volatile compound analysis.

Table 5. Sample types and location

Sample number	Milk Type	Location
1	Goat milk	Karaburun farmer's market
2		Foça farmer's market
3		Efe dairy farm, Çeşme
4		Kıyiege dairy farm, Çeşme
5		Gülbahçe town
6		Baycanlı dairy farm, Çeşme
7		Zeytineli town
8		Karaburun farmer's market
9		Karaburun farmer's market
10		Çomukoğlu dairy farm, Barbaros town
11		Çeşme Ilica farmer's market
12		Çeşme Ilica farmer's market
13		Üçkuyular
14		Zeytineli town
15		Germiyan town
16	Ewe milk	Reisdere farmer's market
17	Cow milk	Çeşme Ilica farmer's market
18		Karaburun farmer's market
19		Foça farmer's market, Ayvalık
20		Çeşme farmer's market
21		Balıkesir
22		Polonezköy
23		Balıkesir
24		Polonezköy
25		Polonezköy
26	Goat and ewe milk	Çeşme farmer's market
27		Çeşme Ilica farmer's market
28	Goat and cow milk	Ovacık farmer's market
29		Kemalpaşa-Turgutlu
30	Cow and ewe milk	Foça farmer's market, Ayvalık

3.2. Methods

3.2.1. Chemical and Physical Analysis

3.2.1.1. Total Solid Content

Total solid content of the cheeses were determined gravimetrically by drying a sample to constant weight in an oven at 105°C. Cheese sample (3 g) was crushed with 20 g sea sand and glass stick in predried weighing dish. The difference in weight before and after drying for 4-5 hours at 105°C gives the results of total solid content (Metod 33.2.44; 990.20, AOAC 2006).

$$\text{Total solid content (\%)} = [(\text{Total solid content of cheese(g)} / \text{cheese(g)})] \times 100 \quad (\text{eq. 1})$$

3.2.1.2. Fat Content

Fat contents of milk samples was measured with Lactostar (Funke gerber, Berlin). Fat content of samples were determined by Gerber method. Cheese sample(3 g) was weighed into a butyrometer vessel and then the vessel was filled with 10 ml H₂SO₄ (d: 1.22 g /ml). Plug was inserted into butyrometer and waited until the cheese was melted in 70°C water bath. Then 1 ml amyl alcohol was added. Butyrometer vessel was completed to the level of 35% with H₂SO₄ solution. The butyrometers were placed in water bath for 5 minutes. After that the butyrometers were centrifuged in Gerber centrifuge for 10 min. The oil level was read as percentage oil in cheese from butyrometer vessel (IDF 1997).

Fat content in total solid content was also determined by dividing fat content to total solid content.

3.2.1.3. Protein Content

Protein contents of milk samples was determined by using Lactostar (Funke gerber, Berlin). Protein content of cheese was determined by measuring the total nitrogen content of samples by the Kjeldahl method and multiplying by a conversion factor (6.38) (Fox, et. al. 2000).

3.2.1.3.1. Preparation of Citrate Dispersion for Nitrogen Analysis

Grated sample (10g) was mixed with 50 ml 50°C 0.5 M trisodium citrate. The solution was cooled to room temperature for 60 min on magnetic stirrer. Then, 150 ml deionized water was added into the solution. Ten ml of this solution was used for total nitrogen analysis (Metin 2006).

3.2.1.3.2. Kjeldahl Method

The Kjeldahl tubes containing sample, catalyst, antifoaming agent and 15 ml H₂SO₄ were placed into digestion unit. The digestion was done at 430°C until the solution in tubes became opaque green. After the solution in tubes was cool, the tubes were placed into the distillation unit. Twenty five ml H₃BO₃ and indicator were added into the erlenmayer flask and placed in distillation unit. After distilation, the flask was titrated with 0.1 N HCl.

$$\text{Nitrogen \%} = \frac{14.01 \times (\text{ml HCl used} - \text{ml blank}) \times (\text{Normalite of HCl}) \times 100}{\text{Sample weight (g)} \times 1000} \quad (\text{eq. 2})$$

3.2.1.3.3. pH 4.6 Soluble Nitrogen Fractions

Citrate dispersion (80 ml) was mixed with 11.3 ml 1M HCl at 12°C. The pH of this solution should be between 4.35-4.55. Then, 8.7 ml deionized water was added into the solution and filtered with Whatman no 2 filter paper. The 25 ml of filtered solution was analysed with Kjeldahl method (Metin 2006, Bynum and Barbano 1985).

Ripening index was calculated as percentage of pH 4.6 soluble nitrogen fraction and total nitrogen ratio (Metin 2006).

3.2.1.3.4. Trichloroacetic acid (12 %) Soluble Nitrogen Fractions

Fifty ml pH 4.4 soluble nitrogen fraction was poured into 24 % trichloroacetic acid. The solution was stored at 4°C for one night and filtered with Whatman no 42 filter paper. Then the 50 ml of filtered solution was analysed with Kjeldahl method (Metin 2006, Bynum and Barbano 1985).

3.2.1.4. Salt Content

The salt content of samples was determined by Mohr method. Five g cheese sample was crushed in porcelain mortar with the help of hot distilled water and watery part was transferred into an erlenmayer flask. Same process was repeated 5 times. Then water level was completed to 500 ml with distilled water at room temperature. The solution was filtered and 25 ml of this solution was transferred into the erlenmayer flask and neutralized with 0.1 N NaOH. Then, 0.5 ml of K₂CrO₄ (5% w/v) was added and titrated with 0.1 N AgNO₃ until tile red color was occurred (IDF 1988).

$$\% \text{ Salt} = ((V_1 - V_2) \times 0.585 \times F) / P \quad (\text{eq. 3})$$

V_1 : Used 0.1 N AgNO_3 amount (ml) from experiment with cheese solution

V_2 : Used 0.1 N AgNO_3 amount (ml) from experiment with deionized water

P: Cheese amount included in titration (0.25 g)

F : Factor of 0.1 N AgNO_3

Salt content in total solid content was determined by dividing salt content to total solid content.

3.2.1.5. The pH and Titratable Acidity

The pH of the cheese was determined by mixing cheese and distilled water in the ratio of 1:1 in stomacher and then results were measured with a pH meter (Rehman and Fox 2002).

For determination of titratable acidity, 10 g cheese was weighed and crushed with 105 ml water (40°C) in porcelain mortar. This solution was filtered and 25 ml of filtered solution was used for titration. Three drops of phenolphthalein were added and titrated with 0.1 N NaOH until the first permanent pink color (Metin 2006).

$$\% \text{ lactic acid} = (0.1 \text{ N NaOH amount (ml)} \times 0.009 \times 100) / \text{Cheese amount (g)} \quad (\text{eq. 4})$$

3.2.1.6. Water Activity

The water activity of samples were measured with water activity meter (Hygrolab V3, Bassersdorf). Five gram sample was weighed into the sample cup and placed into Hygrolab water activity meter. Measurements were done at room temperature. When the partial pressure in the air above sample is unchanged, water activity is read from the monitor as % relative humidity of air $\times 100$.

3.2.1.7. Lipolysis Value

The acid degree value (meq KOH/100 g fat) is used as an index of lipolysis (Pillay, et al. 1980). Forty g of sample was crushed with kiselguhr. Then sample was washed with diethylether and filtered for four times. Diethyl ether was separated under vacuum at 60°C and 2 g of oil was weighed into an erlenmayer flask. Forty ml ether:alcohol (1:1) was added in to a flask. The free fatty acids were titrated with 0.1 N alcoholic KOH using methanolic phenolphthalein as the indicator.

Acid degree value = $(282 \times (\text{ml KOH used} - \text{ml blank}) \times \text{factor}) / (\text{gram oil} \times 100)$ (eq. 5)

3.2.2. Volatile Compound Analysis

For the extraction of volatile compounds solid-phase microextraction method was used. For this purpose, a fibre, provided by Supelco (57348-U, PA, USA) coated with the following sorbent material: Divinylbenzene/Carboxen/Polydimethylsiloxane was used. Samples were defrosted at 4°C before the day of analysis. The outer surfaces of samples were removed and the samples were grated. Three grams of grated samples were weighed into a 15 ml headspace vial, and a PTFE/butyl septum was immediately sealed with an aluminium crimp seal. Sample was equilibrated at 60°C at 500 rpm for 30 min. Then, fibre was inserted into headspace of the vial using SPME fibre holder. The sample was agitated at 500 rpm at 60 °C while the fiber was inside the vial. After 30 minutes, the fibre was inserted into the Gas chromatography injector and held for 5 min. The temperature of the injector port was 250°C. Perkin Elmer Clarus 600 Gas chromatography/olfactometry (Massachusetts, USA) equipped with flame ionization detector was used. The oven was temperature programmed as follows: hold at 40 °C for 6 minutes, then the temperature was raised to 100°C (5 °C/min, held 2 min) to a final temperature 250 °C (10°C/min, held for 4 min). Carrier gas was He with 1 ml/min flow rate. 30m×0.25 mm ID-BP20×0.25 capillary column was used.

The gas chromatography/olfactometry analysis was performed by one person, and aroma intensity was recorded as strong, medium, weak or not detected. A number of 9 was assigned to the strong aroma intensity, 6 to medium intensity, 3 to weak intensity and 0 to not detected (Qian and Reineccius 2003).

The changes in volatile composition during cheese production and ripening were investigated with GC/MS (Agilent 6890). Oven temperature was programmed as : hold at 40 °C for 6 minutes, then the temperature was raised to 100°C (5 °C/min, held 2 min) to a final temperature 220 °C (10°C/min, held for 5 min). Inlet temperature was 220 °C.

The analysis was done duplicate. Identification was done with comparing GC/MS mass spectral data, retention time and aroma with standards and Massa Spectral Library.

3.2.3. Texture Analysis

Cheese samples were cut into 25 mm cubes and stored in a refrigerator at 4°C on the day before analysis. Samples were taken from the refrigerator immediately prior to testing. TA.XT-Plus (Godalming, UK) was used. Samples were compressed between two stainless steel plates using a texture analyser with a 50 kg force load cell and a 75 mm compression plate. A double-bite compression cycle was carried out, with a rest period of 3 s between bites. Figure 6 shows the force versus time × speed curve from a two-bite compression test.

Samples were compressed to 30% of their original height at a speed of 1mm⁻¹ during each bite. Each cheese was tested in duplicate. Texture Profile Analysis (TPA) of compression testing was done. TPA parameters were derived from this test are defined in Table 6 (Everard, et al. 2007B).

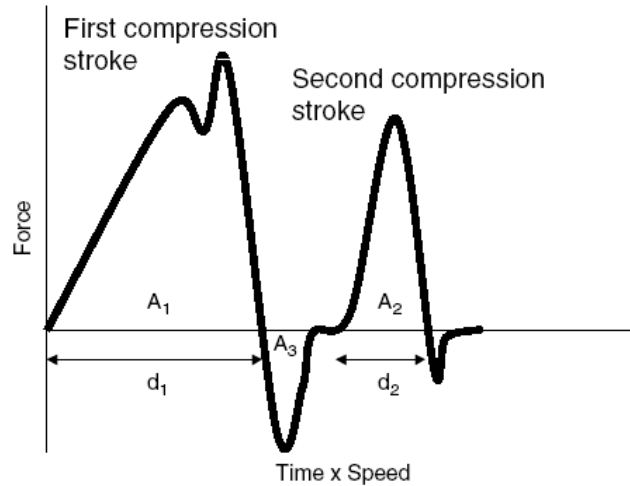


Figure 6. Force *versus* time \times speed curve from a two-bite compression test (Source: Everard, et al. 2007A).

Table 6. Texture profile analysis (TPA) parameters derived from force *versus* time \times speed curve (Source: Everard, et al. 2007A).

Parameter (units)	Definition
Rheological firmness, firmness (N)	Force at a given deformation (strain = 0.7) which is known as hardness.
Springiness (–)	Degree to which a sample returns to its original size after compression. It can be determined, in a double-bite compression test, as springiness = d_2/d_1 , where d_1 is the displacement at the end of the first compression stroke (or bite) and d_2 is the displacement from the point of contact on the second compression stroke to the end of the second compression stroke
Cohesiveness (–)	Resistance of sample separation into parts. Cohesiveness = A_2/A_1 , where A_2 is the area under the force–displacement curve corresponding to the second compression stroke and A_1 is the area under the force-displacement curve corresponding to the first compression stroke.
Adhesiveness (N mm)	Area (A_3) under the negative part of the force–displacement curve immediately after the first compression stroke, i.e. the work of suction during withdrawal of the compression plate
Chewiness (N)	Measure of the force required to masticate a solid food. (firmness \times cohesiveness \times springiness)

3.2.4. Sensory Analysis

A roundtable discussion with a five-member panel was conducted to identify the descriptive flavor terms for the sepet cheeses. Table 7 shows the samples which used in sensory analysis.

Panel members were selected based on willingness to participate and time available. The panelists were staff and graduate students in the Department of Food Engineering at Çanakkale Onsekiz Mart University. The panelists' ages ranged from 24 to 38 years. The panelists identified and defined the flavor terms from representative cheeses. During the training sessions, real food samples, chemicals were used to identify descriptors. The panelist received about 30 h of training during identification and definition of descriptive terms. Table 8 shows the descriptive terms used to define sepet cheese flavor. The panelists quantified the descriptive terms using 25-point product specific scales anchored on the left with “not” and on the right with “very.”

Table 7. Samples analyzed in sensorial analysis

Sample number	Milk type
1	Goat milk
8	
11	
9	
14	
28	Goat+cow milk
17	Cow milk
21	
22	
16	Ewe milk

Table 8. Language used to evaluate cheese flavor: term definitions and preparation of reference materials (Source: Reilly and York 2001, Meilgaard, et al. 1999).

Descriptor	Definition	Reference
Cooked	Aromatics associated with cooked milk	Milk heated to 85°C for 30 min
Whey	Aromatics associated with whey powder	Solubilize 5 g whey powder in 100 mL water
Creamy	Aromatics associated with milkfat	Cream or butter
Free fatty acids	Aromatics associated with butyric acid	10 mL butyric acid in methanol
Moisty cloth	Aroma associated with wet cloth	Moisty cloth
Store (Fridge)	Aromatics associated with warehouse	Long time stored cheese
Fruity	Aromatics associated with different fruits	Fresh pineapple (Ethyl hexanoate, 20 ppm)
Nutty	The nut-like aromatic associated with different nuts	Lightly toasted unsalted nuts wheat germ, unsalted wheat thins, roasted peanut oil extract
Metallic	Aroma associated with tin cans	Ferrous sulfate
Animal-like	Aromatics associated with barns and stock	5% Na-caseinate solution in water
Sulfurous	Aromatics associated with sulfurous compounds	Boiled mashed egg
Sour	Taste sensation elicited by acids	0.08% citric acid solution in water
Bitter	Taste sensation elicited by caffeine	0.08% caffeine solution in water
Salty	Taste sensation elicited by salts	0.5% sodium chloride solution in water
Sweet	Taste sensation elicited by sugars	2% sucrose solution in water
Umami	Chemical feeling factor elicited by certain peptides and nucleotides	1% monosodium glutamate solution in water
Acrid	Burning, irritating, pungent	Burnt wood, smoke
Bite	Chemical feeling factor elicited by carbonation on the tongue	Soda water

The panelists were provided with water, unsalted bread and expectoration cups to cleanse the palate between samples. The cheeses were presented in plastic plates and coded with three-digit numbers. Five samples were evaluated in each session. Panelists evaluated each cheese twice. Duplicate samples were served in different sessions (Yüceer, et al. 2007).

3.2.5. Microbiological Analysis

3.2.5.1. Sampling Procedure

Ten ml sample for milk and 10 g sample for cheese and curd was taken and homogenized in sterile 90 ml of 2 % sodium citrate at 45°C. Serial dilutions in sterile 0.1 % peptone water were prepared for bacterial analysis (Psoni, et al. 2003). Pour plate method was used for all bacteria enumeration except *Staphylococcus aureus* enumeration and incubated as inverse for all bacteria enumeration except mould and yeast enumeration. Two measurements were carried out and average values were represented. After the incubation, the plates with colony forming units (CFU) ranging from 30 and 300 were selected for isolation and enumeration. After the colony counting , the numbers were expressed in logarithmic scales ($\log \text{CFUg}^{-1}$).

3.2.5.2. Total Aerobic Count

Skim milk plate count agar was used for total aerobic count. Plates were incubated at 32°C for 72 h (Mucchetti, et al. 2008).

3.2.5.3. *Lactobacillus* spp. Enumeration

MRS agar was used for the enumeration of *Lactobacillus* spp.. Plates were incubated at 37°C for 48 h in sealed jar containing anaerogen sachet (Mucchetti, et al. 2008).

3.2.5.4. *Lactococcus* spp. Enumeration

M 17 agar was used for the enumeration of *Lactococcus* spp.. Plates were incubated at 37°C for 48 h (Mucchetti, et al. 2008).

3.2.5.5. *Enterococcus* spp. Enumeration

Kanamycin esculin azide agar was used for the enumeration of *Enterococcus* spp.. Plates were incubated at 37°C for 48 h. (Dolci, et al. 2007).

3.2.5.6. *Staphylococcus aureus* Enumeration

Baird Parker agar supplemented with egg yolk tellurite medium and spread plate method was used for the enumeration. Plates were incubated at 37°C for 48 h (Mucchetti, et al. 2008).

3.2.5.7. Yeast and Mould Enumeration

Yeast glucose chloromophenical agar was used for yeast and mould enumeration. Plates were incubated at 25°C for 5 days (Mucchetti, et al. 2008).

3.2.5.8. Coliform Bacteria Enumeration

Double layer violet red bile agar was used for the enumeration of coliforms. Plates were incubated at 37°C for 24 h (Mucchetti, et al. 2008).

3.2.5.9. Psychrotrophic Bacteria Enumeration

Skim milk plate count agar was used for the enumeration of psychrotrophic bacteria. Plates were incubated at 7°C for 10 days (Özdemir and Demirci 2006).

3.2.6. Phenotypic Identification of Lactic Acid Bacteria

3.2.6.1. Isolation of Lactic Acid Bacteria

Bacteria which were enumerated on MRS agar (pH 6.2-6.8), M 17 agar (pH 7.15) and Kanamycin esculin azide agar (pH 7.2) were isolated. Twelve randomly selected colonies of LAB were isolated from each plates that have 30-300 colonies. They were purified by two subsequent subcultures on Kanamycin esculin azide, M17 and MRS agar.

3.2.6.2. Long Term Preservation of the Isolates

Isolates that were purified were stored in MRS and M17 broth medium which contained 20% (v/v) glycerol as frozen stocks at -80°C before being subjected to identification analysis. Glycerol stock samples were prepared by mixing 0.5 ml of overnight cultures, and 40% glycerol.

3.2.6.3. Phenotypic Identification

Phenotypic identification was done according to Devriese et al. (2006), Hertel and Hames (2006) and Abdi et al. (2006), Teuber and Geis (2006) after the following tests.

3.2.6.3.1. Gram Staining

The overnight cultures were streaked on to M17, MRS, and Kanamycin esculin azide agar and incubated for 24 h. The cultures were taken from the plates aseptically and fixed on to a microscope slide with sterile % 0.9 NaCl by exposure to flame 2-3 times for 1-2 sec. The slide was flooded with crystal violet solution for up to 1 min and washed off with tap water and drained. Then, the slide was flooded with Gram's Iodine solution for about 1 min and washed off with tap water. After that excess water was removed from slide and flooded with 95% alcohol for 15 sec and wash off with tap water. After draining the slide was applied with safranin solution for 30 sec and washed off with tap water. Finally, the slide was drained and dried by blotting onto cotton towels. All slides of bacteria were examined under the oil immersion lens by light microscopy (Olympus DP25, Japan). Lactic acid bacteria are Gram positive. Therefore, Gram positive bacteria that have the blue-purple color under light microscopy after

gram staining were selected for further identification analysis. Cell shapes and arrangements were also examined by light microscope.

3.2.6.3.2. Catalase Test

The catalase test is used to detect the presence of catalase enzymes by the decomposition of hydrogen peroxide to release oxygen and water. In order to confirm catalase status of the isolates, overnight cultures were streaked onto M17, MRS, and Kanamycin esculin azide agar and incubated at 30°C for 24 h. 3% hydrogen peroxide solution was dropped onto randomly chosen colony. Lactic acid bacteria are catalase negative, so catalase negative microorganism that did not produce gas bubbles were chosen for further identification analysis.

3.2.6.3.3. Physiological and Biochemical Identification

Tests applied on all isolates were ability to grow at different temperatures and broth that contains different concentrations of NaCl, in 2%, 4% and 6.5% NaCl broth, CO₂ production from glucose, hydrolysis of arginine, sugar fermentation. For all tests non-inoculated media was used as a negative control. Each isolate was activated in 5 ml MRS broth for 24 h at 30°C before use. Therefore, overnight cultures were used during all the identification procedures.

3.2.6.3.3.1. Gas Production from Glucose

In order to identify heterofermentative and homofermentative isolates, CO₂ production from glucose test was applied. Fifty µl of overnight cultures were inoculated into the 8 ml citrate lacking MRS broths (Pepton 10.0 g/l, lab-lemco meat extract 10.0

g/l, yeast extract 5.0 g/l, D (-) glucose 20.0 g/l, tween 80 1 ml, K₂HPO₄ 2.0 g/l, Sodium acetate 5.0 g/l, MgSO₄.7H₂O 0.2 g/l, MnSO₄.4H₂O 0.05 g/l, deionized water 1000 ml at pH 6.2-6.6) and inverted Durham tubes and incubated for 5 days at 30°C (Bulut 2003). The isolates which produced gas in Durham tubes were identified as heterofermentative and the others were identified as homofermentative lactic acid bacteria.

3.2.6.3.3.2. Growth at Different Temperatures

After inoculation of 50 µl of overnight cultures into 5 ml MRS broth containing bromocresol purple (0.004 g/l) as pH indicator and they were incubated for 7 days at 10°C, 40 °C or 45 °C for cocci shaped isolates and 15°C, 45°C for rod shaped isolates. Cells which showed color change from purple to yellow were detected since they are able to grow at that temperature (Guessas and Kihal 2004, Bulut 2003).

3.2.6.3.3.3 Arginine Hydrolysis

For cocci shaped isolates, 8 ml of Reddy broth (Peptone 5.0 g/l, yeast extract 5.0 g/l, K₂HPO₄ 1.0 g/l, arginine hydrochloride 5.0 g/l, sodium citrate 20.0 g/l, bromocresol purple 0.002 g/l, deionized water 1000 ml at pH 6.2) and inverted Durham tubes were used for arginine hydrolysis, gas production from citrate tests (Bulut 2003). Fifty µl of overnight cultures were inoculated into the Reddy broth and incubated at 30°C for 5 days. At end of the 5th day, the cultures which utilize arginine, assume violet color by producing ammonia, but the cultures which do not utilize arginine assume a deep-yellow color by producing lactic acid only. In addition to arginine hydrolysis, gas accumulation in inverted Durham tubes indicated citrate utilization.

