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M.Sc. in Food Engineering

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REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

INVESTIGATION OF BIOGENIC AMINES IN KASHAR CHEESE

M.Sc. THESIS

IN

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BY

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Supervisor

Prof. Dr. Hüseyin BOZKURT

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Nimo Hussein YUSSUF

ABSTRACT

INVESTIGATION OF BIOGENIC AMINES IN KASHAR CHEESE

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M.Sc. in Food Engineering
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The aim of this study was to investigate the growth of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp *Bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis,* mold and yeast, and Total Aerobic Mesophilic Bacteria (TAMB) as function of four different temperatures in Kashar cheese. Effects of temperature like 5°C, 15°C, 25°C and 37°C were studied after packaging of samples for 105 days of storage time. Microorganism numbers were significantly ($p<0.05$) increased with storage time corresponding to temperature. Physicochemical results showed that dry mater changed from 48.50±0.07 to 60.00±0.00; pH reduced from 6.61±0.02 to 5.05±0.02. The moisture content of kashar cheese increased with increasing storage time. This can be due to during fresh stage (0, 15, 30 and 45 days) the sample has high salt concentration that is why increased with increasing storage time, also the pH decreased with increasing storage time. Lactic acid bacteria (LAB) count increased from 3.52±0.27 log cfu/g up to 6.69±0.01 log cfu/g with increasing storage time and stored temperature. The total aerobic mesophilic bacteria count ranged from 4.39 to 6.91 log cfu/g during ripening, whereas mold and yeast from 4.15±0.14 up to 2.92±0.03 log cfu/g. Storage time had significant effect ($p<0.05$) on microbial level of kashar cheese. The consumers have to check leaflet sheet of kashar cheese especially the cheese that produced from raw milk is more likely spoilage than industrial one**.**

KeyWords: Kashar Cheese, Biogenic Amines

ÖZET

KAŞAR PEYNİRLERİNDE BİYOJENİK AMİNLERİN İNCELENMESİ

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76 sayfa

Bu çalışmanın amacı, Kaşar peynirinde *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp *bulgaricus, Lactobacillus acidophilus, Bifidobacterium lactis*, küf ve maya ve Toplam Aerobik Mezofilik Bakteriler (TAMB) gelişimlerinin dört farklı sıcaklığın fonksiyonu olarak. sıcaklığın etkilerinin incelenmesidir. Örneklerde sıcaklığın etkisi paketlendikten sonar 5°C, 15°C, 25°C ve 37°C gibi sıcaklıklarda 105 gün süreyle saklanarak incelenmiştir. Mikroorganizma sayıları, saklama sürecinde sıcaklığa bağlı olarak önemli ölçüde artmıştır ($p < 0.05$). Fizyokimyasal sonuçlar kuru materyalin 48,50 ± 0,07'den 60,00 ± 0,00'a değiştiğini; pH 6,61 ± 0,02'den 5,05 ± 0,02'ye düştüğünü göstermiştir. Kaşar peynir nem oranı taze aşamada artan depolama süresi ile artmıştır. Bunun nedeni taze aşama (0, 15, 30 ve 45 günde) sırasında numunenin yüksek tuz konsantrasyonuna sahip olmasıdır, bu nedenle artan depolama süresi ile artmış, ayrıca artan depolama süresi ile pH azalmıştır. Laktik Asit Bacterileri (LAB) sayısı 3.52 ± 0.27 log kob/g dan 6.69 ± 0.01 log kob/g'a, artan depolama süresi ve sıcaklığı ile artmıştır. Toplam aerobik mezofilik bakteri sayısı olgunlaşma sırasında 4.39 ile 6.91 log kob/g arasında değişirken, küf ve maya sayısı 4.15 ± 0.14 log kob/g ile 2.92 ± 0.03 log kob/g arasında değişmiştir. Depolama süresi, kaşar peyniri mikrobiyal düzeyi üzerinde anlamlı etkiye ($p<0.05$) sahiptir. Tüketiciler ürünleri tüketmeden önce, özellikle çiğ sütten elde edilen endüstriyel olandan daha fazla bozulma olduğundan dolayı, kaşar peyniri broşürünü kontrol etmelidirler.

Anahtar Kelimeler: Kaşar Peyniri, Biyojenik Aminler.

"Dedicated to my family"

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LIST OF SYMBOLS

α	Alfa
β	Beta

LIST OF ABBREVIATIONS

TAMB	Total Aerobic Mesophilic Bacteria
LAB	Lactic Acid Bacteria
MC	Moisture Content
BAs	Biogenic Amines
HIS	Histamine
TYR	Tyramine
TRY	Tryptamine
CAD	Cadaverine

CHAPTER I
INTRODUCTION

1.1 Background of the Study

Kashar cheese is one of most produced and consumed cheese in Turkey. Similar cheeses are commonly manufactured in Balkan countries and known as kashkaval. Due to high pH value and low acid lactic acid concentration, Kashar cheese is very delicate to microbial spoilage. However, it has high NaCl content in the aqueous phase. For this reason, it has long shelf life compared to other cheeses (Yilmaz and Dagdemir, 2012).

Today a wide range of intervention aims to ensure food safety. Some of these interventions are directly trying to specify amelioration risk in food engineering and human health. Lipolytic and proteolytic activities can tolerate different microbiological growth.