For rod shaped isolates, fifty µl overnight cultures were inoculated into 5 ml arginine MRS broth (Peptone 10.0 g/l, yeast extract 5.0 g/l, tween 80 1 ml, K₂HPO₄ 2.0 g/l, sodium acetate 5.0 g/l, sodium citrate 2.0 g/l, MgSO₄.7H₂O 0.2g/l, MnSO₄.4H₂O 0.05 g/l, arginine 1.5 g/l, deionized water 1000 ml at pH 6.2 - 6.6) and were incubated

at 30°C for 5 days (Bulut 2003). After the incubation, ammonia production was detected by using sterile Nessler reagent. After 100 µl of culture broth were pipetted into each well of the microtitre plates, immediately 100 µl of Nessler reagent were added. Ammonia production was decided due to the immediate orange color formation.

3.2.6.3.3.4. Growth at Different NaCl Concentrations

Ability to grow at 2%, 4% or 6.5% NaCl concentrations was tested for all cocci shaped isolates. Ability to grow at 6.5% NaCl concentrations was tested for rod shaped isolates. Fifty µl of overnight cultures were inoculated into 5 ml MRS broth containing 2, 4, 6 % NaCl and bromocresol purples (0.004 g/l) as pH indicator. Then the test media was incubated at 30°C for 7 days. The change of the color from purple to yellow indicated that cells could grow at that concentration (Guessas and Kihal 2004, Bulut 2003).

3.2.6.3.3.5. Carbohydrate Fermentations

In order to classify the genus to species carbohydrate fermentation including L(+)-arabinose, D(+) galactose, lactose, maltose, D-mannitol, raffinose, sucrose, D(-) salicin, sorbitol, D(+) trehalose, D(+)xylose, glycerol, D(+)mannose, D(-)ribose were tested. Overnight cultures were centrifuged for 10 min at 10000 rpm. Pelleted cells were washed and mixed with MRS broth without glucose containing bromocresol purple as the pH indicator (Peptone 10.0 g/l, lab-Lemco meat extract 10.0 g/l, yeast extract 5.0 g/l, tween 80 1 ml, K₂HPO₄ 2.0g/l, sodium acetate 5.0 g/l, triammonium citrate 2.0 g/l, MgSO₄.7H₂O 0.2 g/l, MnSO₄.4H₂O 0.05 g/l, bromocresol purple 0.04 g/l, deionized water 1000 ml at pH 6.2- 6.6) for two times. Forty µl of filter sterilized (0.25µm, Millipore) 10% sugar solutions were pipetted into 96-well microtitre plates. Then, 160 µl suspended cells were added onto sugar solutions (Bulut 2003). After 24 h incubation at 30°C, the results were read at 590 and 615 nm absorbance in Thermo vario scan flash.

Sugar fermentation was decided according to color change from purple to yellow and turbidity. Glucose fermentation was done as a positive control. Samples without sugar were used as negative control.

3.2.7. Statistical Analysis

Mean values, standard deviations, maximum and minimum values were calculated for all the determined parameters. Pearson's correlation coefficients were also calculated to determine linear relations between the characteristics of Sepet cheeses. Analysis of variance was performed to investigate the differences ($p < 0.05$) in characteristics during production and ripening. Student-Newman-Keuls range test was also applied for comparison of the mean values during production and ripening. Multidimensional Scaling (MDS) method was applied in this study to provide a visual representation of the pattern of similarities or distances among a set of objects. Since different scales were used to measure characteristics of sepet cheeses, values were standardized before computing proximities. SPSS (version 13; SPSS Institute Inc., Chicago, IL) was used for all statistical analysis.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Chemical and Physical Characterization of Sepet Cheeses

Thirty cheese samples collected from several farmer's market, dairy farms and towns were analyzed to determine the quality characteristics of sepet cheeses. Changes during cheese production and ripening were also investigated. Sepet cheese samples show similar characteristics with tulum, kp, mihali cheeses in terms of their inoculation temperatures and with white cheese in terms of storage in brine. Therefore results were compared with these cheeses and some other traditional cheeses.

4.1.1. Total Solid Contents of Sepet Cheeses

Total solid contents of all sepet cheese samples were given in Table 9. The average total solid content of sepet cheeses is 54.33 %. Minimum and maximum values for sepet cheese total solid content are 44.56 %, 64.39 %, respectively. Total solid contents change according to type and time of syneresis, type of salting, curd size, preripening (nc 2004). Figure 8 shows the similarities of sepet cheese samples according to chemical and physical characteristics. Samples 1, 11, 10 placed away from other cheese samples since their total solid contents were lower than the total solid contents of other sepet cheese samples.

Table 9. Results of chemical and physical analysis (mean value±standard deviation)

Sample number	pH	Acidity (%)	NaCl (%)	Fat (%)	Total solid content (%)	Water activity
1	5.35±0.07	1.74±0.06	8.62±0.16	15.05±0.07	45.03±0.65	0.82±0.00
2	5.46±0.01	1.99±0.16	11.47±0.99	25.25±1.06	53.77±0.06	0.78±0.00
3	4.96±0.06	2.65±0.12	11.35±0.17	22.50±0.71	57.50±0.23	0.76±0.00
4	5.24±0.01	1.98±0.25	10.73±0.95	21.00±1.41	49.78±0.75	0.75±0.01
5	5.29±0.01	1.89±0.13	5.79±0.25	22.00±0.00	55.72±0.35	0.80±0.00
6	5.46±0.02	1.35±0.13	9.07±0.25	25.50±0.71	50.51±0.76	0.79±0.00
7	5.33±0.01	1.15±0.10	8.07±0.50	27.75±1.06	62.00±0.03	0.78±0.00
8	5.45±0.01	1.46±0.23	8.53±0.10	27.25±0.35	63.32±0.21	0.73±0.00
9	5.44±0.00	1.65±0.03	8.00±0.25	26.50±0.71	64.39±0.41	0.75±0.00
10	6.07±0.00	0.60±0.08	4.19±0.35	26.00±0.00	44.56±0.60	0.81±0.00
11	5.19±0.02	1.79±0.08	8.25±0.10	27.25±0.35	48.78±0.29	0.78±0.00
12	5.27±0.02	1.87±0.04	7.79±0.05	26.75±0.35	49.99±0.00	0.78±0.00
13	5.74±0.01	1.06±0.04	4.37±0.20	25.50±0.71	53.73±0.07	0.80±0.00
14	6.21±0.02	0.89±0.05	9.46±0.24	27.50±0.35	53.56±0.31	0.79±0.00
15	6.10±0.00	1.07±0.05	10.95±0.11	26.13±0.35	56.88±0.17	0.78±0.00
16	5.18±0.01	2.11±0.08	9.09±0.30	23.75±0.35	48.82±0.18	0.79±0.00
17	6.03±0.01	0.92±0.08	7.40±0.10	26.50±0.00	53.78±0.01	0.80±0.00
18	5.73±0.04	0.98±0.15	3.38±0.20	25.25±0.35	47.93±0.08	0.90±0.00
19	5.50±0.01	2.10±0.17	3.74±0.66	25.50±0.71	48.06±0.14	0.88±0.00
20	5.47±0.03	2.03±0.10	2.81±0.33	29.00±0.71	56.05±0.14	0.89±0.00
21	5.37±0.00	2.83±0.07	4.91±0.17	24.75±0.35	55.18±0.44	0.89±0.00
22	6.47±0.01	0.81±0.13	5.32±0.08	27.50±0.71	58.05±0.06	0.88±0.00
23	6.45±0.02	0.87±0.03	6.21±0.06	27.25±0.35	58.44±0.30	0.88±0.00
24	5.56±0.01	2.25±0.02	3.19±0.53	24.75±0.35	55.77±0.10	0.90±0.00
25	5.69±0.01	2.01±0.05	5.49±0.38	24.00±0.00	56.61±0.22	0.90±0.00
26	5.11±0.01	2.27±0.05	13.69±0.50	19.50±0.71	50.73±0.56	0.75±0.00
27	5.07±0.01	2.28±0.15	7.75±0.40	28.25±0.35	57.81±0.17	0.76±0.00
28	5.24±0.01	1.76±0.03	4.93±0.20	26.50±0.71	58.46±0.15	0.82±0.00
29	6.41±0.00	0.70±0.08	5.57±0.30	25.00±0.00	61.53±0.09	0.81±0.00
30	5.39±0.01	2.85±0.21	2.81±0.66	23.50±1.41	53.18±0.31	0.90±0.00
Minimum	4.96	0.60	2.81	15.05	44.56	0.74
Maximum	6.47	2.85	13.69	29.00	64.39	0.90
Average	5.57	1.66	7.10	25.11	54.33	0.82

Total solid contents of sepet cheeses showed similarities with Sıkma cheese, Tulum cheese, and K p cheese. K p cheese had 51.49 % total solid content (G ven and Karaca 2004). Ceylan et al.(2004) found average 53 % total solid content for Sıkma cheeses. Kamber (2008A) reviewed that total solid contents of Tulum cheese samples changed from 63.45 % to 30 %. Kınık et al.(1999), B ke (1981), Karakaş and Korukluođlu (2006) also investigated some properties of sepet cheese samples and total solid content values were in agreement with our results.

The changes in total solid content during Sepet cheese production and ripening were also investigated. Table 10 shows the changes in physical and chemical characteristics of sepet cheese samples during production and ripening. Cheese is rich in fat and casein constituents of milk, which are retained in the curd during production, and it contains relatively small amounts of water soluble constituents (whey proteins, lactose, and water-soluble vitamins), which partition mainly into the whey (Fox, et al. 2000). As a result, Total solid content increased significantly during cheese production ($p<0.001$). The total solid contents of curd and whey mixtures were 33.13 % for Zeytineli cheese and 32.56 % for Germiyan cheese. These values increased to 47.13 %, 48.60% at 1st day, 49.77 %, 54.32 % at 1st month, 51.93 %, 55.88 % at 2nd month, 52.29 %, 56.70 % at 3rd month, 53.07%, 56.63% at 4th month and 53.57 %, 56.89 % at 6th month for Zeytineli cheese and Germiyan cheese, respectively. However, the increase in ripening of Germiyan cheese was not found statistically significant ($p>0.05$). During ripening, increase in salt content cause a sligth increase in total solid content (Fox, et al. 2000).

4.1.2. Fat Contents of Sepet Cheeses

Fat plays several important functions in cheese: it effects cheese firmness, adhesiveness, mouth-feel, and flavor (Fox, et al. 2000). Fat contents of all sepet cheese samples are given in Table 9. Figure 8 shows the similarities of sepet cheese samples based on chemical and physical characteristics. Sample 1 did not grouped together with other cheeses since its fat content was lower than the fat contents of the other cheeses.

The average fat content of Sepet cheese samples was 25.11 %. Maximum fat content was 29 % and minimum fat content was 15.05 %. Variations in fat contents are related to variations in source of milk, types of clotting and syneresis. Turkish tulum cheese standard TS-3001 (2006) suggest that 1st class whole fat tulum cheese should have minimum 45 % fat in total solid content. Sepet cheeses contain approximately 46.21 % fat in total solid content. Only 8 sepet cheese samples had slightly lower fat content in total solid content than 45 %. Therefore, sepet cheeses could be considered in whole fat cheese class. Also, fat content of Sepet cheese are similar with fat content of Sıkma cheese and higher than the fat contents of Küp and Mihaliç cheeses. Ceylan et al. (2003) found 43.87 % fat in dry matter in Sıkma cheese. Mihaliç cheese and Küp cheese had 35.6-39.7 % fat in total solid content, 13.56 % fat contents, respectively (Kamber 2008C, Güven and Karaca 2004).

Table 12 shows the correlation coefficients between chemical and physical characteristics of sepet cheese samples. Fat and total solid contents of sepet cheese samples were correlated significantly because cheese is concentrated form of milk constituents (Blom and Weren 2002). Kınık et al. (1999), Büke (1981), Karakaş and Korukluoğlu (2006) found 23.86 %, 27.53 %, 24.50 % fat for sepet cheese samples, respectively. These results showed similarities with our results.

During cheese production, fat content increased significantly due to increase in total solid content (Table 10). Fat content of both Zeytineli milk and Germiyan milk was 3.22 %. Then, these values increased to 22.00 % fat content during cheese production and reached 27.50 % at the end of 6th month for Zeytineli cheese. Also, fat content increased to 26.13 % value for Germiyan cheese at the end of ripening. The changes during cheese production and ripening were statistically significant ($p < 0.001$). However fat content in total solid content during ripening did not change significantly ($p > 0.05$) for Zeytineli and Germiyan cheeses.

Table 10. Chemical and physical changes during production and ripening

Sample	Location	pH	Acidity (%)	NaCl (%)	Fat (%)	Fat in total solid (%)	Total solid content (%)	Water activity	Protein(%)	Nitrogen in Total solid (%)	pH 4,4 soluble nitrogen(%)	% 12 TCA soluble nitrogen (%)	Ripening Index
Goat milk		6.68±0.04 ^a	0.05±0.00 ^a	-	5.22±0.00 ^a	-	-	0.97±0.00 ^a	3.65±0.00 ^a	-	-	-	-
Curd+whey		6.39±0.01 ^b	0.47±0.01 ^b	0.82±0.17 ^a	14.25±0.35 ^b	43.01±0.78 ^a	33.13±0.22 ^a	0.93±0.00 ^b	17.82±0.14 ^b	8.91±0.12 ^a	0.03±0.01 ^a	0.01±0.01 ^a	1.26±0.49 ^a
1st day cheese		6.24±0.01 ^c	0.83±0.01 ^c	1.89±0.03 ^b	22.00±0.71 ^c	46.68±1.29 ^b	47.13±0.21 ^b	0.92±0.00 ^c	26.01±0.01 ^c	8.65±0.04 ^b	0.09±0.01 ^b	0.03±0.00 ^b	2.21±0.17 ^b
1st month cheese		6.17±0.01 ^d	0.93±0.01 ^d	8.95±0.10 ^c	25.00±0.35 ^d	50.73±0.78 ^c	49.77±0.07 ^c	0.88±0.00 ^d	27.66±0.05 ^d	8.72±0.03 ^b	0.17±0.00 ^c	0.06±0.00 ^c	4.06±0.06 ^c
2nd month cheese		6.15±0.00 ^d	0.96±0.06 ^{cd}	8.89±0.00 ^c	26.25±0.35 ^d	51.54±0.68 ^c	51.93±0.02 ^d	0.87±0.00 ^e	28.27±0.16 ^c	8.70±0.04 ^b	0.23±0.00 ^d	0.06±0.00 ^c	5.34±0.06 ^d
3rd month cheese		6.15±0.01 ^d	0.98±0.04 ^{cd}	9.01±0.17 ^c	26.75±0.35 ^d	51.16±0.43 ^c	52.29±0.25 ^e	0.85±0.00 ^f	28.60±0.07 ^f	8.57±0.06 ^b	0.24±0.01 ^{cd}	0.07±0.01 ^d	5.40±0.17 ^d
4th month cheese		6.15±0.04 ^d	1.03±0.01 ^{de}	9.22±0.16 ^{cd}	27.25±0.35 ^d	51.35±0.81 ^c	53.07±0.15 ^f	0.81±0.00 ^g	28.96±0.07 ^g	8.55±0.02 ^b	0.25±0.00 ^{cd}	0.08±0.01 ^{cd}	5.70±0.08 ^d
6th month cheese		6.10±0.03 ^d	1.07±0.01 ^f	9.46±0.10 ^d	27.50±0.00 ^f	51.34±0.30 ^c	53.57±0.31 ^g	0.79±0.00 ^h	29.23±0.03 ^h	8.55±0.04 ^b	0.26±0.00 ^e	0.09±0.00 ^e	5.88±0.09 ^d
Goat milk		6.80±0.01 ^a	0.05±0.00 ^a	-	5.22±0.00 ^a	-	-	0.97±0.00 ^a	3.67±0.00 ^a	-	-	-	-
Curd+whey		6.47±0.02 ^b	0.37±0.01 ^b	0.76±0.08 ^a	13.50±0.35 ^b	41.45±0.86 ^a	32.56±0.17 ^a	0.94±0.00 ^b	18.60±0.28 ^b	9.02±0.09 ^a	0.03±0.01 ^a	0.01±0.00 ^a	1.08±0.50 ^a
1st day cheese		6.36±0.01 ^c	0.74±0.01 ^c	2.12±0.14 ^b	20.25±0.35 ^c	41.66±0.31 ^a	48.60±0.49 ^b	0.92±0.00 ^c	27.54±0.19 ^c	8.90±0.03 ^a	0.09±0.00 ^b	0.04±0.00 ^b	2.16±0.03 ^b
1st month cheese		6.25±0.01 ^d	0.84±0.00 ^d	9.15±0.20 ^c	23.5±0.00 ^d	43.26±0.36 ^{ab}	54.32±0.45 ^c	0.87±0.00 ^d	29.19±0.10 ^d	8.49±0.10 ^c	0.17±0.01 ^c	0.06±0.01 ^{bc}	3.73±0.34 ^c
2nd month cheese		6.24±0.00 ^d	0.86±0.01 ^d	9.98±0.24 ^d	25.25±0.35 ^c	45.19±0.74 ^b	55.88±0.13 ^d	0.84±0.00 ^e	29.89±0.08 ^e	8.35±0.04 ^c	0.23±0.00 ^d	0.08±0.01 ^{cd}	4.99±0.09 ^d
3rd month cheese		6.24±0.01 ^d	0.86±0.01 ^d	10.56±0.05 ^e	25.75±0.35 ^c	45.41±0.29 ^b	56.70±0.42 ^d	0.81±0.00 ^f	30.26±0.05 ^f	8.33±0.05 ^c	0.23±0.01 ^d	0.08±0.00 ^d	4.84±0.31 ^d
4th month cheese		6.23±0.00 ^d	0.87±0.01 ^d	10.79±0.17 ^e	25.75±0.35 ^c	45.47±0.96 ^b	56.63±0.41 ^d	0.79±0.00 ^g	30.42±0.14 ^f	8.35±0.10 ^{bc}	0.25±0.01 ^d	0.09±0.01 ^d	5.07±0.09 ^d
6th month cheese		6.21±0.01 ^d	0.89±0.01 ^e	10.95±0.11 ^e	26.13±0.18 ^e	45.93±0.41 ^b	56.89±0.12 ^d	0.78±0.00 ^h	30.89±0.08 ^g	8.48±0.04 ^b	0.26±0.02 ^d	0.09±0.00 ^d	5.20±0.43 ^d

(a-g) means within columns are significantly different (P < 0.05) according to the Student-Newman-Keuls test.

4.1.3. Proteolysis Values of Sepet Cheeses

Fox et al. (2000) reviewed that proteolysis during ripening is essential in most cheese varieties. The extent of proteolysis varies from very limited (e.g., Mozzarella) to very extensive (e.g., Blue varieties). Tarakçı and Kuçukoner (2006) reviewed that these peptides and amino acids are contributed mainly by the action of microorganisms on the caseins and their peptides.

Sepet cheese samples were analyzed to determine protein contents and ripening indices. Table 11 shows the protein contents and nitrogen fraction values of sepet cheeses. The average protein content of sepet cheese was 28.99 %. Maximum protein content was 33.69 %. Minimum protein content was 24.40 %. Variations in protein contents were related to variations in the composition of milk and production methods. These protein content values were found to be nearly the same with Cheddar cheese. Fox et al. (2000) reported that Cheddar cheese had 31.5% protein content. Kamber (2008A,C) also reviewed that mihaliç cheese had 24.8 - 26.5 % protein content and protein contents of tulum cheeses changed from 19.1 % to 40 %. Protein content values of sepet cheeses were higher than protein content of Mihaliç cheese and some Tulum cheese samples. The protein content values of sepet cheese samples were higher than values that are reported in previous studies related to sepet cheeses. Previous studies reported that sepet cheese had 18.49 - 24.22 % protein content (Kınık, et al. 1999, Büke 1981).

Protein degradation is a part of the cheese aging process. Protease activity of milk-clotting enzymes and proteases from starter and nonstarter bacteria carry out the normal protein breakdown in cheese (Bynum and Barbano 1985). pH 4.6 soluble and 12% trichloroacetic acid soluble nitrogen fractions were indices of proteolysis. Average pH 4.6 soluble fraction was 0.49 %. Minimum value was 0.16 % and maximum value was 1.56 % for pH 4.6 soluble fraction of sepet cheese. Average trichloroacetic acid soluble nitrogen fraction was 0.39 %. Minimum value was 0.09 % and maximum value was 1.66% for trichloroacetic acid soluble nitrogen fraction values of sepet cheeses. Maximum ripening index for sepet cheeses was 34.53. Minimum ripening index was 3.32. The average ripening index was 10.84 for sepet cheese samples. Nineteen sepet cheese samples had lower ripening index value than average value.

Table 11. Proteolysis values of sepet cheeses (mean value±standard deviation)

Sample number	Milk Type	Total nitrogen (%)	Protein (%)	pH 4.6 soluble nitrogen fraction (%)	12% trichloroacetic acid soluble nitrogen farction (%)	Ripening Index
1	Goat	4.94±0.04	31.55±0.25	0.16±0.00	0.16±0.00	3.32
2		4.80±0.16	30.65±1.01	0.37±0.06	0.36±0.04	7.73
3		4.05±0.04	25.83±0.25	0.40±0.10	1.66±0.10	9.86
4		3.88±0.04	24.75±0.25	0.50±0.05	0.65±0.02	13.00
5		4.10±0.04	26.18±0.25	1.00±0.04	0.79±0.02	24.40
6		4.61±0.12	29.40±0.76	0.92±0.04	0.54±0.02	19.90
7		4.33±0.04	27.61±0.25	0.23±0.02	0.15±0.02	5.34
8		4.36±0.08	27.79±0.51	0.34±0.02	0.20±0.01	7.88
9		4.30±0.08	27.43±0.51	0.37±0.02	0.27±0.04	8.63
10		4.66±0.12	29.76±0.76	0.81±0.08	0.92±0.04	17.30
11		5.28±0.20	33.69±1.26	0.44±0.00	0.20±0.02	8.36
12		5.00±0.20	31.90±1.26	0.36±0.04	0.19±0.00	7.14
13		4.55±0.04	29.04±0.25	0.40±0.10	0.18±0.03	8.77
14		4.58±0.17	29.23±0.12	0.26±0.00	0.09±0.01	5.88
15		4.84±0.22	30.89±0.11	0.26±0.20	0.09±0.02	5.22
16	Ewe	4.26±0.04	27.17±0.25	0.96±0.08	0.37±0.06	22.50
17	Cow	4.92±0.08	31.37±0.51	0.55±0.04	0.34±0.02	11.30
18		4.50±0.12	28.69±0.76	0.27±0.04	0.19±0.04	6.07
19		4.75±0.16	30.29±1.01	0.33±0.04	0.29±0.06	6.93
20		4.75±0.16	30.29±1.01	0.21±0.03	0.18±0.02	4.42
21		4.52±0.08	28.86±0.51	1.56±0.04	1.27±0.06	34.53
22		4.80±0.08	30.65±0.51	1.24±0.02	0.61±0.00	25.80
23		4.56±0.10	29.13±0.63	0.56±0.05	0.38±0.03	12.30
24		4.46±0.08	28.51±0.50	0.33±0.04	0.29±0.02	7.39
25		4.88±0.04	31.19±0.25	0.36±0.02	0.36±0.02	7.37
26		4.44±0.12	28.33±0.76	0.32±0.02	0.27±0.04	7.10
27	Goat+ewe	4.36±0.08	27.79±0.51	0.48±0.06	0.40±0.06	11.10
28	Goat+cow	4.64±0.07	29.58±0.51	0.20±0.02	0.19±0.04	4.38
29		4.36±0.08	27.79±0.51	0.32±0.02	0.15±0.02	7.23
30	Cow+ewe	3.82±0.12	24.40±0.76	0.16±0.04	0.15±0.02	4.21
Maximum		5.28	33.69	1.56	1.66	34.53
Minimum		3.82	24.40	0.16	0.09	3.32
Average		4.54±0.33	28.99±2.12	0.49±0.34	0.39±0.36	10.84

Sample 21, 22, 5, 16, 10 had relatively higher ripening indices than of the other cheeses. Therefore, they were placed away from the other cheese samples in Figure 8. Table 12 shows the correlation coefficients between chemical and physical characteristics of sepet cheese samples. It was found that ripening index and lypolysis values of sepet cheese samples were correlated significantly with 0.541 ($p < 0.01$) correlation factor. Both ripening index and lypolysis values are related to biochemical events during ripening.

During production and ripening period, at the beginning Zeytineli milk had 3.65 % and Germiyan milk had 3.67 % protein content. Then these values increased to 26.01 % and 27.54 % during cheese production due to concentration of milk constituents into cheese. During ripening period these values reached to 29.23 % and 30.89 % for Zeytineli cheese and Germiyan cheese, respectively. Slight increase during ripening also investigated in the study concluded by Horne et al. (2005). pH 4.6 soluble nitrogen fractions and 12 % trichloroacetic soluble fractions increased during production and ripening. Increase in pH 4.6 soluble fractions were higher than 12 % trichloroacetic acid soluble nitrogen fractions because while 12 % trichloroacetic acid soluble nitrogen fractions is indication of the amount of small peptides and amino acids present in cheese, the pH 4.6 soluble fraction contains all proteins except casein, all peptides, amino acids, and amine (Metin 2006).

4.1.4. Salt Contents of Sepet Cheeses

Salt plays a major role in the texture, flavor, and microbial quality of cheese (Günasekaran and Ak 2003). Sepet cheeses were analyzed for salt content and it was found that average salt content of sepet cheeses was 7.10 %. Salt contents of sepet cheeses were given in Table 9. The maximum salt content of sepet cheeses was 13.69 % and the minimum salt content was 2.81 %. TS 591 (2006) and TS 3001 (2006) stated that salt content of white cheese in total solid content should not exceed 10 % and salt content of 1st class tulum cheese in total solid content should not exceed 6 %. The salt contents in dry matter of 17 sepet cheeses were higher than of white cheese limits. Only 3 cheese samples had salt content in total solid content less than 6 %. Salt contents of

sepet cheese samples showed similarities with salt contents of Mihaliç cheeses. Kamber (2008C) reviewed that salt contents of mihaliç cheeses changed from 7.5 % to 9.3 %. These results were also higher than values that reported in previous studies about sepet cheeses. Kınık et al. (1999), Büke (1981), Karakaş and Korukluoğlu (2006) found 1.062 %, 5.31 %, 1.118 % salt contents for sepet cheese samples.

During ripening, salt content significantly increased up to 1st month ($p < 0.001$) and continued to increase slightly during ripening ($p < 0.05$). At the end of ripening, the salt content of Zeytineli and Germiyan cheeses reached 9.46 % and 10.95 %, respectively.

4.1.5. The pH and Titratable Acidity Values of Sepet Cheeses

The pH values of sepet cheeses were between 4.96 and 6.47. The average pH value of Sepet cheese samples was 5.57. TS 591(2006) stated that white cheese should have minimum 4.5 pH. All sepet cheese samples had higher pH value than 4.5. Table 9 shows the pH values of sepet cheese samples. These values were also in agreement with previous studies. Kınık et al. (1999), Karakaş and Korukluoğlu (2006) found 5.22, 5.46 pH values for sepet cheeses. Demir (2008) investigated characteristics of Çirek cheese and found that Çirek cheese had 5.18 pH value. Polat and Yetişmeyen (2004) also found that Civil cheese had 4.68 pH value. The pH values of sepet cheese samples are nearly the same with Çirek cheese, but higher than of Civil cheese.

Figure 7 shows relations between physical and chemical characteristics and Table 12 also shows the correlation coefficients of chemical and physical characteristics of sepet cheese samples. pH values of sepet cheese samples were correlated significantly with acidity values. Correlation factor was found as -0.762 with p value less than 0.001.

Changes of pH values during sepet cheese production and ripening were investigated. pH values decreased from 6.68 to 6.27 and 6.80 to 6.36 during Zeytineli and Germiyan cheese production, respectively. During ripening, pH values reached to 6.10 and 6.21. Table 10 shows the changes during production and ripening periods. While the changes were found to be significant with p values less than 0.001, changes

from the 1st month to 6th month was not significant ($p>0.05$). Table 13 shows the correlations between chemical and physical changes during production and ripening. All changes were significantly correlated.

SePET cheese samples had maximum 2.85 % and minimum 0.6 % titratable acidity. It was found that average titratable acidity of sePET cheese was 1.66 %. The values of titratable acidity are given in Table 9. These values were higher than previous studies. Kınık et al. (1999), Karakaş and Korukluoğlu (2006) found 1.42 % and 0.72 % titratable acidity for sePET cheeses. TS 591 (2006), TS 3001 (2006) suggest that titratable acidity should not exceed 3 % as lactic acid in cheeses. The titratable acidity values of all sePET cheeses did not exceed 3 %. The titratable acidity values of sePET cheeses are nearly the same with the titratable acidity values of Örgü cheese and higher than of Civil cheese. Türkoğlu et al. (2003) stated that Örgü cheese had 1.11% acidity. Acidity value of civil cheese was found as 0.933 % by Polat and Yetişmeyen (2004). Kamber (2008A,C) reviewed that the titratable acidity values of tulum cheese and mihaliç cheese had 2.60-1.09 %, 1.68-1.43 %, respectively. Titratable acidity values of SePET cheese samples showed similarities with titratable acidity values of tulum and mihaliç cheeses.

Table 12 . Correlation coefficients between chemical and physical characteristics of SePET Cheeses

	pH	Acidity (%)	NaCl (%)	Fat (%)	Total Solid (%)	Water activity	Protein (%)	Ripening index
Acidity (%)	-0.762***	1.000						
NaCl (%)	-0.191	0.061	1.000					
Fat (%)	0.307	-0.318	-0.314	1.000				
Total solid (%)	0.170	-0,085	0.001	0.445*	1.000			
Water activity	0.295	0.087	-0.781***	0.089	-0.122	1.000		
Protein (%)	0.286	-0.328	-0.018	0.225	-0.248	0.140	1.000	
Ripening index	0.027	0.064	-0.073	0.003	-0.069	0.104	-0.140	1.000
Lypolysis	-0.356	0.381	0.324	-0.240	-0.116	-0.173	-0.217	0.541**

Significant *p value < 0.05, **p value < 0.01, ***p value < 0.001

During cheese production titratable acidity significantly increased ($p < 0.001$). Tarakçı and Kuçukoner (2006) stated that the initial increase in acidity is due to lactic acid formation. Through ripening, titratable acidity did not change significantly ($p > 0.01$). Table 10 shows the changes through cheese production and ripening. Calleja et al. (2006) investigated Valdeteja raw goat's milk cheese through production and ripening and found a significant increase through production and these results were in agreement with Sepet cheese production values.

4.1.6. Water Activity Values of Sepet Cheeses

Water activity is a measure of the availability of water for biological functions and relates to water present in a food in free form. Water activity affects the microbial activity during ripening. Fox et al. (2002) stated that water activity value of less than 0.92 is necessary to prevent bacterial growth.

Sepet cheeses had maximum 9.90 and minimum 0.74 water activity values. Average water activity value was 0.82. Eight sepset cheese samples had higher water activity than 0.82. Fox et al. (2002) also mentioned correlation between salt content and water activity correlation. Salt content and water activity values of Sepet cheeses were correlated significantly. The high salty sepset cheeses had lower water activity values. Table 12 shows the correlation coefficients. Table 9 shows the water activity values of Sepet cheeses.

During production and ripening of sepset cheeses, water activity values decreased significantly ($p < 0.001$) due to increase in total solid content and presence of salt. Table 10 shows the changes in salt contents during production and ripening. At the beginning, both Zeytineli goat milk and Germiyan goat milk had 0.97 water activity value. Then, during cheese production these values decreased to 0.92 and 0.94 for Zeytineli and Germiyan cheeses, respectively. At the end of ripening, Zeytineli cheese reached 0.78 and Germiyan cheese reached 0.79 water activity value. Calleja et al. (2002) investigated Valdetaje goat milk cheese during production and ripening and stated decrease in water activity.

Table 13. Correlation coefficients between physical and chemical characteristics throughout the production and ripening of a) Zeytineli sepet cheese, b) Germiyan sepet cheese

Determinations for a)	pH	Acidity (%)	NaCl (%)	Fat (%)	Total solid content (%)	Water activity	Protein(%)
pH	1.000						
Acidity (%)	-0.994***	1.000					
NaCl (%)	-0.910**	0.877**	1.000				
Fat (%)	-0.991***	0.998***	0.910**	1.000			
Total solid (%)	-0.981***	0.994***	0.860*	0.994***	1.000		
Water activity	0.841**	-0.863**	-0.824*	-0.859**	-0.763*	1.000	
Protein (%)	-0.994***	0.985***	0.862*	0.981***	0.999***	-0.794*	1.000
Ripening index	-0.931**	0.913**	0.955**	0.931**	0.896**	-0.905**	0.887**

Determinations for b)	pH	Acidity (%)	NaCl (%)	Fat(%)	Total solid content(%)	Water activity	Protein (%)
pH	1.000						
Acidity (%)	-0.982***	1.000					
NaCl (%)	-0.963***	0.867*	1.000				
Fat (%)	-0.985***	0.993***	0.938**	1.000			
Total solid (%)	-0.977***	0.997***	0.897**	0.991***	1.000		
Water activity	0.842**	-0.851**	-0.935**	-0.896**	-0.838*	1.000	
Protein (%)	-0.992**	0.988***	0.853*	0.981***	0.995***	-0.810**	1.000
Ripening index	-0.961**	0.893**	0.977***	0.964***	0.920**	-0.953**	0.889**

Significant *p value < 0.05, **p value < 0.01, ***p value < 0.001

4.1.7. Lypolysis Values of Sepet Cheeses

Lipolysis is considered to be undesirable in most cheese varieties. However, some cheese varieties are characterized by extensive lipolysis (e.g., Romano, Parmesan, and Blue cheeses) (Fox, et al. 2000). Lypolysis values of sepet cheeses are given in Table 14.

Average lypolysis value of sepet cheeses was 7.31. Maximum value was 41.63 belonging to ewe sepet cheese. Minimum value was 1.64 belonging to cow sepet cheese. The degrees of lypolysis in sepet cheeses varied widely. Lypolysis are related with lipases in cheese originate from milk, rennet preparation (paste), starter, adjunct starter, or nonstarter bacteria which could be different during production of sepet cheese samples (Üçüncü 2004). Kınık et al. (1999) also explained that the reasons of variations in lypolysis values of sepet cheese samples could be originate from sampling at different ripening periods, microbial loads and variations in production methods. Figure 7 shows the relationships between chemical and physical characteristics. Lypolysis and ripening index values were seen to be related in Figure 7.

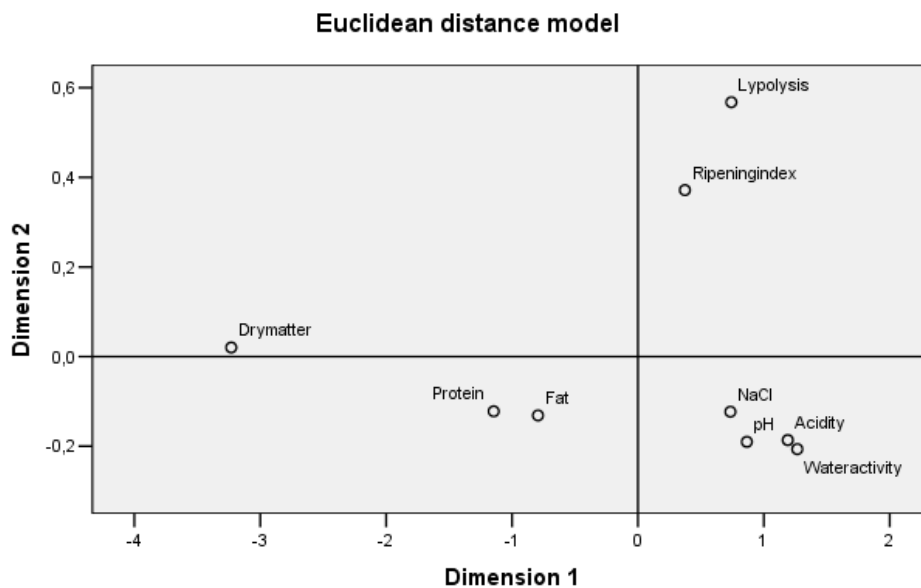


Figure 7. Geometrical representation of sepet cheese chemical and physical characteristics produced by multidimensional scaling (Stress= 0.01518, $R^2= 0.99938$)

Table 14. Lypolysis values of sepet cheeses (mean±standard deviation)

Sample number	Milk Type	Lypolysis (ADV)
1		3.33±0.00
2		2.85±0.09
3		8.00±0.00
4		5.18±0.57
5		14.07±0.16
6		4.95±0.43
7		2.21±0.18
8		9.77±0.15
9		6.74±0.26
10		2.87±0.17
11		6.81±0.16
12		6.61±1.64
13	Goat milk	2.79±0.27
16	Ewe milk	41.63±0.50
17		2.23±0.40
18		2.37±0.20
19		2.25±0.13
20		1.64±0.05
21		21.66±0.01
22	Cow milk	4.07±0.04
26		19.43±2.17
27	Goat and ewe milk	5.79±1.12
28		1.97±0.02
29	Goat and cow milk	1.79±0.09
30	Cow and ewe milk	3.64±0.01
Minimum		1.64
Maximum		41.63
Average		7.31

ADV:Acid degree value

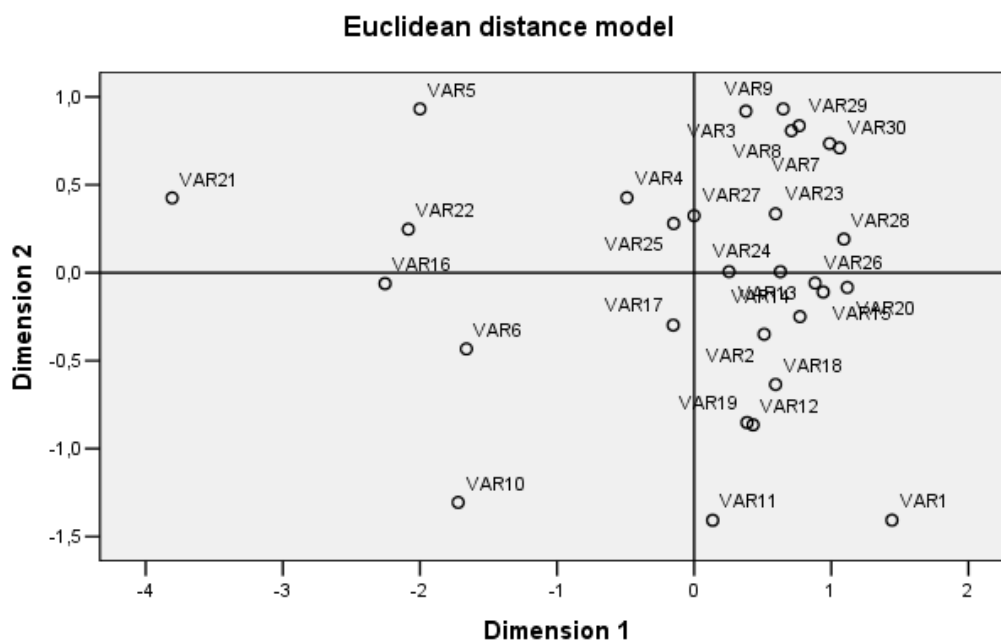


Figure 8. Geometrical representation of sepet cheeses in terms of chemical and physical characteristics by multidimensional scaling. (Each var represents the cheeses in order), (Stress= 0.12053, $R^2= 0.95257$)

4.2. Volatile Compositions of Sepet Cheeses

Aroma is the most important food quality criteria. They are major attributes that influence the selection and consumption of food. Cheese flavour results from the breakdown of milk proteins, fat, lactose, and citrate due to enzymes from microorganisms, coagulants, and milk. Many volatile compounds are potentially involved in cheese flavour: hydrocarbons, alcohols, aldehydes, ketones, esters, fatty acids, lactones, sulphur- and nitrogen-containing compounds (Vitova, et al. 2006).

In sepet cheese samples free fatty acids, esters, aldehydes, and ketones were found in volatile fraction. The volatile fractions of sepet cheese samples are shown in Table 15. The aroma intensity results obtained by Gas chromatography/Olfactometry are shown in Table 16. Figure 9 shows the similarities of samples based on volatile fraction. Variations in volatile fractions are related to milk type, composition of milk, production and ripening methods. Volatile compositions of cheese can be influenced by

lactation which affect the milk composition (Üçüncü 2005). Chifalo et al. (2004) also found quantitative fluctuations for each volatile components of the cheese in relation to the influence of lactation. Samples 3, 20 placed away from the other samples since they had relatively higher decanoic acid fraction than the others. Samples 14, 15, 27 had higher butyric acid fraction, so they were also placed away from the other samples in Figure 9. Samples 12, 8, 22, 7 also placed away since they had lower butyric acid fraction than the other samples. Hexanoic acid, octanoic acid, butyric acid, decanoic acid which were characteristic for sepet cheese samples were placed away from other compounds in Figure 10.

In all sepet cheese samples, free fatty acids were the most abundant volatile compounds of total identified fraction. Hexanoic acid (24.87 %), octanoic acid (24.72 %), decanoic acid (20.05 %), and butyric acid (8.19 %) had the highest percentage values in volatile fraction, respectively. The olfactometry results of free fatty acids were also higher than the other compounds. Free fatty acids in sepet cheese volatile fractions were responsible for the cheesy, lipolyzed aroma. Hexanoic acid, octanoic acid, butyric acid were perceived as a mild to strong goat-like, waxy, cheesy odor. Although, decanoic acid had higher percentage value than butyric acid, odor intensity of butyric acid was higher than decanoic acid. Because, the odor intensity values are related to threshold values. Rychlik et al.(1998) reported that the treshhold value of decanoic acid was 10000 ng/g and threshold value of butyric acid was 1000 ng/g (Whetstine, et al. 2003). Odor intensities of aroma compounds of sepet cheeses were correlated with ripening index and lypolysis value. Odor intensity values of acetic acid, hexanoic acid, octanoic acid, decanoic acid were significantly correlated with ripening index and had correlation factor 0.630 ($p<0.001$), 0.458 ($p<0.05$), 0.535 ($p<0.01$), 0.420 ($p<0.05$), respectively. Moreover, odor intensities of butyric acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid were positively correlated with lypolysis values of sepet cheese samples. The correlation coefficients of odor intensities of butyric acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid with lypolysis were found as 0.661 ($p<0.001$), 0.690 ($p<0.001$), 0.657 ($p<0.001$), 0.576 ($p<0.005$), 0.561 ($p<0.005$), respectively. Ur-Rehman et al. (2000) reported that linear free fatty acids containing four carbon atoms or more are generally produced from lipolysis of milk fat. However, acetic acid originates from oxidation of lactose by lactic acid bacteria under anaerobic conditions, and the catabolism (oxidative deamination and decarboxylation) of alanine

and serine by lactic acid bacteria (Ziino, et al., 2005).

The important esters in Sepet cheese volatile fraction were acetyl acetate (2.11 %), ethyl hexanoate (1.16 %), ethyl decanoate (0.58 %), ethyl octanoate (0.67 %), ethyl butyrate (0.02 %). The odor intensity values of esters changed from medium to weak. These esters appeared to be responsible for the characteristic fruity, green aroma perceived in sepet cheese samples. Zino et al. (2005) stated that most esters have floral and fruity notes and may contribute to cheese aroma by minimizing the sharpness of fatty acids and the bitterness of amines. Odor intensity values of ethyl butyrate, ethyl hexanoate, acetyl acetate, ethyl octanoate, ethyl decanoate were also significantly correlated with lypolysis values and had correlation factor 0.691 ($p < 0.001$), 0.426 ($p < 0.05$), 0.456 ($p < 0.05$), 0.500 ($p < 0.05$), 0.441 ($p < 0.05$), respectively. Engels et al. (1997) reported that esters are mainly produced by enzymatic or chemical reaction of fatty acids with primary alcohols (Ziino, et al. 2005).

Aldehydes such as octanal, nonanal, decenal, (E,Z)-2,6-nonadienal, (E)-2-decenal, (E)-2-nonenal, 2-3 butanedial were found in sepet cheeses. Although their fractions were low, they affected the aroma of sepet cheeses. These compounds have green and fatty aromas and probably contribute to the overall flavor of the goat cheese in many complex ways (Whestine, et al. 2003). Vitova et al. (2006) mentioned that straight-chain aldehydes may result from β -oxidation of unsaturated fatty acids or from amino acids by Strecker degradation. Branched chain aldehydes probably originate from amino acid degradation via enzymatic as well as nonenzymatic, e.g. Strecker degradation, processes. The odor intensities of (E,Z)-2,6-nonadienal was significantly correlated with ripening index and had 0.389 ($p < 0.05$) correlation factor.

2-Nonanone and 2-tridecanone were ketones found in Sepet cheeses volatile fraction. The formation of methyl ketones is a result of enzymatic oxidation of free fatty acids to β -ketoacids and their consequent decarboxylation to alkan-2-ones with the loss of one carbon atom (Frank, et al. 2004). The odor intensities of 2-tridecanone was significantly correlated with lypolysis values of sepet cheese samples and correlation factor was 0.585 ($p < 0.01$). Ketones have low perception thresholds and typical odours (Curioni and Bossetb 2002). Therefore, they have important effects on aroma intensity. Aroma intensity values of ketones changed between medium to weak among sepet cheese samples.

Terpenes such as limonene are common in citrus fruits but are not often found in dairy products. Limonene was detected in some Sepet cheeses and also the odor intensities were recorded as weak. Occurrence of D-Limonene is related to animal feeding and increase with green grass feeding (Chiofalo, et al. 2004). Also, δ -decalactone, and γ -dodecalactone, *o*-aminoacetophenone was found in some Sepet cheeses. Whestine et al. (2003) mentioned that they are thermally generated aroma compounds.

The volatile compounds identified were compared to previously reported cheese aroma compounds and similarities were found between aroma compounds of sepet cheese, blue cheese and Parmigiano-Reggiano cheese. Butanoic acid was generally a major aroma impact compound with its characteristic cheesy sharp aroma (Frank, et al. 2004). Butanoic acid (butyric acid) was also found in all sepet cheese samples. In Blue cheese samples, Frank et al. (2004) found hexanoic acid and perceived as very mild to strong sharp goat-like smell. Also they were reported that ketones, especially 2-heptanone (musty, sweet, moldy, varnish) and 2-nonanone (floral fruity, peach) were important in the blue cheese aroma. Qian and Reineccius (2002) found that butanoic, hexanoic, octanoic, and decanoic acids were the major free fatty acids contributing to cheesy, lipolyzed aroma and ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl propanoate, ethyl pentanoate, ethyl heptanoate, ethyl decanoate were found to be important in volatile fraction of Parmigiano-Reggiano cheese.

Figure 11 shows the typical GC chromatogram of sepet cheese's volatile compounds. Although most of the volatile compounds cannot be observed in goat milk (data not given), most of the volatile compounds identified were present at all stages of the sepet cheese production and ripening. However, their peak areas changed. The results are given in Tables 17 and 18. The peak areas of volatile compounds of Zeytineli cheese had higher values than Germiyan cheese. Diverse lactic acid bacteria flora can be the reason of this difference. The reasons of the resemblences of volatile compounds can be the same cheese making and ripening methods.

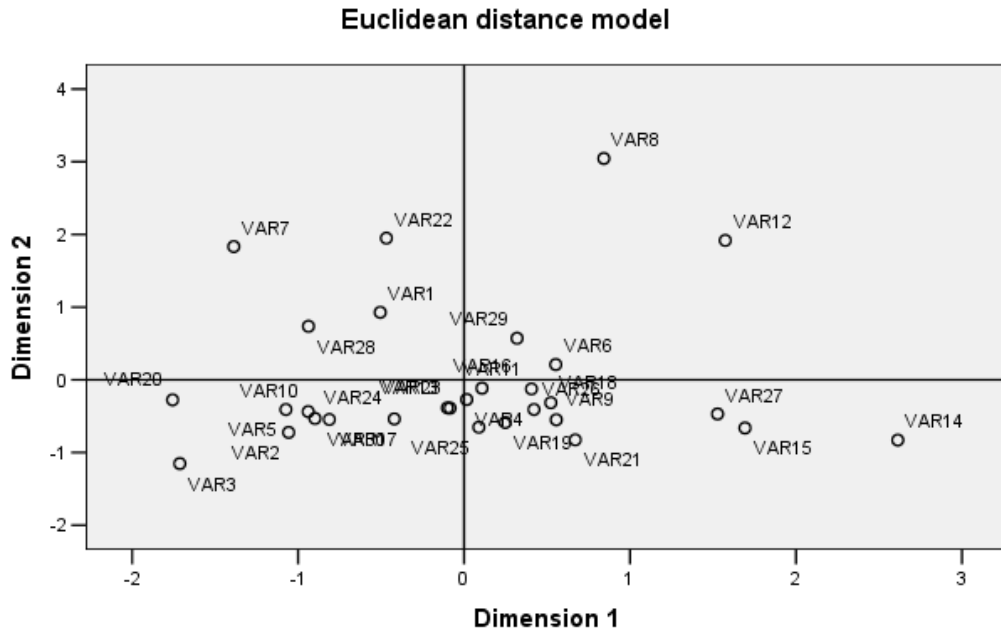


Figure 9. Geometrical representation of cheeses in terms of volatile fraction by multidimensional scaling. (Each var represents the cheeses in order), (Stress= 0.09233, $R^2= 0.98620$)

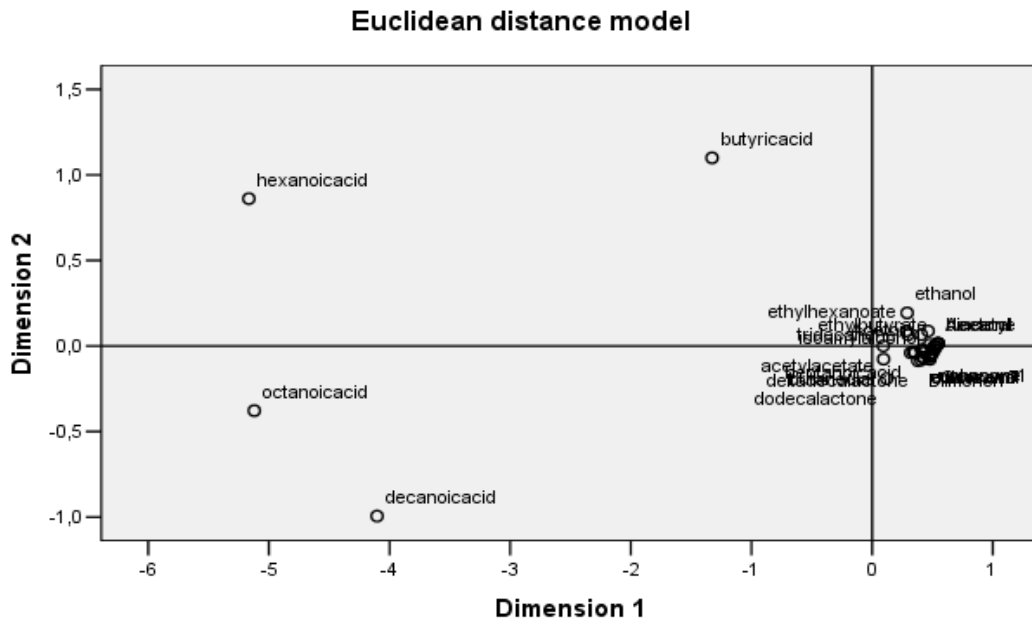


Figure 10. Geometrical representation of cheese volatile fractions produced by multidimensional scaling (Stress= 0.03032, $R^2= 0.99882$)

Table 15. Volatile fractions (area %) of sepet cheese samples

		Goat					
Compound		1	2	3	4	5	6
Free Fatty Acids	Acetic acid	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.8±0.37	0.00±0.00
	Butyric acid	3.10±0.74	8.66±2.94	1.83±0.64	8.64±1.03	5.23±0.33	11.76±1.25
	Hexanoic acid	20.82±1.32	17.91±0.83	24.48±1.35	25.12±2.75	19.48±1.50	24.1±3.97
	Heptanoic acid	1.52±1.70	0.17±0.12	0.29±0.02	0.26±0.01	0.26±0.01	0.59±0.09
	Octanoic acid	30.64±3.33	26.03±0.76	29.74±1.79	25.70±1.7	27.44±0.55	23.85±0.33
	4-methyl octanoic acid	5.22±0.95	0.34±0.09	0.35±0.03	0.00±0.00	0.00±0.00	0.00±0.00
	Decanoic acid	14.68±2.74	28.50±1.89	34.63±0.16	21.52±0.61	27.55±0.89	17.44±1.25
	Dodecanoic acid	0.00±0.00	1.05±0.09	1.35±0.05	0.74±0.13	1.11±0.07	1.14±0.04
Ketones	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.95±0.11	0.00±0.00	0.00±0.00
	Diacetyl	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Acetoin	0.00±0.00	0.26±0.25	0.00±0.00	0.00±0.00	0.55±0.68	0.28±0.06
	2-nonanone	2.11±0.75	0.28±0.35	0.00±0.00	0.00±0.00	0.04±0.05	0.81±0.54
	2-tridecanone	0.00±0.00	3.59±0.23	0.79±0.85	0.45±0.36	4.53±0.85	0.79±0.44
Aldehydes	Hexanal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Octanal	0.25±0.09	0.14±0.12	0.00±0.00	0.27±0.11	0.17±0.03	0.00±0.00
	Nonanal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.25±0.35
	Decenal	0.11±0.05	0.48±0.08	0.00±0.00	0.14±0.19	0.09±0.05	0.25±0.06
	e-2 nonenal	0.60±0.77	0.14±0.17	0.00±0.00	0.52±0.08	0.06±0.02	0.36±0.21
	e-2, z-6 nonadienal	0.10±0.14	3.04±0.16	0.75±0.34	0.30±0.08	1.28±0.26	0.28±0.27
	2,3-butanedial	2.59±0.41	0.25±0.35	0.00±0.00	0.23±0.32	1.49±0.22	11.79±3.68
	e-2 decenal	0.00±0.00	0.59±0.58	0.14±0.20	1.14±0.05	0.19±0.14	0.12±0.16
Esters	Ethyl butyrate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.16±0.12
	Ethyl hexanoate	0.70±0.39	2.85±0.48	0.39±0.25	0.83±0.19	0.97±0.25	1.19±0.24
	Acetyl acetate	1.32±0.65	0.17±0.07	0.84±0.76	3.56±1.06	0.78±0.14	1.73±0.25
	Ethyl octanoate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Ethyl decanoate	2.85±0.82	0.11±0.04	0.38±0.31	0.31±0.06	0.35±0.23	0.00±0.00
Others	Ethanol	0.72±0.06	0.00±0.00	0.59±0.00	4.51±2.24	1.88±0.23	0.61±0.33
	Isoamylalcohol	0.78±0.11	2.59±1.20	0.86±0.32	2.96±0.61	0.33±0.22	0.00±0.00
	D-limonen	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	δ-decalactone	2.66±0.42	0.15±0.19	0.58±0.12	0.46±0.37	0.24±0.00	0.17±0.18
	γ-dodecalactone	4.58±1.37	0.28±0.03	0.62±0.08	0.42±0.08	0.22±0.00	1.41±0.15
	4-aminoacetophenone	0.55±0.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Unknown 1	0.36±0.45	0.04±0.01	0.00±0.00	0.00±0.00	0.63±0.73	0.00±0.00
	Unknown 2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Unknown 3	1.06±0.47	0.00±0.00	0.00±0.00	0.00±0.00	0.12±0.12	0.00±0.00
	Unknown 4	0.71±0.92	0.08±0.11	0.00±0.00	0.00±0.00	0.44±0.13	0.00±0.00
	Unknown 5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Unknown 6	0.00±0.00	2.07±0.61	0.91±0.09	0.63±0.11	0.37±0.03	0.38±0.14
	Unknown 7	1.96±0.91	0.22±0.31	0.48±0.02	0.35±0.28	0.42±0.01	0.56±0.51
Unknown 8	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	

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Table 15. (cont.) Volatile fractions (area %) of sepet cheese samples

		Goat					
Compound		7	8	9	10	11	12
Free Fatty Acids	Acetic acid	0.00±0.00	0.00±0.00	0.00±0.00	1.49±0.69	0.00±0.00	0.00±0.00
	Butyric acid	0.00±0.00	4.95±0.37	9.31±2.31	1.82±0.45	12.19±2.27	3.33±0.75
	Hexanoic acid	17.22±1.32	18.39±7.83	33.99±3.67	26.65±1.19	26.18±1.94	19.09±0.32
	Heptanoic acid	0.51±0.14	9.29±2.31	0.58±0.04	0.27±0.06	0.61±0.09	0.32±0.25
	Octanoic acid	22.06±0.55	23.36±3.84	25.84±2.69	32.43±1.13	26.3±3.34	19.18±1.10
	4-methyl octanoic acid	0.97±0.08	18.69±0.86	0.22±0.01	0.00±0.00	0.46±0.16	0.08±0.05
	Decanoic acid	15.55±0.61	5.37±1.23	18.63±1.75	22.34±1.41	17.37±3.40	18.07±1.32
	Dodecanoic acid	1.55±0.07	0.00±0.00	0.92±0.08	0.97±0.12	1.32±0.27	0.48±0.08
Ketones	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Diacetyl	0.00±0.00	0.00±0.00	0.99±0.75	0.00±0.00	0.00±0.00	0.57±4.20
	Acetoine	0.00±0.00	0.19±0.05	0.16±0.09	0.40±0.25	0.00±0.00	0.00±0.00
	2-nonanone	0.00±0.00	0.00±0.00	0.16±0.03	0.00±0.00	0.00±0.00	0.13±0.05
	2-tridecanone	7.55±0.42	0.19±0.22	0.29±0.30	0.00±0.00	1.76±0.24	0.00±0.00
Aldehydes	Hexanal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.20±0.05
	Octanal	0.83±0.11	0.14±0.01	0.00±0.00	0.33±0.21	0.00±0.00	0.08±0.00
	Nonanal	0.83±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.17±0.07	0.00±0.00
	Decenal	0.00±0.00	0.26±0.28	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	e-2 nonenal	0.00±0.00	0.57±0.06	0.30±0.36	0.00±0.00	0.58±0.13	2.06±0.25
	e-2, z-6 nonadienal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.21±0.06	0.00±0.00
	2,3-butanedial	15.89±0.33	4.63±1.04	2.05±0.25	0.89±0.81	1.52±0.27	1.13±0.04
e-2 decenal	0.00±0.00	0.38±0.02	0.93±1.30	0.00±0.00	0.93±1.24	0.00±0.00	
Esters	Ethyl butyrate	0.00±0.00	0.00±0.00	0.09±0.06	0.00±0.00	0.00±0.00	0.00±0.00
	Ethyl hexanoate	0.00±0.00	0.12±0.03	0.21±0.12	3.42±0.34	0.20±0.19	0.25±0.01
	Acetyl acetate	1.30±0.25	4.48±0.81	2.03±1.39	0.00±0.00	4.22±2.70	9.94±1.44
	Ethyl octanoate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.28±0.05	4.17±0.17
	Ethyl decanoate	1.44±0.41	0.00±0.00	1.60±0.61	0.50±0.41	0.53±0.63	0.00±0.00
Others	Ethanol	0.59±0.25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	9.15±0.82
	Isoamylalcohol	0.00±0.00	0.00±0.00	0.38±0.22	0.40±0.25	0.00±0.00	0.20±0.05
	D-limonen	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	δ-decalactone	0.00±0.00	2.46±0.02	0.33±0.2	0.74±0.39	0.84±0.13	0.14±0.01
	γ-dodecalactone	2.54±0.25	3.15±4.45	0.51±0.26	1.07±0.42	1.23±0.37	0.16±0.04
	4-aminoacetophenone	1.24±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.30±0.15	0.05±0.03
	Unknown 1	0.00±0.00	0.09±0.12	0.00±0.00	3.35±4.62	0.00±0.00	0.00±0.00
	Unknown 2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.04
	Unknown 3	0.00±0.00	0.00±0.00	0.24±0.04	0.00±0.00	0.00±0.00	0.08±0.03
	Unknown 4	0.00±0.00	0.45±0.37	0.00±0.00	0.52±0.25	0.55±0.71	0.00±0.00
	Unknown 5	0.00±0.00	0.21±0.03	0.00±0.00	0.00±0.00	0.00±0.00	1.94±0.71
	Unknown 6	0.00±0.00	0.56±0.79	0.00±0.00	1.33±0.81	0.74±0.16	0.00±0.00
Unknown 7	0.00±0.00	1.98±2.80	0.55±0.02	0.37±0.31	0.22±0.30	0.13±0.02	
Unknown 8	9.91±0.51	0.10±0.04	0.00±0.00	0.72±0.47	0.29±0.05	0.07±0.00	

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Table 15. (cont.) Volatile fractions (area %) of sepet cheese samples

	Compound	Goat			Ewe	Cow	
		13	14	15	16	17	18
Free Fatty Acids	Acetic acid	0.00±0.00	5.29±1.05	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	Butyric acid	6.86±1.32	20.24±2.24	16.43±1.05	10,47±2,15	6.05±1.94	13.76±1.09
	Hexanoic acid	28.96±1.65	35.00±1.73	31.49±1.94	25,65±0,73	22.09±4.58	26.85±1.14
	Heptanoic acid	0.42±0.06	0.00±0.00	0.00±0.00	0,58±0,77	0.34±0.02	0.00±0.00
	Octanoic acid	25.98±3.23	16.46±0.87	19.23±1.01	26,44±2,16	27.58±2.55	27.26±2.64
	4-methyl octanoic acid	0.21±0.23	0.00±0.00	0.00±0.00	0,95±1,29	0.51±0.04	0.00±0.00
	Decanoic acid	22.24±2.29	9.01±0.59	12.60±1.29	20,22±3,85	27.92±0.49	16.95±0.36
	Dodecanoic acid	0.98±0.13	0.00±0.00	0.00±0.00	0,55±0,28	0.90±0.24	0.00±0.00
Ketones	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	Diacetyl	0.00±0.00	0.00±0.00	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	Acetoine	0.21±0.18	2.61±0.17	0.40±0.08	0,54±0,71	0.00±0.00	1.74±0.82
	2-nonanone	1.04±0.49	0.00±0.00	0.00±0.00	0,68±0,08	0.00±0.00	0.00±0.00
	2-tridecanone	0.34±0.03	1.09±0.06	1.93±0.66	0,47±0,13	0.00±0.00	0.00±0.00
Aldehydes	Hexanal	0.00±0.00	0.00±0.00	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	Octanal	0.11±0.08	0.00±0.00	0.00±0.00	0,04±0,02	0.53±0.16	0.00±0.00
	Nonanal	0.00±0.00	0.00±0.00	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	Decenal	0.00±0.00	0.00±0.00	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	e-2 nonenal	0.00±0.00	0.00±0.00	0.00±0.00	0,04±0,02	0.00±0.00	0.00±0.00
	e-2, z-6 nonadienal	0.00±0.00	0.00±0.00	0.00±0.00	0,08±0,02	0.00±0.00	0.00±0.00
	2,3-butanedial	4.78±1.11	0.00±0.00	0.00±0.00	3,49±0,73	0.00±0.00	0.00±0.00
e-2 decenal	0.00±0.00	0.00±0.00	0.00±0.00	0,09±0,01	1.51±1.39	0.00±0.00	
Esters	Ethyl butyrate	0.13±0.08	0.00±0.00	0.00±0.00	0,16±0,04	0.00±0.00	0.00±0.00
	Ethyl hexanoate	1.37±1.40	2.11±0.54	4.02±0.48	0,67±0,81	0.49±0.56	2.84±1.35
	Acetyl acetate	0.92±0.24	0.00±0.00	0.00±0.00	2,71±1,41	2.10±0.52	1.89±0.66
	Ethyl octanoate	0.32±0.13	1.05±0.03	2.13±0.09	0,14±0,02	0.00±0.00	0.00±0.00
	Ethyl decanoate	1.66±1.66	1.41±0.05	1.42±0.45	0,63±0,03	0.33±0.36	2.08±0.14
Others	Ethanol	0.37±0.08	5.69±3.36	8.99±3.39	0,00±0,00	0.00±0.00	0.00±0.00
	Isoamylalcohol	0.27±0.32	0.00±0.00	0.00±0.00	0,05±0,04	0.00±0.00	0.00±0.00
	D-limonen	0.00±0.00	0.00±0.00	1.24±1.99	0,67±0,84	3.89±3.48	3.17±2.03
	δ-decalactone	0.26±0.01	0.00±0.00	0.00±0.00	0,37±0,03	1.18±0.63	1.01±1.08
	γ-dodecalactone	0.26±0.07	0.00±0.00	0.00±0.00	0,39±0,02	1.61±1.54	2.37±0.30
	4-aminoacetophenone	0.00±0.00	0.00±0.00	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	Unknown 1	0.41±0.47	0.00±0.00	0.00±0.00	0,00±0,00	0.00±0.00	0.00±0.00
	Unknown 2	0.00±0.00	0.00±0.00	0.00±0.00	0,24±0,27	0.00±0.00	0.00±0.00
	Unknown 3	0.34±0.12	0.00±0.00	0.00±0.00	1,20±1,58	0.00±0.00	0.00±0.00
	Unknown 4	0.89±0.17	0.00±0.00	0.00±0.00	1,35±0,50	0.00±0.00	0.00±0.00
	Unknown 5	0.00±0.00	0.00±0.00	0.00±0.00	0,03±0,02	0.23±0.28	0.00±0.00
	Unknown 6	0.21±0.05	0.00±0.00	0.00±0.00	0,19±0,06	1.73±0.81	0.00±0.00
	Unknown 7	0.46±0.00	0.00±0.00	0.00±0.00	0,90±0,28	0.74±0.20	0.00±0.00
Unknown 8	0.00±0.00	0.00±0.00	0.00±0.00	0,00±0,00	0.26±0.21	0.00±0.00	

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Table 15. (cont.) Volatile fractions (area %) of sepet cheese samples

		Cow					
Compound		19	20	21	22	23	24
Free Fatty Acids	Acetic acid	0.00±0.00	0.00±0.00	0.55±0.16	0.00±0.00	0.00±0.00	0.00±0.00
	Butyric acid	13.78±0.89	1.37±1.33	17.35±0.26	1.87±0.31	9.31±2.99	4.28±4.45
	Hexanoic acid	28.74±9.08	18.31±0.58	26.88±2.59	23.00±1.88	24.75±1.12	21.21±1.16
	Heptanoic acid	1.02±1.26	0.76±0.50	0.51±0.09	3.56±0.69	0.61±0.75	0.83±0.39
	Octanoic acid	25.85±11.13	26.15±1.13	21.02±1.03	27.53±0.40	25.32±1.03	20.48±0.58
	4-methyl octanoic acid	0.00±0.00	0.38±0.00	0.58±0.25	8.55±1.85	0.22±0.18	0.43±0.40
	Decanoic acid	24.6±6.57	31.08±0.40	22.17±3.14	9.42±1.46	24.26±0.48	27.2±10.96
	Dodecanoic acid	1.65±1.94	0.71±0.29	1.23±0.27	0.00±0.00	0.76±0.05	1.52±0.63
Ketones	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Diacetyl	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Acetoin	0.68±4.44	0.00±0.00	0.04±0.03	0.23±0.20	0.30±0.05	3.43±0.83
	2-nonanone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.65±0.17
	2-tridecanone	0.00±0.00	0.00±0.00	0.34±0.08	0.00±0.00	0.30±0.13	2.26±2.48
Aldehydes	Hexanal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Octanal	0.56±1.16	0.17±0.16	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Nonanal	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Decenal	0.00±0.00	0.32±0.01	0.06±0.01	0.34±0.38	0.00±0.00	0.66±0.76
	e-2 nonenal	0.00±0.00	0.72±0.56	0.04±0.03	0.10±0.07	1.03±1.04	0.41±0.07
	e-2, z-6 nonadienal	0.00±0.00	0.00±0.00	0.05±0.03	0.28±0.06	0.00±0.00	0.00±0.00
	2,3-butanedial	0.00±0.00	0.00±0.00	1.06±0.29	1.33±1.00	0.00±0.00	0.00±0.00
	e-2 decenal	0.00±0.00	0.45±0.32	0.00±0.00	0.23±0.17	1.99±0.51	0.44±0.24
Esters	Ethyl butyrate	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Ethyl hexanoate	2.40±2.43	1.79±0.94	0.21±0.12	0.37±0.15	0.00±0.00	1.91±0.91
	Acetyl acetate	3.03±7.72	0.78±0.55	4.22±0.17	0.65±0.89	6.40±0.78	1.32±0.32
	Ethyl octanoate	0.00±0.00	0.00±0.00	0.36±0.12	0.00±0.00	2.63±2.88	0.75±0.11
	Ethyl decanoate	0.00±0.00	0.00±0.00	0.08±0.08	1.30±0.18	0.8±0.75	0.48±0.33
Others	Ethanol	0.00±0.00	0.99±0.95	0.23±0.05	0.00±0.00	0.00±0.00	0.00±0.00
	Isoamylalcohol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	D-limonen	0.00±0.00	1.62±1.98	0.00±0.00	0.00±0.00	0.28±0.05	6.56±1.78
	δ-decalactone	0.00±0.00	2.37±3.03	0.55±0.20	4.51±0.73	0.35±0.32	0.00±0.00
	γ-dodecalactone	2.85±1.79	6.77±1.72	0.81±0.23	12.74±0.23	0.47±0.17	2.39±0.77
	4-aminoacetophenone	0.83±2.03	0.87±0.55	0.07±0.00	0.00±0.00	0.00±0.00	0.58±0.62
	Unknown 1	0.00±0.00	0.00±0.00	0.34±0.08	0.00±0.00	0.00±0.00	0.00±0.00
	Unknown 2	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Unknown 3	0.00±0.00	0.00±0.00	0.00±0.00	0.11±0.07	0.00±0.00	0.00±0.00
	Unknown 4	0.00±0.00	1.33±1.21	0.24±0.28	0.00±0.00	0.00±0.00	1.21±1.57
	Unknown 5	0.00±0.00	0.00±0.00	0.41±0.00	0.17±0.07	0.00±0.00	0.00±0.00
	Unknown 6	0.00±0.00	0.61±0.46	0.10±0.00	0.36±0.05	0.00±0.00	0.00±0.00
	Unknown 7	0.00±0.00	1.22±0.06	0.52±0.06	3.34±0.20	0.24±0.34	0.00±0.00
Unknown 8	0.00±0.00	1.22±0.92	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	

(cont. on next page)

Table 15. (cont.) Volatile fractions (area%) of sepet cheese samples

Compound	Cow	Goat-Ewe		Goat-Cow		Ewe-Cow	
	25	26	27	28	29	30	
Free Fatty Acids	Acetic acid	0.00±0.00	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	0.00±0.00
	Butyric acid	6.54±1.25	14,4±1,92	18,66±1,99	1,98±1,05	6,35±0,44	3.68±0.71
	Hexanoic acid	28.12±2.34	26,99±2,30	31,78±1,65	18,05±1,43	25,53±1,19	30.47±4.03
	Heptanoic acid	9.34±0.04	0,28±0,03	0,18±0,21	1,42±0,37	0,00±0,00	1.03±0.12
	Octanoic acid	18.45±0.78	22,02±2,80	20,55±2,81	30,75±1,92	23,25±0,19	27.24±2.17
	4-methyl octanoic acid	0.00±0.00	0,09±0,01	0,00±0,00	3,18±0,69	0,00±0,00	0.00±0.00
	Decanoic acid	25.76±3.2	21,96±0,07	14,21±0,57	18,51±0,40	14,24±0,05	22.86±0.84
	Dodecanoic acid	0.75±0.03	1,20±0,17	0,09±0,02	0,50±0,06	0,83±0,19	1.23±0.12
Ketones	Acetone	0.00±0.00	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	0.00±0.00
	Diacetyl	0.00±0.00	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	0.00±0.00
	Acetoine	0.00±0.00	0,31±0,39	0,37±0,24	2,49±3,35	0,47±0,34	0.91±0.96
	2-nonanone	0.00±0.00	0,08±0,02	0,00±0,00	1,59±0,41	1,01±0,58	0.51±0.13
	2-tridecanone	0.00±0.00	1,93±0,65	2,13±0,93	0,00±0,00	2,68±0,38	1.09±0.76
Aldehydes	Hexanal	0.00±0.00	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	0.00±0.00
	Octanal	0.23±1.16	0,05±0,06	0,00±0,00	0,17±0,09	0,00±0,00	0.74±0.88
	Nonanal	1.03±3.23	0,00±0,00	0,00±0,00	0,00±0,00	0,00±0,00	1.29±0.45
	Decenal	0.00±0.00	0,04±0,04	0,21±0,13	0,27±0,17	0,00±0,00	0.73±0.46
	e-2 nonenal	0.00±0.00	0,37±0,53	1,32±0,17	0,23±0,26	0,00±0,00	0.00±0.00
	e-2, z-6 nonadienal	0.00±0.00	0,20±0,28	0,4±0,11	0,00±0,00	0,00±0,00	0.00±0.00
	2,3-butanedial	0.00±0.00	2,85±0,26	3,24±0,50	1,05±0,21	8,88±1,33	0.00±0.00
e-2 decenal	0.00±0.00	0,40±0,16	1,14±0,57	0,22±0,16	0,00±0,00	2.18±0.47	
Esters	Ethyl butyrate	0.00±0.00	0,08±0,06	0,00±0,00	0,00±0,00	0,00±0,00	0.00±0.00
	Ethyl hexanoate	1.45±0.15	0,58±0,17	0,00±0,00	0,83±0,66	0,84±0,01	1.13±1.07
	Acetyl acetate	3.06±0.76	1,54±0,18	2,42±1,03	1,53±2,09	1,19±0,85	0.00±0.00
	Ethyl octanoate	0.00±0.00	1,84±0,16	1,67±0,30	0,00±0,00	0,00±0,00	0.77±0.48
	Ethyl decanoate	0.00±0.00	0,06±0,05	0,62±0,39	0,33±0,32	1,25±0,02	0.00±0.00
Others	Ethanol	0.00±0.00	0,00±0,00	0,00±0,00	1,23±0,16	2,45±0,15	0.00±0.00
	Isoamylalcohol	0.00±0.00	0,00±0,00	0,00±0,00	0,44±0,39	0,00±0,00	0.00±0.00
	D-limonen	0.00±0.00	0,00±0,00	0,00±0,00	0,33±0,11	2,45±0,12	0.00±0.00
	δ-decalactone	0.00±0.00	0,20±0,00	0,13±0,11	3,43±0,99	1,52±0,76	0.00±0.00
	γ-dodecalactone	4.67±0.14	0,15±0,01	0,11±0,03	4,26±0,52	3,63±1,65	2.91±0.37
	4-aminoacetophenone	0.55±0.33	0,00±0,00	0,00±0,00	1,14±0,37	0,76±0,46	0.00±0.00
	Unknown 1	0.00±0.00	0,14±0,13	0,11±0,02	2,78±3,54	0,00±0,00	0.00±0.00
	Unknown 2	0.00±0.00	0,76±0,77	0,00±0,00	0,00±0,00	0,00±0,00	0.36±0.40
	Unknown 3	0.00±0.00	0,36±0,50	0,63±0,71	0,00±0,00	2,11±0,15	0.00±0.00
	Unknown 4	0.00±0.00	0,57±0,29	0,00±0,00	0,37±0,04	0,00±0,00	0.00±0.00
	Unknown 5	0.00±0.00	0,00±0,00	0,00±0,00	0,00±0,00	0,56±0,10	0.00±0.00
	Unknown 6	0.00±0.00	0,23±0,07	0,00±0,00	0,46±0,19	0,00±0,00	0.00±0.00
Unknown 7	0.00±0.00	0,31±0,09	0,00±0,00	1,98±0,23	0,00±0,00	0.00±0.00	
Unknown 8	0.00±0.00	0,00±0,00	0,00±0,00	0,45±0,60	0,00±0,00	0.87±0.80	

Table 16. Aroma intensities of volatile compounds in sepet cheeses

No	Compound	Odor	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Acetic acid	Vinegar sour	0	0	0	0	4	0	0	0	0	2	0	0	4	0	0
2	Butyric acid	Chessy, sharp	5	4	3	7	4	4	0	5	4	3	5	6	5	3	2
3	Hexanoic acid	Goaty	6	5	5	6	9	6	3	9	8	4	5	7	9	4	3
4	Heptanoic acid	Goaty,cheesy	2	0	1	1	2	1	1	6	3	1	1	1	1	0	0
5	Octanoic acid	Waxy	6	6	6	8	9	6	3	9	8	6	6	7	9	4	3
6	4-methyl octanoic acid	Goaty, sour	4	1	1	0	0	0	1	9	1	0	3	1	2	0	0
7	Decanoic acid	Sour/waxy	4	4	4	6	6	4	1	3	4	3	4	5	6	2	2
8	Dodecanoic acid	Soapy	2	1	0	2	2	1	0	0	1	0	2	3	3	0	0
9	Acetone	Alcoholic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Diacetyl	Buttery	0	0	0	0	0	0	0	0	2	0	0	2	0	0	0
11	Acetoine	Buttery	0	0	0	0	3	1	0	1	1	1	0	0	2	1	0
12	2-nonanone	Fruity, floral	2	2	0	0	1	2	0	0	3	0	0	1	4	0	0
13	2-tridecanone	Fatty	0	2	2	2	3	2	0	1	1	0	3	0	2	1	1
14	Hexanal	Woody	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
15	Octanal	Sweet, citrus	0	0	0	1	0	0	1	1	0	0	0	0	1	0	0
16	Nonanal	Hay/sweet	0	0	0	0	0	1	2	0	0	0	1	0	0	0	0
17	Decenal	Floral, waxy	1	2	0	0	0	1	0	3	0	0	0	0	0	0	0
18	e-2 nonenal	Fatty, cucumber	1	1	0	1	0	2	0	3	1	0	1	4	0	0	0
19	e-2, z-6 nonadienal	Hay/fatty	1	3	2	1	4	1	0	0	0	0	3	0	0	0	0
20	2,3-butanedial	Cheesy	3	1	0	2	3	4	3	5	2	1	2	3	4	0	0
21	e-2 decenal	Hay/fatty	0	1	1	3	1	1	0	2	2	0	2	0	0	0	0
22	Ethyl butyrate	Fruity	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
23	Ethyl hexanoate	Orange, sour	2	2	2	3	2	3	0	2	2	3	0	2	3	1	1
24	Acetyl acetate	Green	2	2	2	5	2	3	1	4	4	0	3	4	0	2	0
25	Ethyl octanoate	Fruity	0	0	0	0	0	0	0	0	0	0	1	4	2	1	1
26	Ethyl decanoate	Fruity	2	1	2	3	2	0	1	0	4	2	1	0	4	1	3
27	Ethanol	Alcoholic	1	0	0	1	0	2	1	0	0	0	0	4	1	3	3
28	Isoamylalcohol	Buttery	1	1	1	3	1	0	0	0	2	0	0	1	0	0	0
29	D-limonen	Sour	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	δ -decalactone	Peach	1	0	0	1	1	1	0	4	2	0	1	0	1	0	0
31	γ -dodecalactone	Coconut	2	0	0	1	1	1	0	4	1	0	2	0	2	0	0
32	4-aminoacetophenone	Grape	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	Unknown 1	Sweet	0	0	0	0	2	0	0	1	0	3	0	0	1	0	0
34	Unknown 2	Sweet	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
35	Unknown 3	Sweet	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0
36	Unknown 4	Sour	1	0	0	0	1	0	0	2	0	1	1	0	3	0	0
37	Unknown 5	Fatty	0	0	0	0	0	0	0	2	0	0	0	4	0	0	0
38	Unknown 6	Fruity	0	2	1	1	2	1	0	2	0	2	2	0	0	0	0
39	Unknown 7	Fuity	2	1	0	1	1	2	0	4	2	1	1	1	2	0	0
40	Unknown 8	Sour	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table 16. (cont.) Aroma intensities of volatile compounds in sepet cheeses

No	Compound	Odor	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	Acetic acid	Vinegar sour	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
2	Butyric acid	Chessy, sharp	8	5	4	4	3	8	3	4	4	3	8	6	3	3	4
3	Hexanoic acid	Goaty	10	6	4	4	5	9	6	5	4	3	9	8	5	3	4
4	Heptanoic acid	Goaty,cheesy	2	1	0	1	1	2	4	0	1	1	1	0	1	0	1
5	Octanoic acid	Waxy	10	6	4	4	6	9	8	6	4	3	9	6	6	3	4
6	4-methyl octanoic acid	Goaty, sour	4	2	0	0	1	3	8	0	0	0	2	0	3	0	0
7	Decanoic acid	Sour/waxy	6	5	2	3	3	6	2	3	3	2	6	4	3	2	2
8	Dodecanoic acid	Soapy	3	1	0	1	1	3	0	0	0	0	3	0	1	0	0
9	Acetone	Alcoholic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	Diacetyl	Buttery	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	Acetoine	Buttery	1	0	1	1	0	1	1	1	2	0	1	1	4	1	0
12	2-nonanone	Fruity, floral	4	0	0	0	0	0	0	0	2	0	1	0	3	1	1
13	2-tridecanone	Fatty	3	0	0	0	0	2	0	3	0	0	4	3	0	1	1
14	Hexanal	Woody	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	Octanal	Sweet, citrus	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
16	Nonanal	Hay/sweet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
17	Decenal	Floral, waxy	0	0	0	0	1	1	1	1	1	0	1	1	1	0	1
18	e-2 nonenal	Fatty, cucumber	1	0	0	0	1	1	1	3	1	0	3	3	1	0	0
19	e-2, z-6 nonadienal	Hay/fatty	1	0	0	0	0	1	3	1	0	0	4	1	0	0	0
20	2,3-butanedial	Cheesy	4	0	0	0	0	4	2	2	0	0	5	2	2	2	0
21	e-2 decenal	Hay/fatty	1	2	0	0	2	0	1	2	0	0	3	2	1	0	2
22	Ethyl butyrate	Fruity	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0
23	Ethyl hexanoate	Orange, sour	4	2	2	2	2	2	2	0	2	1	3	0	2	2	1
24	Acetyl acetate	Green	4	2	1	2	2	4	2	3	1	1	4	3	3	2	0
25	Ethyl octanoate	Fruity	2	0	0	0	0	3	0	2	0	0	4	2	0	0	1
26	Ethyl decanoate	Fruity	4	1	1	0	0	2	1	2	1	0	2	2	2	1	0
27	Ethanol	Alcoholic	0	0	0	0	1	1	0	0	0	0	0	0	2	0	0
28	Isoamylalcohol	Buttery	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
29	D-limonen	Sour	0	1	1	0	1	0	0	0	2	0	0	0	0	1	0
30	δ -decalactone	Peach	2	1	0	0	1	2	4	0	0	0	0	0	2	0	0
31	γ -dodecalactone	Coconut	2	2	1	1	2	3	4	0	1	0	0	0	2	0	1
32	4-aminoacetophenone	Grape	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
33	Unknown 1	Sweet	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0
34	Unknown 2	Sweet	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
35	Unknown 3	Sweet	3	0	0	0	0	0	0	0	0	0	0	2	0	1	0
36	Unknown 4	Sour	3	0	0	0	1	1	0	0	1	0	3	0	1	0	0
37	Unknown 5	Fatty	0	1	0	0	0	4	1	0	0	0	0	0	0	0	0
38	Unknown 6	Fruity	2	2	0	0	1	2	1	0	0	0	5	0	1	0	0
39	Unknown 7	Fuity	3	1	0	0	1	2	3	0	0	0	1	0	2	0	0
40	Unknown 8	Sour	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Among the volatile compounds, free fatty acids were quantitatively the dominant family present. The most abundant compound in volatile fraction is hexanoic acid, followed closely, in decreasing order of peak area, by butyric acid, octanoic acid, and decanoic acid. All free fatty acids increased during production and ripening. Most esters showed a significant increase until the first month. The important esters in sepet cheese were ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl butanoate, and ethyl acetate. Only ethyl acetate did not increase during ripening. Alcohols such as ethanol and 1-propanol also significantly increased until first month in Zeytineli volatile composition. Methyl ketones, 2-heptanone and 2-tridecanone, increased until first month significantly and continued to increase slightly upto 6th month.

Ziino et al.(2005) investigated volatile composition changes of sicilan cheeses during ripening and concluded that fatty acids and esters increased during ripening. Pinho et al.(2001) also found that the largest peak areas of most ewe cheese volatile compounds were observed after ripening and explained that this could be attributed to the high fat content of ewe milk and the extent of lipolysis common during ripening of ewe cheese. These results were in agreement with sepet cheese volatile composition changes.

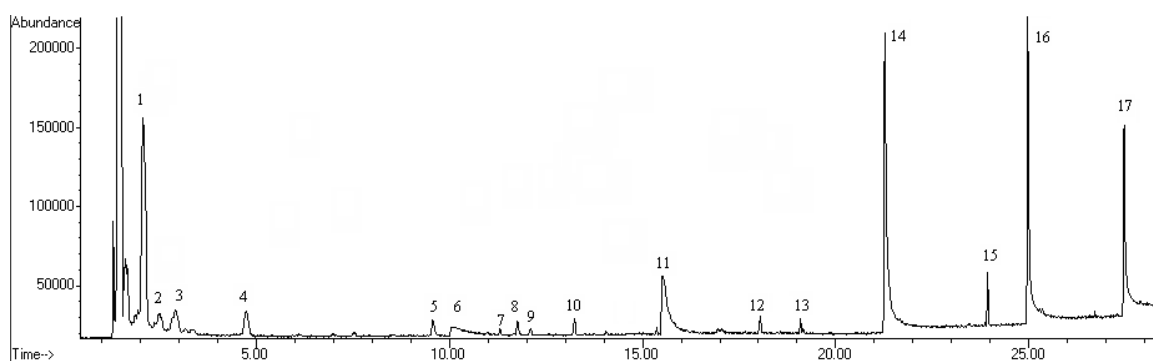


Figure 11. Typical GC chromatogram of Sepet Cheese's volatile compounds (1.Ethanol, 2.Ethyl acetate, 3.1-propanol, 4.Unknown2, 5. Acetoin, 6.Acetic acid, 7.D-limonene, 8.2-heptanone, 9.Unknown3, 10.Ethyl hexanoate, 11.Butyric acid, 12.2-tridecanone, 13.Ethyl octanoate, 14.Hexanoic acid, 15.Ethyl decanoate, 16.Octanoic acid, 17.Decanoic acid)

Table 17. Volatile compounds (area/10⁵) during production and ripening (Germiyan)

No	Compound	Curd+Whey	Cheese				P value
			1st day	1st month	3rd month	6th month	
1	Ethanol	12.68±4.27 ^a	51.43±1.30 ^a	48.62±9.37 ^a	28.77±14.15 ^a	33.43±15.24 ^a	0.062
2	Acetone*	0.00±0.00 ^a	0.00±0.00 ^a	4.15±5.87 ^a	24.16±10.92 ^b	0.00±0.00 ^a	0.025
3	unknown1	0.00±0.00 ^a	1.67±2.36 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.486
4	Ethyl acetate	0.00±0.00 ^a	1.78±2.51 ^a	1.94±2.75 ^a	1.11±0.47 ^a	1.58±2.23 ^a	0.854
5	unknown2	0.00±0.00 ^a	1.94±2.74 ^a	1.83±2.59 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.598
6	1-propanol	0.25±0.35 ^a	4.22±1.85 ^a	4.68±0.90 ^a	5.37±0.76 ^a	5.25±2.20 ^a	0.062
7	2-butanol	0.00±0.00 ^a	0.00±0.00 ^a	0.74±1.04 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.486
8	unknown3*	0.00±0.00 ^a	0.36±0.51 ^a	3.41±0.09 ^b	1.72±1.30 ^{ab}	1.51±0.01 ^{ab}	0.016
9	unknown4	1.05±1.48 ^a	6.72±2.18 ^a	4.77±1.61 ^a	2.52±0.17 ^a	4.21±2.49 ^a	0.131
10	α-pinenene	1.21±1.62 ^a	1.08±1.52 ^a	1.14±1.62 ^a	5.85±3.84 ^a	8.26±9.31 ^a	0.475
11	nonanal	0.73±1.03 ^a	0.67±0.95 ^a	0.00±0.00 ^a	2.80±3.96 ^a	4.41±6.23 ^a	0.682
12	Allylacetate	0.00±0.00 ^a	0.50±0.70 ^a	0.34±0.49 ^a	1.20±0.59 ^a	2.07±1.51 ^a	0.226
15	Acetoin	0.26±0.37 ^a	2.00±2.83 ^a	1.67±2.36 ^a	1.04±1.47 ^a	1.03±1.45 ^a	0.899
17	D-limonene	0.00±0.00 ^a	0.19±0.26 ^a	0.44±0.62 ^a	2.01±2.84 ^a	3.19±1.99 ^a	0.321
18	2-heptanone*	0.80±0.33 ^a	3.33±0.97 ^a	6.19±0.30 ^{ab}	7.95±0.11 ^{ab}	12.30±4.76 ^b	0.021
19	Unknown6	0.00±0.00 ^a	0.47±0.67 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.486
20	Ethyl hexanoate*	0.23±0.33 ^a	2.15±1.11 ^{ab}	4.39±1.47 ^{ab}	7.11±1.41 ^{ab}	11.82±4.36 ^b	0.041
21	benzene, 1,2-dichloro	0.00±0.00 ^a	0.96±1.36 ^a	0.89±1.26 ^a	1.37±1.93 ^a	1.41±1.99 ^a	0.874
22	Butyric acid*	4.72±1.60 ^a	10.20±3.74 ^{ab}	24.13±4.87 ^{bc}	31.11±1.64 ^c	42.20±11.05 ^c	0.006
24	2-tridecanone*	0.96±0.33 ^a	1.49±0.62 ^a	2.40±0.41 ^{ab}	4.56±0.49 ^b	5.46±0.95 ^b	0.017
25	Ethyl octanoate*	0.12±0.17 ^a	1.07±0.12 ^{ab}	2.30±0.63 ^{ab}	3.47±0.58 ^{bc}	5.59±1.68 ^c	0.008
26	Hexanoic acid*	5.58±1.65 ^a	16.53±4.13 ^a	63.52±5.75 ^b	63.15±2.57 ^b	80.88±7.94 ^c	0.000
27	Ethyl decanoate*	0.75±1.06 ^a	2.24±0.22 ^{ab}	3.13±0.31 ^b	3.15±0.33 ^b	4.16±0.75 ^b	0.019
28	Octanoic acid*	6.46±0.45 ^a	18.11±8.37 ^a	44.61±7.50 ^b	41.55±2.74 ^b	49.39±1.01 ^b	0.002
29	Decanoic acid*	3.76±1.79 ^a	9.85±1.31 ^a	21.77±5.17 ^b	32.50±5.46 ^b	32.60±3.29 ^b	0.002

*Changes within a row are significant (p<0.05).

(a–c) means within rows are significantly different (p<0.05) according to the Student–Newman–Keuls test.

Table 18. Volatile compounds (area/10⁵) during production and ripening (Zeytineli)

No	Compound	Curd+Whey	Cheese				P value
			1st day	1st month	3rd month	6th month	
1	Ethanol*	14.36±7.64 ^a	4.08±5.77 ^a	75.78±15.63 ^b	58.27±28.84 ^{ab}	57.69±6.41 ^{ab}	0.021
2	Acetone	12.04±8.92 ^a	10.81±8.30 ^a	3.95±5.58 ^a	15.22±15.19 ^a	0.00±0.00 ^a	0.506
4	Ethyl acetate	0.00±0.00 ^a	0.00±0.00 ^a	5.75±0.49 ^a	4.33±3.17 ^a	4.53±0.41 ^a	0.027
5	unknown2	0.00±0.00 ^a	0.00±0.00 ^a	3.29±4.65 ^a	5.43±7.67 ^a	0.00±0.00 ^a	0.584
6	1-propanol	1.17±1.65 ^a	0.47±0.66 ^a	9.46±3.49 ^b	13.28±1.98 ^b	11.72±0.18 ^b	0.003
8	unknown3*	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.11±0.19 ^b	0.000
9	unknown4*	0.00±0.00 ^a	0.68±0.96 ^a	13.09±2.27 ^b	16.09±7.41 ^b	5.08±0.53 ^{ab}	0.018
10	α-pienene	1.11±1.57 ^a	1.19±1.67 ^a	3.47±0.57 ^a	1.70±2.41 ^a	3.47±1.30 ^a	0.454
11	nonanal	0.74±1.04 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.486
12	Allylacetate	0.00±0.00 ^a	0.00±0.00 ^a	2.45±0.45 ^a	1.13±1.59 ^a	1.91±0.25 ^a	0.069
13	unknown5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	2.09±1.60 ^a	0.00±0.00 ^a	0.106
15	Acetoin*	0.00±0.00 ^a	0.00±0.00 ^a	3.15±1.13 ^{ab}	10.87±5.80 ^b	10.90±1.37 ^b	0.020
16	Acetic acid*	0.00±0.00 ^a	0.00±0.00 ^a	6.43±4.36 ^a	13.39±4.78 ^{ab}	22.02±6.42 ^b	0.011
17	D-limonene*	0.00±0.00 ^a	0.00±0.00 ^a	2.26±0.59 ^a	1.81±1.12 ^a	0.00±0.00 ^a	0.022
18	2-heptanone*	1.64±0.22 ^a	2.05±0.12 ^a	5.04±0.73 ^b	5.28±0.84 ^b	6.81±0.48 ^b	0.001
19	Unknown6	0.00±0.00 ^a	0.00±0.00 ^a	0.98±1.38 ^a	1.03±1.45 ^a	0.00±0.00 ^a	0.598
20	Ethyl hexanoate*	0.94±1.33 ^a	2.89±0.92 ^b	6.08±0.04 ^c	6.28±0.12 ^c	8.75±0.33 ^d	0.001
21	Unknown7	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.15±1.62 ^a	0.00±0.00 ^a	0.486
22	benzene, 1,2-dichloro*	0.00±0.00 ^a	0.39±0.55 ^a	1.31±0.25 ^{ab}	1.80±0.63 ^b	0.00±0.00 ^a	0.019
23	Butyric acid*	0.00±0.00 ^a	4.71±1.72 ^a	60.96±4.43 ^b	72.17±8.29 ^b	84.16±2.24 ^c	0.000
26	2-tridecanone*	0.45±0.64 ^a	1.43±0.62 ^a	3.90±0.08 ^b	4.66±0.73 ^b	4.53±0.45 ^b	0.002
27	Ethyl octanoate*	0.00±0.00 ^a	0.94±0.84 ^a	3.67±0.04 ^b	3.94±0.29 ^b	4.37±0.26 ^b	0.000
28	Hexanoic acid*	8.20±2.12 ^a	15.18±4.82 ^a	95.19±2.74 ^b	125.72±16.45 ^c	145.52±1.97 ^c	0.000
29	Ethyl decanoate*	0.83±1.17 ^a	2.93±1.28 ^{ab}	4.87±0.59 ^b	5.78±0.15 ^b	5.89±0.58 ^b	0.008
30	Octanoic acid*	9.01±1.66 ^a	11.32±0.64 ^a	57.99±0.26 ^b	66.62±4.53 ^c	68.45±3.29 ^c	0.000
31	Decanoic acid*	4.39±0.18 ^a	13.35±3.64 ^a	39.92±5.44 ^b	44.40±13.37 ^b	37.46±5.91 ^b	0.008

*Changes within a row are significant (p<0.05).

(a–d) means within rows are significantly different (p<0.05) according to the Student–Newman–Keuls test.

4.3. Texture Profiles of Sepet Cheeses

Gunasekaran and Ak (2003) reported that the textural attributes of foods play a major role in consumer appeal, buying decisions, and eventual consumption. For some foods, texture is more important to consumers than flavor and color.

The results of texture parameters obtained from texture profile analysis are given in Table 19. Average firmness value of sepet cheeses was 207.59 N. Maximum and minimum firmness values were 540.55 and 65.55 N, respectively.

Pinho et al. (2004) found that Terrincho Ewe cheese had 1810.8 N firmness. Gutiérrez (2004) also found that cheddar cheese containing gamma-oryzanol reached 171 N firmness at the end of ripening. Therefore, firmness value of sepet cheese was less than firmness of Terrincho Ewe cheese and higher than cheddar cheese containing gamma-oryzanol.

The average springiness, cohesiveness, adhesiveness and chewiness values of sepet cheese samples were 0.57, 0.59, 0.82, 58.29, respectively. Minimum value for springiness was 0.42 and maximum value was 0.76. Minimum cohesiveness of sepet cheeses was 0.21. Maximum value for cohesiveness was 1.56. Pollard et al. (2003) investigated cheddar cheese cohesiveness and springiness during ripening and found average 0.79 springiness value and 0.69 cohesiveness value. Pinho et al. (2004) also found that Terrincho Ewe cheese had 0.98 springiness value and 0.49 cohesiveness value. Sepet cheese had lower springiness value than Terrincho Ewe cheese and cheddar cheese. Cohesiveness value of sepet cheese was lower than of cheddar cheese and higher than of Terrincho Ewe cheese .

Some sepet cheeses did not show any adhesiveness. The maximum adhesiveness value was 14.34 Nmm. Maximum and minimum chewiness values were 123.84 N and 29.40 N, respectively. Terrincho Ewe cheese reached 991.7 N chewiness value at the end of ripening (Pinho, et al. 2004). Sepet cheese chewiness value was less than of Terrincho Ewe cheese.

Table 19. Texture profile parameters of sepet cheeses (mean values±standard deviation)

Sample number	Firmness (N)	Springiness(-)	Cohesiveness(-)	Adhesiveness (Nmm)	Chewiness (N)
1	141.24±02.21	0.72±0.07	0.41±0.09	0.00±00.00	36.47±04.66
2	182.39±00.52	0.44±0.01	0.48±0.08	0.00±00.00	38.36±07.93
3	540.55±26.17	0.64±0.00	0.37±0.00	0.00±00.00	123.84±00.00
4	84.89±08.68	0.53±0.04	0.72±0.09	0.00±00.00	32.14±01.78
5	217.41±02.43	0.52±0.03	0.49±0.02	0.00±00.00	54.96±02.29
6	179.46±23.22	0.57±0.01	0.46±0.03	0.00±00.00	46.99±02.57
7	279.90±42.54	0.63±0.07	0.41±0.03	0.00±00.00	72.12±01.36
8	306.35±02.25	0.57±0.03	0.43±0.02	0.00±00.00	76.40±09.12
9	504.11±00.00	0.51±0.00	0.21±0.00	0.00±00.00	53.97±00.00
10	87.71±08.06	0.58±0.01	0.58±0.04	0.00±00.00	29.40±05.01
11	148.34±19.56	0.49±0.02	0.73±0.10	0.00±00.00	52.91±01.70
12	253.33±27.76	0.52±0.03	0.61±0.12	0.00±00.00	81.78±22.38
13	283.69±21.70	0.49±0.01	0.66±0.22	0.00±00.00	93.17±39.72
14	149.02±12.08	0.81±0.05	0.40±0.07	0.00±00.00	49.61±15.65
15	184.60±13.48	0.82±0.02	0.34±0.00	0.00±00.00	53.07±04.55
16	86.38±07.01	0.42±0.05	1.56±0.30	0.00±00.00	55.87±00.61
17	254.12±41.63	0.62±0.04	0.28±0.01	0.00±00.00	44.83±09.04
18	76.00±02.51	0.51±0.04	0.96±0.12	0.00±00.00	37.38±08.80
19	112.98±07.17	0.76±0.03	0.72±0.08	0.00±00.00	69.83±03.05
20	250.00±13.20	0.48±0.15	0.51±0.03	0.00±00.00	61.00±19.54
21	65.55±07.39	0.52±0.07	0.94±0.14	14.34±04.75	31.85±05.11
22	111.16±05.43	0.49±0.00	0.76±0.04	10.37±14.67	41.41±04.10
23	73.39±03.72	0.44±0.01	0.90±0.02	0.00±00.00	29.47±01.26
24	104.04±01.13	0.46±0.02	0.83±0.06	0.00±00.00	40.45±04.40
25	114.80±06.68	0.48±0.02	0.91±0.06	0.00±00.00	50.74±01.86
26	284.25±00.67	0.60±0.01	0.57±0.08	0.00±00.00	97.46±15.15
27	352.22±04.26	0.61±0.01	0.51±0.05	0.00±00.00	109.28±07.12
28	255.36±00.00	0.55±0.00	0.57±0.00	0.00±00.00	79.63±00.00
29	391.53±00.00	0.76±0.00	0.22±0.00	0.00±00.00	67.06±00.00
30	153.15±10.10	0.68±0.02	0.36±0.13	0.00±00.00	37.29±09.91
Minimum	65.55	0.42	0.21	0.00	29.40
Maximum	540.55	0.76	1.56	14.34	123.84
Average	207.59	0.57	0.59	0.82	58.29

Texture is affected during production and ripening. Network structure of curd is critically affected by composition of milk and technological conditions of coagulation (Fox, et al. 2000; Gunasekaran and Ak 2003). Therefore, cheese production technology cause varieties in cheese texture. Moreover, there are effects of moisture, pH, salt content, proteolysis which is affected by ripening conditions on cheese texture (Lawrance, et al. 1987). Figure 12 shows the typical texture profile plot of sepet cheese samples.

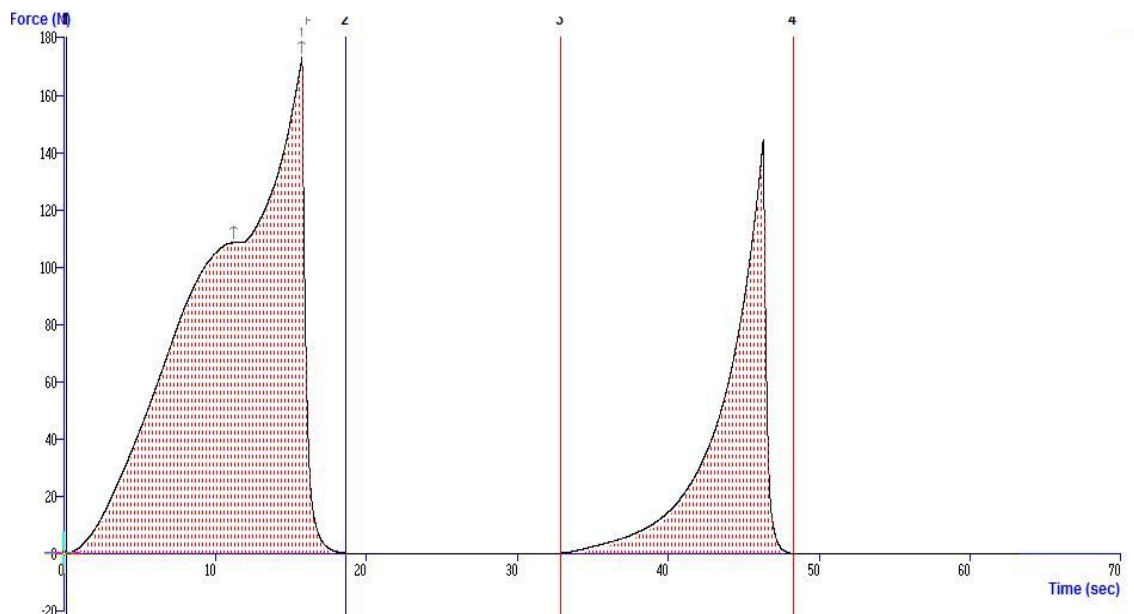


Figure 12. Typical texture profile plot of sepet cheese

Figure 13 shows the similarities between samples in terms of textural characteristics. All samples clustered together except 3, 16, 21. Sample 3 had highest firmness value. Sample 16 had highest cohesiveness value and lowest springness value. Sample 21 also had highest adhesiveness value.

Table 20 shows the correlations between textural parameters of sepet cheese samples. There was found that there was a significant correlation between firmness and chewiness. It was also found a significant correlations between firmness and cohesiveness. There was also a inverse correlation between springiness and cohesiveness with -0.568 ($p < 0.001$) coefficient. Moreover, chemical and physical

characteristics and textural characteristics of sepet cheese samples were correlated (Table 21). Figure 14 shows the relations of textural characteristics. Firmness and chewiness values seemed to be related in Figure 14.

It was found that there was a significant correlation between total solid content and firmness. Firmness and water activity were also correlated significantly. The cheese with higher water activity had lower firmness value. Positive correlation was found between firmness and dry matter and correlation coefficient was found as 0.573 ($p < 0.01$) for firmness-dry matter. International Dairy Federation classify cheeses according to firmness on the basis of water in fat free substances (IDF General Secretariat 1981). The water contents in fat free substance of sepet cheese samples were found to be significantly correlated with firmness and found -0.593 correlation coefficient ($p < 0.001$) and concluded that sepet cheese samples were semi hard cheese. It was also found that water activity were inversely correlated with firmness and chewiness and positively correlated with cohesiveness. Water activity of sepet cheese samples were related to salt content and total solid content. Fox et al. (2000) explained that salting affects cheese texture due to protein solubility and protein conformation and cause hard cheese. Fat in total solid content also significantly negatively correlated with firmness. Gwartney et al. (2006), Gunasekaran and Ak (2003) stated that lower fat cheeses had more compact protein matrix and harder texture than whole fat cheeses.

Table 20. Correlation coefficients between textural characteristics of sepet cheeses

	Firmness	Springiness	Cohesiveness	Adhesiveness	Chewiness
Firmness	1.000				
Springiness	0.192	1.000			
Cohesiveness	-0.627***	-0.568***	1.000		
Adhesiveness	-0.267	-0.158	0.254	1.000	
Chewiness	0.735***	0.168	-0.226	-0.246	1.000

*p value < 0.05, **p value < 0.01, ***p value < 0.001

Table 21. Correlation coefficients between textural and physical, chemical characteristics of sepet cheeses

	Firmness	Springiness	Cohesiveness	Adhesiveness	Chewiness
pH	-0.258	0.204	-0.085	0.162	-0.444*
Acidity (%)	0.074	-0.163	0.181	0.133	0.241
NaCl (%)	0.293	0.174	-0.191	-0.186	0.280
Fat (%)	0.090	-0.144	-0.038	0.074	0.063
Fat in total solid content (%)	-0.354*	-0.140	0.250	-0.024	-0.162
Dry matter (%)	0.573***	-0.012	-0.358	0.107	0.284
Water in fat free substances (%)	-0.593***	0.042	0.379*	-0.090	-0.283
Water activity	-0.553**	-0.163	0.380*	0.362*	-0.460**
Protein (%)	-0.255	-0.044	0.057	0.078	-0.154
Ripening index	-0.293	-0.355*	0.414*	0.714***	-0.266
Lypolysis	-0.168	-0.369	0.689***	0.232	0.047

*p value < 0.05, **p value < 0.01, ***p value < 0.001

It was also found that there was a correlation between ripening index, lypolysis and textural characteristics. There was a significant inverse correlation between springiness and ripening index ($p < 0.05$). These result was in agreement with the results of Gunasekaran and Ak. (2003) study. They were reported that decrease in springiness occurred during ripening with the effects of proteolytic breakdown of the protein matrix. Moreover, significant correlation (-0.443 correlation coefficient with $p < 0.05$) between pH and chewiness was observed. There were also significant correlations between cohesiveness-ripening index and lypolysis-cohesiveness. Lypolysis and ripening indices were also significantly correlated since both of them were related to biochemical events during ripening. Second compression required much more force than first bite in texture analysis of some cheeses which had higher cohesiveness values than of the other samples. This could be related to the increase in the holes of cheese texture. Holes in cheese texture can occur with the activity of heterofermentative lactic acid bacteria or propionic acid bacteria during ripening (Üçüncü 2004).

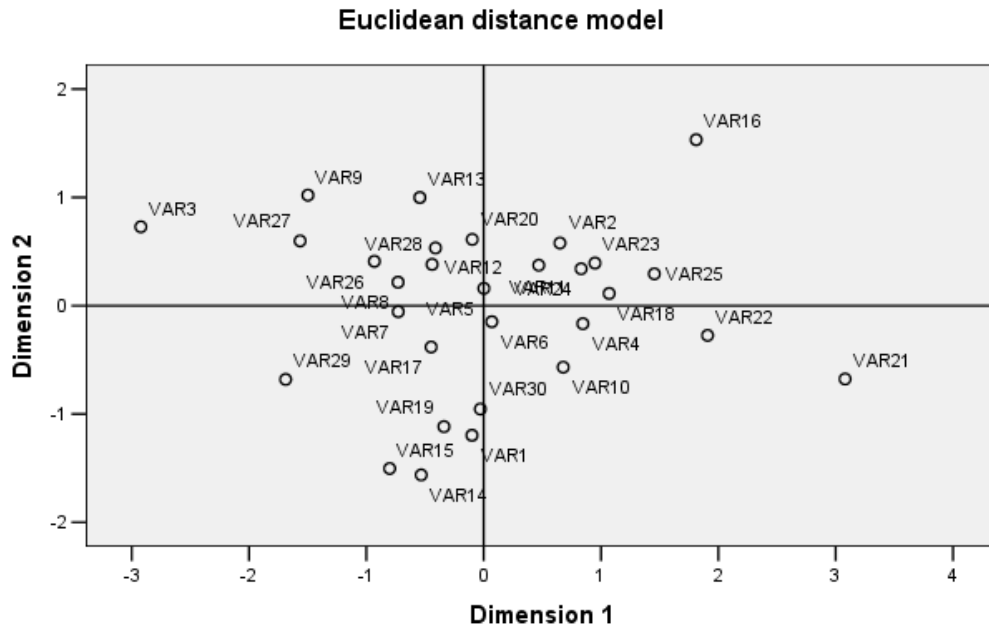


Figure 13. Geometrical representation of cheeses in terms of textural characteristics by multidimensional scaling. (Each var represents the cheeses in order), (Stress= 0.10269, $R^2= 0.95541$)

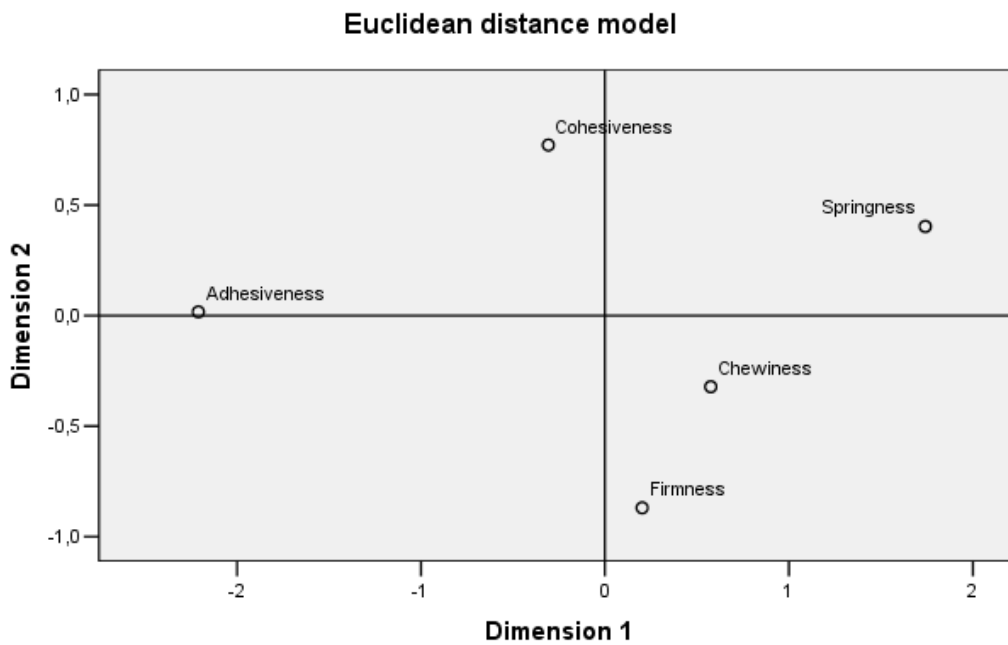


Figure 14. Geometrical representation of cheese textural characteristics produced by multidimensional scaling (Stress= 0.00320, $R^2= 0.9993$)

4.4. Sensory Analysis of Sepet Cheeses

Sensorial analysis was done to determine flavor profile of sepet cheeses. Flavor profile analysis contains vocabulary development and rating sessions and carried out during group discussions (Murray, et al. 2001).

Typical flavor profile diagram are given in Figure 15. Results for flavor profile analysis are given in Table 22. As a result of descriptive sensory analysis of sepet cheeses, predominant basic taste was salty for all sepet cheese samples. Other basic tastes which were perceived by panelists were bite, sour, umami, sweet in decreasing order. Free fatty acid, animal like, sulfurous, creamy, whey-like, cooked, moisty cloth aromatic terms were also found as characteristic for sepet cheeses in decreasing order.

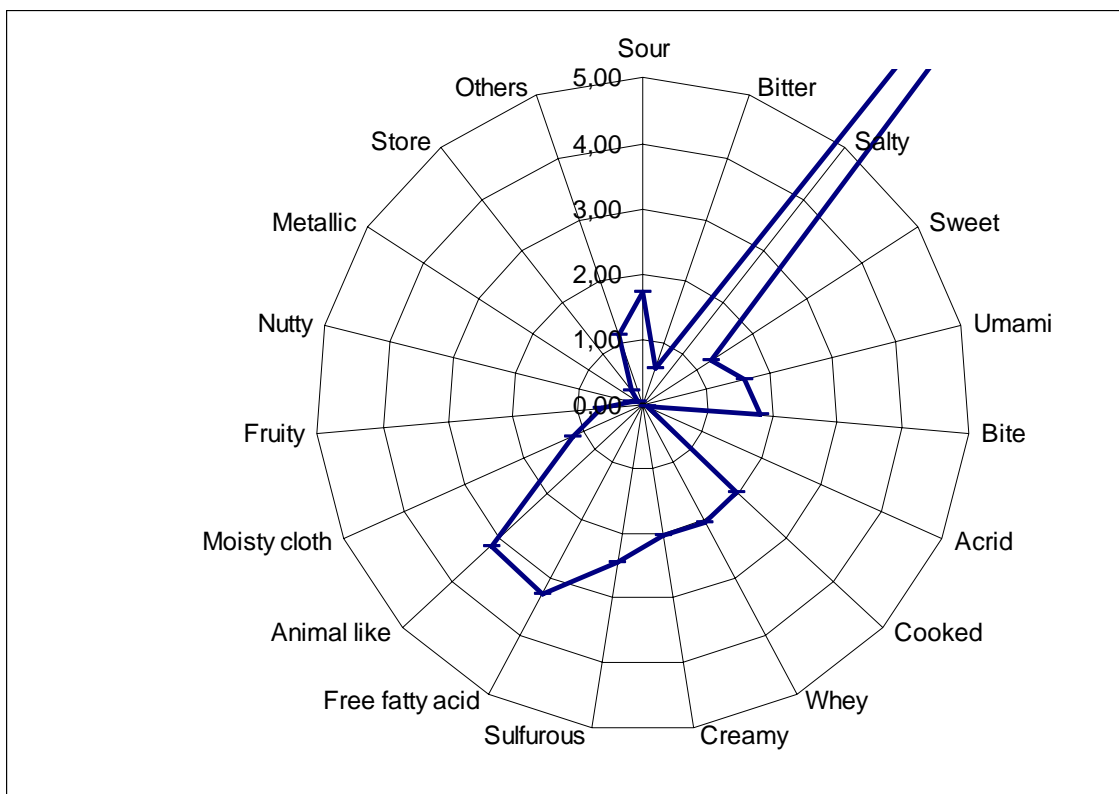


Figure 15. Typical flavor profile diagram of sepet cheese

Some of these terms were also found as characteristic for Cheddar and Ezine cheese. Cooked, whey, diacetyl, milkfat/lactone, fruity aromatics, sulfur aromatics, free fatty acid, brothy aromatics, nutty nutlike, cowy/phenolic descriptive terms used for cheddar cheese (Drake 2004). Free fatty acids, whey, creamy, goaty, fermented was characteristic flavors of Ezine cheese. (Yüceer, et al. 2006). Beuquier et al. (1997) determined that acid, salty, bitter terms were characteristic for Swiss type cheese (Beuquier and Buchin 2004). Hough et al.(1996) used milky-creamy, sweet, sour, lypolysis terms for sensorial characterisation of Reggianito grating cheese (Delahunty and Drake 2004).

Table 22. Descriptive sensory analysis results (mean value±standard deviation)

Basic tastes	Sample number										Average
	1	8	11	9	14	28	17	21	22	16	
Sour	1.43	1.98	1.75	1.83	1.63	1.80	1.63	1.13	1.55	2.48	1.72±0.36
Bitter	0.40	0.93	0.95	0.55	0.30	0.45	0.58	0.15	0.43	1.08	0.58±0.31
Salty	19.25	17.25	21.50	19.55	19.65	20.85	23.20	8.65	19.85	21.00	19.08±3.99
Sweet	1.65	1.30	1.15	1.05	1.33	1.23	1.05	1.53	1.24	0.98	1.25±0.21
Umami	1.30	1.55	1.40	2.00	1.95	1.33	1.30	1.80	1.63	1.65	1.59±0.26
Bite	1.43	2.40	2.58	2.00	1.13	1.10	1.93	0.80	1.15	3.50	1.80±0.84
Acrid	0.10	0.10	0.10	0.00	0.00	0.00	0.05	0.00	0.35	0.10	0.08±0.11
Aromatics											
Cooked	1.95	1.90	1.65	1.85	2.30	2.10	1.85	2.13	1.85	1.85	1.94±0.19
Whey	1.93	1.78	1.88	1.88	2.20	2.53	1.85	2.20	2.38	1.68	2.03±0.28
Creamy	1.83	1.83	1.83	1.83	2.20	2.10	1.90	2.13	2.70	1.90	2.02±0.28
Sulfurous	2.25	3.03	2.35	2.60	2.00	2.08	2.23	2.33	2.33	3.13	2.43±0.38
Free fatty acid	2.98	4.70	2.93	5.35	2.38	2.20	2.90	2.28	2.55	4.43	3.27±1.13
Animal like	3.20	3.48	3.30	3.13	2.75	2.63	3.63	3.28	2.33	3.85	3.16±0.47
Moisty cloth	0.73	0.88	0.95	1.73	1.20	1.45	1.03	1.60	1.00	1.05	1.16±0.33
Fruity	0.53	0.85	0.60	0.60	0.48	0.58	0.63	0.65	0.38	1.00	0.63±0.18
Nutty	0.15	0.05	0.10	0.15	0.20	0.20	0.05	0.50	0.15	0.15	0.17±0.13
Metallic	0.10	0.10	0.05	0.18	0.05	0.00	0.05	0.00	0.05	0.10	0.07±0.05
Store	0.30	0.20	0.20	0.30	0.35	0.18	0.20	0.58	0.05	0.30	0.27±0.14
Others	1.03	1.53	1.30	1.10	1.00	1.15	1.05	1.10	0.85	1.43	1.15±0.21

4.5. Microbial Characterization of Sepet Cheeses

In microbiological analysis of the sepet cheese samples, minimum total aerobic mesophilic bacteria count was 5.54 log cfu/g and maximum value was found to be 9.03 log cfu/g. Minimum and maximum lactococcus bacteria counts were 5.52 log cfu/g and 8.89 log cfu/g. The results of lactobacilli enumeration were minimum 5.51 log cfu/g and maximum 8.74 log cfu/g. Enterococci counts were found in between 5.51 log cfu/g and 8.88 log cfu/g. Maximum yeast, mold, and coliform counts were 5.41 log cfu/g, 2.59 log cfu/g, 5.51 log cfu/g, respectively. Some of the sepet cheese samples did not contain yeast, mold, coliform. Eleven sepet cheese samples had *Staphylococcus aureus* with maximum 4.61 log cfu/g. Hamid and Owni (2007) stated that high counts of *S. aureus* found in some cheese samples might be attributed to the high initial numbers of *S. aureus* in milk or contamination during processing. Maximum microbial counts for psychrotrophic bacteria was 7.23 log cfu/g and minimum value was 3.18 log cfu/g. Average total aerobic bacteria, lactococci, lactobacilli, enterococci, yeast, mold, psychrotrophic, coliform bacteria, and *S.aureus* microbial counts were 7.64 , 7.38, 7.38, 6.99, 3.22, 0.95, 2.72, 5.37, and 1.25 log cfu/g, respectively in sepet cheese samples. The microbial counts for all sepet cheese samples are given in Table 23. Similarities of sepet cheese samples are shown in Figure 16. Figure 17 shows that lactococci, enterococci, lactobacilli, total aerobic bacteria counts of sepet cheese samples were related with each other. The 16, 15, 27, 8, 9 samples were placed away from the other samples in Figure 16, because their microbial counts lower than microbial counts of the other cheese samples. Total aerobic bacteria counts of sepet cheese samples were similar with results of Kınık et al. (1999) study, but higher than of Karakaş and Korukluoğlu (2006) study. Previous studies found that sepet cheese samples had 4.26 - 7.05 log cfu/g total aerobic bacteria (Kınık, et al. 1999; Karakaş and Korukluoğlu 2006). Kınık et al. (1999) also found 2.678 log cfu/g Staphlococci in sepet cheese samples. Moreover, mold and yeast counts shows similarities with Kınık et al. (1999) study. However, Karakaş and Korukluoğlu found relatively higher yeast counts in sepet cheese samples.

According to the Turkish Standard TS 3001, Tulum Cheese should not contain any *Escherichia coli*, *Salmonella*, or *S. aureus* and should have a maximum number

of 10^2 cfu/g for coliform bacteria and yeast and mould. According to the standard, in this study, *S. aureus*, coliform bacteria, yeast counts of sepet cheese samples were found to be higher than the value given in the standard. When the results were compared with previous traditional cheese studies, coliform bacteria counts were seen to be lower than Örgü and Sıkma cheeses. Coliform bacteria counts for Örgü cheese and Sıkma cheeses were 3.73 log cfu/g and 5.99 log cfu/g, respectively by Turkoglu et al. (2003) and Ceylan et al. (2003). Kamber (2008C) reviewed that mihaliç cheese had 3.30 log cfu/g coliform bacteria counts, 5.9 log cfu/g lactococci and 7.69 log cfu/g total aerobic bacteria counts. Sepet cheese samples showed similarities with mihaliç cheese based on total aerobic bacteria counts. Kamber and Çelik (2007) investigated the microbiological characteristics of gorcola cheese and found that gorcola cheese had 1.9×10^7 cfu/g total aerobic mesophilic bacteria count, 2.9×10^4 cfu/g the lactic acid bacteria count, 1.0×10^7 cfu/g the lactococcus count, 1.5×10^4 cfu/g coliform count, and 1.4×10^5 cfu/g yeast-mold count. Yeast and mold counts of sepet cheeses were lower than gorcola cheese. Lactococci and total aerobic mesophilic bacteria counts of sepet cheese samples were higher than of the gorcola cheese.

Table 24 shows the correlation coefficients between microbial counts of sepet cheese samples. The total aerobic mesophilic bacteria counts were significantly correlated with coliform and lactococci, lactobacilli, and enterococci counts. Lactococci, lactobacilli and enterococci bacteria counts were also significantly correlated with each other. These results were in agreement with previous studies. Ceylan et al.(2003) found significant correlation between total aerobic, lactic acid and coliform bacteria counts. Turkoglu et al.(2003) also found significant correlation with coliform and total aerobic bacteria counts. Moreover, the results of microbiological, physical and chemical analysis were correlated and results are given in Table 25. Total aerobic mesophilic bacteria, lactococci, lactobacilli, enterococci, coliform, *S. aureus* bacteria counts were significantly correlated with water activity values. Sepet cheese samples with lower water activity values had lower total aerobic mesophilic bacteria, lactococci, lactobacilli, enterococci, coliform, *S. aureus* bacteria counts than of the other cheese samples. Coliform bacteria counts and NaCl contents in total solid content of sepet cheese samples were significantly correlated. The effect of salt content on coliform was related to the role of NaCl in increasing osmotic pressure and lowering water activity.

Table 23. Microbial counts of sepet cheeses (mean values±standard deviation)

Sample number	Total aerobic bacteria log cfu/g	Lactococci log cfu/g	Lactobacilli log cfu/g	Enterococci log cfu/g	Yeast log cfu/g	Mold log cfu/g	Coliform log cfu/g	Psychrotrophic bacteria log cfu/g	<i>S.aureus</i> log cfu/g
1	7.86±0.02	7.82±0.02	7.82±0.04	7.62±0.13	3.03±0.03	1.65±0.07	3.02±0.15	6.07±0.03	<10 cfu/g
2	7.98±0.05	7.95±0.01	7.93±0.01	7.53±0.06	5.16±0.02	1.78±0.34	3.61±0.01	7.00±0.03	<10 cfu/g
3	8.70±0.05	8.13±0.01	7.61±0.08	7.18±0.01	3.24±0.07	1.40±0.12	2.62±0.08	5.18±0.01	<10 cfu/g
4	7.30±0.02	7.20±0.05	6.92±0.03	5.69±0.02	3.50±0.03	1.18±0.21	2.13±0.12	5.14±0.03	<10 cfu/g
5	7.53±0.02	6.91±0.01	6.88±0.04	6.69±0.08	5.41±0.09	<10 cfu/g	3.13±0.03	6.28±0.01	<10 cfu/g
6	8.04±0.02	8.02±0.01	7.78±0.02	7.79±0.05	4.07±0.03	<10 cfu/g	4.93±0.01	6.84±0.12	<10 cfu/g
7	8.56±0.03	8.24±0.05	8.68±0.05	8.10±0.02	4.72±0.05	1.18±0.21	3.46±0	7.23±0.03	<10 cfu/g
8	5.54±0.04	5.52±0.00	5.51±0.01	5.54±0.04	3.38±0.00	<10 cfu/g	<10 cfu/g	5.17±0.02	<10 cfu/g
9	5.70±0.09	5.53±0.02	5.72±0.01	5.65±0.03	3.17±0.03	<10 cfu/g	<10 cfu/g	4.87±0.10	<10 cfu/g
10	8.87±0.02	8.89±0.01	8.74±0.02	8.53±0.01	4.60±0.05	<10 cfu/g	5.48±0.02	6.90±0.04	3.59±0.06
11	7.77±0.05	7.72±0.06	7.61±0.03	7.21±0.03	2.63±0.08	2.59±0.06	1.60±0.16	5.38±0.01	<10 cfu/g
12	8.14±0.02	7.94±0.00	7.66±0.04	6.80±0.05	2.94±0.03	2.57±0.07	1.70±0.12	5.19±0.05	<10 cfu/g
13	5.61±0.09	5.59±0.06	7.84±0.04	5.59±0.06	2.31±0.08	1.54±0.09	1.48±0.21	4.67±0.17	3.45±0.09
14	6.95±0.01	6.91±0.02	7.14±0.01	7.95±0.02	2.30±0.15	2.30±0.33	<10 cfu/g	4.95±0.02	<10 cfu/g
15	6.00±0.01	5.87±0.02	5.69±0.06	6.16±0.04	<10 cfu/g	<10 cfu/g	<10 cfu/g	4.97±0.01	<10 cfu/g
16	5.60±0.04	5.60±0.05	5.63±0.01	5.51±0.01	<10 cfu/g	<10 cfu/g	<10 cfu/g	4.55±0.06	<10 cfu/g
17	6.94±0.03	6.61±0.10	7.13±0.03	5.89±0.01	4.64±0.12	<10 cfu/g	1.60±0.16	3.22±0.08	<10 cfu/g
18	7.14±0.02	6.93±0.01	7.13±0.01	6.91±0.03	4.16±0.03	<10 cfu/g	1.81±0.14	6.17±0.02	3.77±0.05
19	8.75±0.05	8.74±0.01	8.57±0.03	8.88±0.08	3.52±0.02	1.48±0.21	4.63±0.12	6.90±0.05	3.95±0.07
20	8.21±0.01	8.16±0.01	7.78±0.03	7.06±0.14	4.61±0.08	<10 cfu/g	5.18±0.09	5.57±0.03	3.81±0.25
21	8.04±0.03	7.69±0.04	7.82±0.06	7.11±0.01	2.50±0.03	<10 cfu/g	3.26±0.02	5.29±0.07	2.60±0.16
22	9.03±0.03	8.85±0.02	8.22±0.06	7.82±0.01	3.14±0.05	1.81±0.05	4.87±0.03	3.20±0.21	<10 cfu/g
23	8.98±0.01	8.02±0.02	8.14±0.01	7.91±0.01	2.60±0.07	1.65±0.06	3.10±0.02	5.41±0.04	<10 cfu/g
24	8.90±0.02	7.85±0.02	7.92±0.02	7.14±0.02	2.47±0.04	<10 cfu/g	2.52±0.01	4.90±0.01	3.41±0.04
25	8.95±0.01	7.78±0.01	7.85±0.02	7.03±0.03	2.73±0.01	2.54±0.08	3.41±0.04	3.18±0.13	2.65±0.06
26	8.56±0.02	8.26±0.03	7.70±0.09	8.13±0.01	3.27±0.02	1.54±0.09	4.07±0.02	5.96±0.03	3.49±0.04
27	5.80±0.05	5.59±0.01	5.54±0.04	5.70±0.04	3.43±0.04	<10 cfu/g	<10 cfu/g	3.18±0.02	<10 cfu/g
28	8.51±0.05	8.14±0.02	8.11±0.02	7.61±0.02	4.06±0.03	<10 cfu/g	5.31±0.02	6.88±0.02	<10 cfu/g
29	6.66±0.04	6.23±0.05	5.88±0.02	5.55±0.04	1.90±0.08	1.54±0.09	3.43±0.03	5.15±0.07	2.30±0.34
30	8.86±0.02	8.80±0.05	8.72±0.05	7.63±0.04	3.21±0.05	1.81±0.14	5.51±0.03	5.70±0.02	4.61±0.02
Min.	5.54	5.52	5.51	5.51	<10 cfu/g	<10 cfu/g	<10 cfu/g	3.18	<10 cfu/g
Max.	9.03	8.89	8.74	8.88	5.41	2.59	5.51	7.23	4.61

Min. : Minimum
Max.: Maximum

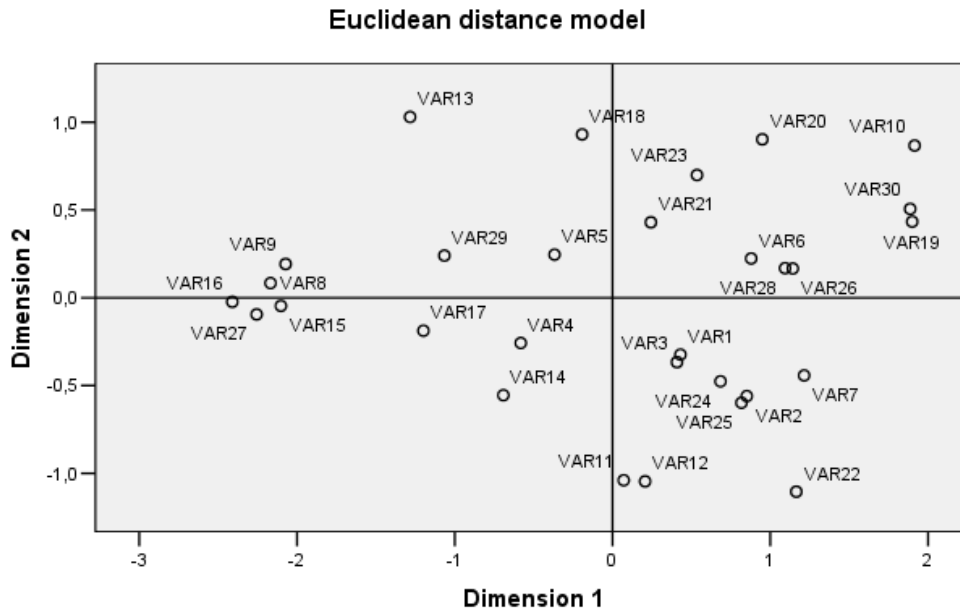


Figure 16. Geometrical representation of cheeses in terms of microbial characteristics by multidimensional scaling. (Each var represents the cheeses in order), (Stress= 0.13743, $R^2= 0.92$)

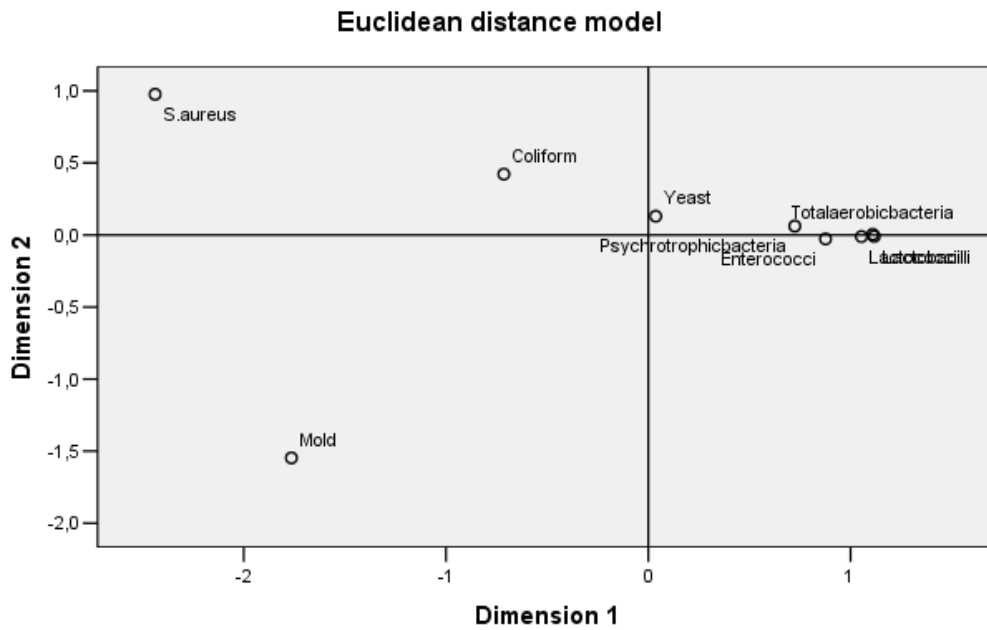


Figure 17. Geometrical representation of microbial characteristics produced by multidimensional scaling (Stress= 0.0063, $R^2= 0.99988$)

Table 24. Correlation coefficients between microbial counts of sepet cheese samples

	Total aerobic bacteria log cfu/g	Lactococci log cfu/g	Lactobacilli log cfu/g	Enterococci log cfu/g	Yeast log cfu/g	Mold log cfu/g	Coliform log cfu/g	Psychrotrophic bacteria log cfu/g
Lactococci log cfu/g	0.964***	1.000						
Lactobacilli log cfu/g	0.861***	0.891***	1.000					
Enterococci log cfu/g	0.824***	0.875***	0.818***	1.000				
Yeast log cfu/g	0.375*	0.411*	0.424*	0.341	1.000			
Mold log cfu/g	0.349	0.353	0.377*	0.308	-0,130	1.000		
Coliform log cfu/g	0.803***	0.840***	0.774***	0.678***	0.486**	0.108	1.000	
Psychrotrophic bacteria log cfu/g	0.327	0.430*	0.421*	0.547**	0.411*	-0.085	0.471**	1.000
<i>S. aureus</i> log cfu/g	0.289	0.295	0.389*	0.250	0.050	-0.013	0.444*	0.145

Significant *p value < 0.05, **p value < 0.01, ***p value < 0.001)

Table 25. Correlation coefficients between microbial counts (logarithmic units) and physico-chemical characteristics of sepet cheeses

	Total aerobic bacteria Log cfu/g	Lactococci log cfu/g	Lactobacilli log cfu/g	Enterococci log cfu/g	Yeast log cfu/g	Mold log cfu/g	Coliform log cfu/g	Psychrotrophic bacteria log cfu/g	<i>S. aureus</i> log cfu/g
pH	-0,019	-0,054	-0,014	0.055	-0.255	0.087	-0,001	-0.240	0.038
Acidity (%)	0.199	0.184	0.084	0.040	-0.003	0.048	0.085	0.011	0.180
Fat (%)	-0.122	-0,116	-0.071	-0.082	0.051	-0.178	-0.099	-0.160	-0.052
Dry matter (%)	-0.219	-0.340	-0.352	-0.344	-0.038	-0.164	-0.199	-0.268	-0.265
Water activity	0.531**	0.464*	0.507**	0.374*	-0.002	0.036	0.507**	-0.045	0.618**
Protein (%)	0.123	0.150	0.177	0.224	-0.078	0.237	0.013	-0.079	-0.104
Ripening index	0.020	0.015	-0.029	-0.042	-0.023	-0.296	0.086	-0.150	-0.141
Lypolysis	-0.290	-0.300	-0.389	-0.241	-0.582**	-0.231	-0.357	-0.170	-0.135
Salt in total solid content (%)	-0.188	-0.128	-0.260	-0.038	-0.164	0.232	-0.534**	0.049	-0.341
Fat in total solid content (%)	0.058	0.161	0.211	0.200	0.091	-0.056	0.068	0.081	0.186

(*p value < 0.05, **p value < 0.01, ***p value < 0.001)

The microbial counts during production and ripening periods of sepet cheeses were also investigated. The changes of microbial counts are given in Table 26. The number of all bacterial counts increased during cheese production. Then the total aerobic, lactococci, lactobacilli, enterococci counts decreased slightly during Germiyan cheese and Zeytineli cheese ripening. The Staphylococci and coliform counts decreased and they were under 10 cfu/g during ripening of both cheeses. Initial psychrotrophic bacteria counts for Zeytineli and Germiyan cheese production periods were 5.56 and 6.12 log cfu/g and decreased to 4.95 and 4.98 log cfu/g, respectively. Yeast and mold counts were less than 10 cfu/g at the end of Germiyan cheese ripening. Although yeast and mold counts in Zeytineli cheese decreased until the first month, their levels could not decreased below 10 cfu/g.

These results are similar with the study of Kılıç et al. (2006). They found significant decrease in staphylococci and coliform counts during Turkish fresh goat cheese ripening. The similar increase and decrease trend were also found by Cabezas et al. (2007) and Calleja et al. (2002).

Tables 27 and 28 shows the correlation between microbial counts and their relation with chemical and physical changes during production and ripening. Lactobacilli, total aerobic bacteria and lactococci counts were correlated significantly during both Germiyan and Zeytineli cheese production and ripening periods. Staphylococci - yeast and coliform - yeast counts were significantly correlated. Also, Staphylococci counts were significantly correlated with pH and titratable acidity values which obtained during production and ripening of Zeytineli and Germiyan cheeses. Coliform counts also sinificantly correlated with pH and titratable acidity during Germiyan cheese production and ripening and these results showed that increasing in acidity and decrease in pH cause decrease in Staphylococci and coliform counts. Staphylococci, yeast, and coliform counts were significantly inversely correlated with ripening index, NaCl content, and significantly positively correlated with water activity. Since mold and yeast could grow at wide range of pH and acidity, the most important reason of decrease in yeast and mold counts during ripening were anaerobic conditions (Jay 1996).

Table 26. Microbiological counts during production and ripening

Sample	Location	Total aerobic Bacteria log cfu/g	Lactococci log cfu/g	Lactobacilli log cfu/g	Enterococci log cfu/g	Yeast log cfu/g	Mold log cfu/g	Coliform log cfu/g	Psychrotrophic Bacteria log cfu/g	Staphylococci log cfu/g	
Goat milk	Zeytineli	5.60±0.05 ^a	5.29±0.08 ^a	5.27±0.02 ^a	5.35±0.01 ^a	3.03±0.01 ^c	1.39±0.12 ^a	3.23±0.07 ^c	5.56±0.01 ^b	2.48±0.04 ^b	
Curd		6.70±0.04 ^b	6.40±0.07 ^b	6.15±0.03 ^b	6.55±0.06 ^b	3.98±0.01 ^d	1.96±0.17 ^a	3.56±0.08 ^d	6.62±0.09 ^{de}	2.62±0.03 ^c	
1st day cheese		7.74±0.03 ^f	7.61±0.01 ^f	7.11±0.01 ^c	6.71±0.12 ^c	4.02±0.03 ^d	2.22±0.02 ^b	5.30±0.05 ^e	6.54±0.03 ^d	2.53±0.02 ^c	
1st month cheese		7.48±0.01 ^e	7.51±0.05 ^e	7.53±0.02 ^e	7.39±0.00 ^e	2.64±0.05 ^b	1.74±0.06 ^a	1.59±0.16 ^b	5.01±0.04 ^a	<10 cfu/g ^a	
2nd month cheese		7.86±0.03 ^g	7.69±0.02 ^f	7.66±0.02 ^f	7.64±0.12 ^f	2.70±0.07 ^b	1.74±0.06 ^a	<10 cfu/g ^a	6.71±0.05 ^c	<10 cfu/g ^a	
3rd month cheese		7.23±0.02 ^d	7.25±0.01 ^d	7.17±0.01 ^d	7.13±0.03 ^d	2.90±0.02 ^c	<10 cfu/g ^a	1.65±0.07 ^b	6.07±0.03 ^c	<10 cfu/g ^a	
4th month cheese		6.91±0.04 ^c	6.89±0.03 ^c	7.11±0.02 ^c	8.16±0.05 ^h	2.25±0.10 ^a	1.38±1.30 ^a	<10 cfu/g ^a	5.00±0.01 ^a	<10 cfu/g ^a	
6th month cheese		6.96±0.01 ^c	6.91±0.02 ^c	7.14±0.01 ^{cd}	7.95±0.02 ^g	2.29±0.16 ^a	1.23±1.00 ^a	<10 cfu/g ^a	4.95±0.02 ^a	<10 cfu/g ^a	
Goat milk		Germiyan	6.23±0.10 ^c	5.42±0.05 ^a	5.00±0.02 ^a	5.10±0.01 ^b	2.70±0.04 ^b	<10 cfu/g ^a	4.07±0.02 ^c	6.12±0.02 ^e	2.15±0.15 ^b
Curd			6.47±0.01 ^d	5.92±0.03 ^c	6.02±0.08 ^d	6.59±0.01 ^e	3.05±0.05 ^c	<10 cfu/g ^a	4.74±0.02 ^d	6.97±0.04 ^f	2.44±0.09 ^c
1st day cheese	6.70±0.04 ^e		7.13±0.02 ^e	7.27±0.01 ^f	6.71±0.25 ^e	3.79±0.05 ^d	<10 cfu/g ^a	4.80±0.17 ^d	6.06±0.03 ^{de}	2.32±0.03 ^c	
1st month cheese	6.62±0.03 ^e		7.12±0.02 ^e	6.71±0.05 ^e	6.21±0.02 ^d	<10 cfu/g ^a	<10 cfu/g ^a	1.39±0.12 ^b	5.40±0.02 ^c	<10 cfu/g ^a	
2nd month cheese	6.46±0.05 ^d		6.30±0.00 ^d	6.07±0.03 ^d	5.62±0.07 ^c	<10 cfu/g ^a	<10 cfu/g ^a	<10 cfu/g ^a	6.02±0.03 ^d	<10 cfu/g ^a	
3rd month cheese	5.97±0.03 ^a		5.70±0.05 ^b	5.14±0.03 ^b	4.79±0.04 ^a	<10 cfu/g ^a	<10 cfu/g ^a	<10 cfu/g ^a	5.12±0.03 ^b	<10 cfu/g ^a	
4th month cheese	6.11±0.00 ^b		5.85±0.02 ^c	5.76±0.06 ^c	6.26±0.02 ^d	<10 cfu/g ^a	<10 cfu/g ^a	<10 cfu/g ^a	4.99±0.02 ^a	<10 cfu/g ^a	
6th month cheese	6.00±0.01 ^a		5.87±0.02 ^c	5.70±0.06 ^c	6.16±0.04 ^d	<10 cfu/g ^a	<10 cfu/g ^a	<10 cfu/g ^a	4.98±0.01 ^a	<10 cfu/g ^a	

(a–g) means within column are significantly different ($P < 0.05$) according to the Student–Newman–Keuls test.

Table 27. Correlation coefficients between microbial counts throughout the production and ripening of a) Zeytineli sepet cheese, b) Germiyan sepet cheese

Description for a)	Total aerobic bacteria Log cfu/g	Lactococci log cfu/g	Lactobacilli log cfu/g	Enterococci log cfu/g	Yeast log cfu/g	Coliform log cfu/g	Psychrotrophic bacteria log cfu/g
Total aerobic bacteria log cfu/g	1.000						
Lactococci log cfu/g	0.990***	1.000					
Lactobacilli log cfu/g	0.916**	0.954***	1.000				
Enterococci log cfu/g	0.617	0.678	0.838**	1.000			
Yeast log cfu/g	0.023	-0.076	-0.362	-0.610	1.000		
Mold log cfu/g	0.277	0.177	-0.001	-0.167	0.594		
Coliform log cfu/g	-0.147	-0.214	-0.483	-0.748*	0.912**	1.000	
Psychrotrophic bacteria log cfu/g	0.310	0.192	-0.042	-0.333	0.742*	0.484	1.000
Staphylococci log cfu/g	-0.429	-0.522	-0.741*	-0.820*	0.854**	0.890**	0.474

Description for b)	Total aerobic bacteria log cfu/g	Lactococci log cfu/g	Lactobacilli log cfu/g	Enterococci log cfu/g	Yeast log cfu/g	Coliform log cfu/g	Psychrotrophic bacteria log cfu/g
Total aerobic bacteria log cfu/g	1.000						
Lactococci log cfu/g	0.812*	1.000					
Lactobacilli log cfu/g	0.838**	0.948***	1.000				
Enterococci log cfu/g	0.561	0.576	0.775*	1.000			
Yeast log cfu/g	0.503	0.121	0.290	0.336	1.000		
Coliform log cfu/g	0.580	0.175	0.311	0.353	0.971***	1.000	
Psychrotrophic bacteria log cfu/g	0.597	0.059	0.204	0.248	0.764*	0.800**	1.000
Staphylococci log cfu/g	0.449	0.009	0.179	0.283	0.987***	0.977***	0.813*

*p value < 0.05, **p value < 0.01, ***p value < 0.001

Table 28. Correlation coefficients between microbial counts (logarithmic units) and chemical-physical determinations throughout the production and ripening of a) Zeytineli sepet cheese, b) Germiyan sepet cheese

Description for a)		pH	Acidity(%)	NaCl(%)	Fat(%)	Total Solid (%)	Water activity	Protein(%)	Ripening index	Nitrogen in total solid (%)
Total aerobic bacteria log cfu/g		-0.807*	0.782*	0.100	0.783*	0.369	-0.377	0.841**	0.057	-0.087
Lactococci log cfu/g		-0.862**	0.846**	0.286	0.848**	0.548	-0.467	0.891**	0.225	-0.273
Lactobacilli log cfu/g		-0.944***	0.939**	0.729	0.946***	0.839*	-0.672	0.946***	0.658	-0.529
Enterococci log cfu/g		-0.927**	0.933**	0.847*	0.930**	0.769*	-0.923**	0.906**	0.882*	-0.673
Yeast log cfu/g		0.413	-0.466	-0.958**	-0.489	-0.789*	0.729*	-0.330	-0.930**	0.644
Mold log cfu/g		0.151	-0.187	-0.689	-0.218	-0.539	0.468	-0.086	-0.731	0.617
Coliform log cfu/g		0.555	-0.573	-0.887**	-0.597	-0.634	0.796*	-0.481	-0.885**	0.462
Psychrotrophic bacteria log cfu/g		0.147	-0.206	-0.635	-0.200	-0.519	0.499	-0.086	-0.554	0.535
Staphylococci log cfu/g		0.768*	-0.790*	-0.997***	-0.817*	-0.822*	0.842**	-0.712*	-0.940**	0.618
Description for b)		pH	Acidity(%)	NaCl(%)	Fat(%)	Total Solid (%)	Water activity	Protein(%)	Ripening index	Nitrogen in total solid (%)
Total aerobic bacteria log(cfu/g)		0.106	-0.108	-0.645	-0.198	-0.442	0.589	-0.053	-0.665	0.587
Lactococci log(cfu/g)		-0.376	0.413	-0.330	0.312	-0.019	-0.096	0.437	-0.360	0.311
Lactobacilli log(cfu/g)		-0.331	0.343	-0.557	0.247	-0.245	0.157	0.400	-0.547	0.551
Enterococci log(cfu/g)		-0.231	-0.173	-0.626	0.116	-0.526	0.104	0.265	-0.614	0.710
Yeast log(cfu/g)		0.681	-0.683	-0.967***	-0.736*	-0.776*	0.857**	-0.610	-0.904**	0.943**
Coliform log(cfu/g)		0.718*	-0.936*	-0.987***	-0.792*	-0.843*	0.925**	-0.663	-0.976***	0.974***
Psychrotrophic bacteria log(cfu/g)		0.590	-0.669	-0.861*	-0.687	-0.887**	0.844**	-0.575	-0.844*	0.798*
Staphylococci log(cfu/g)		0.756*	-0.777*	-0.989***	-0.816*	-0.864*	0.893**	-0.702	-0.941**	0.969***

*p value < 0.05, **p value < 0.01, ***p value < 0.001

4.6. Phenotypic Identification of Lactic Acid Bacteria Flora In Sepet Cheeses

Lactic acid bacteria are essential for fermentation and are acceptable in very large numbers mainly in natural cheese (Hamid and Owni 2007).

Changes were followed in lactic acid bacteria population during cheese production and ripening period up to 90 days. Isolates were chosen for identification after subculturing, Gram staining and catalase test.

According to identification results, percentage of cocci and rods in samples of milk and cheeses of different ripening period was fluctuating. The percentage of lactic acid bacteria during cheese production and ripening is shown in Table 29. Raw milk had a considerably greater number of cocci than rods. During cheese production, the number of cocci was decreased whereas the number of rods was increased. However, during ripening, the number of cocci was again increased while the number of rods was decreased.

The phenotypic identification results are given in Table 30. According to Teuber and Geis (2006), the isolated *Lactococcus* spp. in Sepet cheese were related to the species *Lactococcus lactis* subsp *lactis*. Psoni et al. (2003) reported that the main lactococci species, *Lactococcus lactis* subsp *lactis* were, decreased during ripening of Batzos cheese, in a similar way as has been observed by other authors for different varieties of raw goat's milk cheeses, mainly due to the inhibitory effect of high NaCl content. The two sepet cheeses, in which the lactic acid bacteria were isolated, reached approximately 10 % NaCl content at the end of the first month. As a result of inhibitory effect of salt, *Lactococcus* spp. decreased during ripening of both cheeses. The Sepet cheeses were collected in summer and Psoni et al. (2003) found that lactococci were less frequently isolated from cheeses made in spring and summer. Therefore, in contrast to Bulut (2003), Guessas and Kihal (2004), the predominant lactic acid bacteria was not *Lactococcus lactis* subsp *lactis* in Sepet cheese. Bulut (2003) also found atypical characteristic of some lactococcal strains caused difficulties to distinguish them from *Enterococcus faecium*.

Heterofermentative and homofermentative *Lactobacillus* spp. were isolated during ripening of both cheeses. Biochemical tests were found to be not enough to classify heterofermentative *Lactobacillus* spp. at species level. According to Hertel

and Hames (2006) and Abdi et al. (2006), facultative homofermentative *Lactobacillus* spp. isolates in Sepet cheese were closely related to *Lactobacillus plantarum* and *Lactobacillus casei* spp. *rhamnosus*. Tserovska et al. (2002) also found *L. plantarum* in goat cheeses.

Only *Streptococcus thermophilus* was isolated from milk. *Leuconostoc* spp. were isolated during cheese production and ripening, however their quantities were less than the others.

Enterococcus spp. was predominant during Sepet cheese production and ripening. Fox et al. (2000) stated that Enterococci had ability to grow at wide range of temperature, salt content and found in many Mediterranean cheeses. According to Devriese et al. (2006), Enterococci isolates in Sepet cheese were closely related to *Enterococcus durans* and *Enterococcus faecium*. Bulut (2003) and Psoni et al. (2003) also isolated *E. durans*, *E. faecium* from cheeses.

Table 30. Biochemical test results of isolates

Shape	2% NaCl	4% NaCl	6.5% NaCl	10 °C	15 °C	40 °C	45 °C	Arginine hydrolysis	CO ₂ in red dy broth	CO ₂ from glucose	Arabiose	Galactose	Lactose	Ribose	Sorbitol	Salicin	Raffinose	Mannitol	Mannose	Xylose	Maltose	Trehalose	Glycerol	Sucrose	Glucose	All isolates of <i>Lactococcus</i> spp		
																										<i>Streptococcus thermophilus</i>	Homofermentative <i>Lactobacillus</i> spp.	
cocci	+	-	-	ND	+	+	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
cocci	+	-	-	ND	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
rod	ND	ND	+	ND	+	ND	+	+	ND	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rod	ND	ND	+	ND	+	ND	+	+	ND	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
rod	ND	ND	+	ND	+	ND	+	+	ND	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rod	ND	ND	+	ND	+	ND	+	-	ND	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rod	ND	ND	+	ND	+	ND	+	-	ND	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rod	ND	ND	+	ND	+	ND	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rod	ND	ND	+	ND	+	ND	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rod	ND	ND	+	ND	+	ND	+	+	ND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cocci	+	+	+	ND	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cocci	+	+	+	ND	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cocci	+	+	+	ND	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cocci	+	+	+	ND	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cocci	+	+	+	ND	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

ND: Not determined

CHAPTER 5

CONCLUSION

Investigation of production methods and their quality characteristics during production and ripening contribute to manufacture traditional cheeses in large scale and then export throughout the continent and protect diversity of tradition. Sepet cheese is a traditional cheese and specific to Aegean region. In the production of sepet cheese usually goat milk is used. In Ödemis and Söke, a blend of cow's and ewe's milk can be used. Baskets are used in the shaping of sepet cheeses. Therefore, this traditional cheese is named as sepet cheese.

Determination of physical, chemical, microbiological, organoleptic, and aroma characteristics of traditional sepet cheeses were the objectives of this study.

Results obtained during this study were summarized below:

1) Average chemical characteristics of traditional sepet cheeses were found as 54.33 % \pm 5.17 total solid content, 0.82 \pm 0.05 water activity, 25.11 % \pm 2.86 fat content, 1.66 % \pm 0.64 acidity, 5.58 \pm 0.45 pH, 28.99 % \pm 2.12 protein content, and 7.09% \pm 2.89 NaCl content.

2) 0.49 % \pm 0.34 pH 4.6 soluble nitrogen fraction, 0.39 % \pm 0.35 trichloroacetic acid soluble nitrogen fraction, 7.31 \pm 8.91 lypolysis value was determined for sepet cheese samples.

3) Acidity, total solid content, fat content and protein content increased significantly during cheese making and slightly increased during ripening. While water activity, and pH decreased during cheese production, trichloroacetic acid and pH 4.6 soluble nitrogen fractions increased during ripening.

4) Descriptive sensory analysis was done to determine the flavor profile of sepet cheeses. Traditional sepet cheeses were described with free fatty acid, cooked, creamy, whey, animal like, sulfurous aromatic descriptives with high salty basic taste.

5) Average firmness, springiness, cohesiveness, adhesiveness and chewiness values of traditional sepet cheese samples were found as 207.59 \pm 123.46 N, 0.57 \pm 0.11, 0.59 \pm 0.27, 0.82 \pm 3.17 Nmm, 58.29 \pm 24.44 N, respectively.

6) Volatile composition analysis was done to determine odor active compounds. Free fatty acids were the most abundant volatile compounds of total identified fraction. Hexanoic acid, octanoic acid, decanoic acid, and butyric acid had the highest percentage values in volatile fraction of traditional sepet cheeses, respectively. Moreover, they had the highest aroma intensities in volatile compounds.

7) Changes in volatile composition during cheese production and ripening were determined and found that most of volatile compounds increased during cheese production and ripening.

8) Average total aerobic, lactococci, lactobacilli, enterococci, psychrotrophic bacteria, *Staphylococcus aureus*, yeast, mold, coliform bacteria counts were 7.64 ± 1.18 , 7.38 ± 1.10 , 7.38 ± 0.99 , 6.99 ± 0.99 , 5.37 ± 1.15 , 1.25 ± 1.72 , 3.22 ± 1.25 , 0.95 ± 0.961 , 2.72 ± 1.82 log cfu/g, respectively.

9) All bacterial counts increased during cheese production. Then the total aerobic, lactococci, lactobacilli, and enterococci counts decreased slightly during ripening and staphylococci and coliform counts decreased below 10 cfu/g during ripening.

10) According to phenotypic identification, isolates were closely related to *Lactococcus lactis* subsp. *lactis*, *Lactobacillus casei* spp. *rhamnosus*, *L. plantarum*, heterofermentative *Lactobacillus* spp., *Streptococcus thermophilus*, *Leuconostoc* spp., *Enterococcus durans* and *E. faecium*. At the beginning of cheese production, the percentages of lactic acid bacteria isolates were 23%, 52.94%, 17.64%, 5.88%, 0.00% for *Lactococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., respectively. At the end of ripening, these values reached 0.00%, 60%, 0.00%, 13.33%, 26.67% for *Lactococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., respectively.

Cocolin et al. (2007) reported that stressed or injured cells were not recovered in selective media and that cells present in low numbers were very often inhibited by more abundant microbial populations. For these reasons, culture-independent methods such as PCR denaturing gradient gel electrophoresis and fluorescence in situ hybridization were useful tools that allow monitoring of the microbial populations without cultivation. For further study, culture independent molecular methods can be used to evaluate changes in microbial species arising during production and ripening of sepet cheese. Most of the chemical, physical, volatile, textural characteristics were correlated significantly with ripening index and lipolysis. Therefore, sensory analysis, based on consumer acceptance, can be done during ripening to determine the optimum ripening time for a further study.

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APPENDICES

Appendix A

Table A.1. Chemical Used

No	Chemical	Code
1	AgNO ₃	Merck 1.0512.0025
2	Potassium chromate	Merck 1.04951.1000
3	NaOH	Riedel-de Haen 06203
4	Phenol ftalein	Merck 1.07233.0100
5	D (+) - Trehalose	SIGMA T9531 - 5 gr
6	Sulfuric acid	Merck 1.00729.2500
7	n-Amyl Alcohol	Merck 8.07500.1000
8	Boric acid	Sigma B6768
9	HCl	Reidel-de Haen 07102
10	Trichloroacetic acid	Merck 1.00807.1000
11	Trisodium citrate dihydrate	Merck 1.06448.1000
12	Silicon antifoaming agent	Merck 1.07743.0100
13	Kjeltabs-catalysts	Delta
14	Filter paper(Whatman No:2)	Schleicher&Schuell 597 ref.no: 10311811
15	Filter paper(Whatman No: 42)	ISOLab
16	M17 agar	Merck 1.15108.0500
17	MRS agar	Fluka 69964
18	Violet Red Bile agar	Difco 211695
19	Plate Count agar	Merck 1.05463.0500
20	Skim milk	Difco 232100
21	Potassium tellurite %3,5	Oxoid SR0030J
22	Baird Parker agar base	Difco 276840
23	Kanamycin esculin azide agar	Merck 1.05222.0500
24	Yeast extract glucose chloromphenical agar	Difco 219001
25	Acetic acid	Merck 100063
26	Butyric acid	Merck 800457.0100
27	Caproic acid	Merck 800198005
28	Heptanoic acid	Merck 8075820100
29	Caprylic acid	Merck 800192100

(cont. on next page)

Table A.1.cont. Chemical Used

No	Chemical	Code
30	4-Methyl octanoic acid	Aldrich 35.7502
31	2-Nonanone	Merck 8187900025
32	Ethyl butyrate	Merck 8005000100
33	Ethyl caproate	Merck 8001900005
34	Hexanal	Merck 8026720005
35	Decanal	Merck 8032110100
36	δ -Decalactone	A11756.25
37	Acetoin	Merck 8206640100
38	Methional	Merck 8411680010
39	(E)-2-nonenal	Aldrich 255653
40	(E,Z)-2,6-nonadienal	Aldrich 294675
41	(E)-2-decenal	Aldrich 36658, 10 ml
42	(E,E)-2,4-nonadienal	Merck 843810.0005
43	o-Aminoacetophenone	Merck 800273
44	γ -Dodecalactone	Aldrich W.240001.100
45	L-Arginine Monohydrochloride	MERCK 1.01543.0050 gr
46	tri-Sodium Citrate Dihydrate	MERCK 1.06448.1000 gr
47	Triammonium citrate	Fluka 09723
48	MnSO ₄ .4H ₂ O	MERCK 1.102786.1000 gr
49	L(+)-Arabinose	ALDRICH A91906 - 25 gr
50	D(+)-Galactose	MERCK 1.04062.0050 gr
51	Maltose monohydrate	MERCK 1.05911.1000gr
52	Lactose	SIGMA L3750 - 100 gr
53	D-Mannitol	DIFCO 217020 500 gr
54	D- Mannose	MERCK 1.05388.0025 gr
55	Raffinose pentahydrate	MERCK 1.07419.0050 gr
56	D (+) -Xylose	MERCK 1.08689.0100 gr
57	D (-) - Ribose	SIGMA R7500 - 5 gr
58	D (+) - Galactose	ALDRICH 112593 - 5 gr
59	L (+) - Arabinose	ALDRICH A91906 - 25 gr
60	MRS broth	Fluka 69966
61	M17 broth	Biolab M1B20500
62	Bacteriological peptone	Oxoid LP 0037
63	Lablemco powder	Oxoid LP0029
64	Yeast extract	Fluka 70161
65	Peptone water	Merck 1.07228.0500
66	D- Glucose	Merck 1.08342.1000

Appendix B

Sensory Evaluation Sheet

Sensory Evaluation of Sepet Cheese: Aroma and taste

Name:

Date:

	Cheese (#)				
Aromatics					
Cooky					
Whey					
Creamy					
Sulfurous					
Free fatty acids					
Animal-like					
Moisty cloth					
Fruity					
Nutty					
Metallic					
Store(fridge)					
Others(mould, yeast)					
Basic tastes					
Sour					
Bitter					
Salty					
Sweet					
Umami					
Bite					
Acrid					