Formation of lipolysis in cheese is due to triglycerides caused rancidity. On other hand, inhibition of enzymes by heating can produce awful smell and unwanted taste. The other important issue is the type of microorganisms, isolated from cheese. These mesophilic microorganisms may come into existence because of the microflora associated with the milk used to make cheese or contamination during manufacturing cheese deliberately in starter culture they are mainly mesophilic organisms. They come from different sources and contaminate the product during production and ripening of cheese (Var et al., 2005).

Lactic acid bacteria can tolerate the cheese by producing microbial decarboxylation of the amino acids to form biogenic amines by microbial decarboxylation. Also, there are some other factors that can influence forming of biogenic amines like enzymes, temperature, pH value, salt and moisture content (Var et al., 2005). According to (Tabasco et al., 2007), The one of the most food bone diseases that causes biogenic amines is Histamine. It has been associated with consumption of

100-180mg of histamine. The major products of carbohydrate catabolism are organic acids. Homo-fermentative bacteria, like lactic acid bacteria, ferment glucose to multiple products such as acetic, formic and propionic acid, acetaldehyde, ethanol and carbon dioxide.

1.2 Importance of this Study

During this study, it was tried to figure out the problems that are encountered with Kashar cheese during manufacturing and after storage by focusing the shelf life of Kashar cheese and microbiological changes that can happen during ripening. Microbiological status can involve the formation of biogenic amines. Therefore, the result of this study will help Food Engineers and food quality control centers to improve the quality of Kashar cheese.

Objectives of this study were:

1. To determine the effect of temperature and time on chemical and physical properties during ripening of kashar cheese.
2. To investigate effect of temperature and time on the forming of biogenic amines during ripening and storage of kashar cheese.
3. To determine effect of temperature and time on microbiological changes during ripening and storage of kashar cheese.

CHAPTER II
LITERATURE REVIEW

2.1 Milk

Milk is a white liquid and full of nutrients, which is produced by the all mammals. The ages, humans begun to consume milk from cows, goats and camels for nourishment (Jenness, 1988). The composition of milk is water, lipids, sugar, proteins and salt. It may contain more than 105 unique molecules. The type of sugar found in milk is mainly lactose formed by the association one particle of D-galactose (connected by its semiacetyl capacity) and one molecule of D-glucose (submitted by its hydroxyl 4 position). It has a β-galactoside 1,4 bond (which is hydrolyzed by a β-galactosidase) and is a 4-D-glucopyranosyl-β-D-galactopyranose (Armstrong and Yates, 1963). Lactose unlike other form of sugar does not have sweet taste. The protein in milk has good quality and good for human growth. It contains essential amino acids likewise it gives fundamental amino acids those our body can't produce itself. In milk, fat is the principle source of energy. Goat and bovine milk are low in polyunsaturated fats that are important for human digestion (Armstrong and Yates, 1963. Fat is available in milk as a combination of fat cells in the form of emulsion. The union of the fat substance of milk can be found in little cells suspended in water, which allows them to be easily used. The unsaturated fats are lipid particles containing two forms either cis or trans unsaturated fat. It is likely to recognize the cis unsaturated fats and trans unsaturated fats, most unsaturated fats in our eating regimen are in the cis structure, and a lower extent are in the trans structure (Guetouache et al., 2014). The fat substance of goat milk is significant according to the other mammals. Milk triglycerides are all most over 95% of complete lipids. Phospholipids include 30 to 40 mg/100 ml of dairy animal's milk, which contains 8 to 10 mg of lipid. Cholesterol is present 10 to 20 mg per 100 ml, the greater part of which is in the free form. During the homogenization of the milk, the fat globule increments in number and impressively diminishes in width (under 1 micron). As we know the business of cheese increasing day by day. In Turkey, manufacturing of

cheeses also increased with different kind of cheeses according to the customer needs and quality of production (Guetouache et al., 2014).

2.2 Cheese

Human life being important, milk and milk products have an important place in food engineering. Cheese is one of these products, it is a milk product whose history dates back many years ago and has the widest range of Kashar Cheese (Çetinkaya, 2015). Cheese is manufactured from raw milk and it has good nutrients either in ripening or unripening produced by boiling the milk, adding starter culture, coagulating the proteolytic bacteria, filtering the clot and separating it from the whey by salting and suppressing the curd (Yetişmeyen et al., 2008). According to global sale it has 30 percent value of the dairy products. Cheese making is a process that has been going on for several thousand years. Documents linked to cheese manufacturing go back to 6000-7000 BC (Dayısıoğlu et. al., 1998). It is believed that the first production of cheese was created between the rivers Tigris and Euphrates in Mesopotamia. Today these regions are famous for cheese productions (Kamber, 2008). In Turkey has huge milk production and cheese delivery, compared to other countries those have not actual number of cheese generation. The aims behind these are rural enterprises being dispersed; generally bringing up animals will be a marginal rather than a distinct company; and similarly, generation is frequently accomplished on a small scale, in dairy ranches or in family activities.

Moreover, since big amounts of dairies operate on a periodic basis and in fields that change from one year to the next, neither their capacity nor the quantities of cheese they generate has been accurately made it. Amount of Turkey’s milk production is around 11 million tons per year and 40 percent (4–5 million) of milk yields were treated as cheese, which is around 700–800 thousand tons per year (Özer et al., 2002). All most 3000 businesses with a daily capacity ranging from several hundred liters to 10–15 tons were discovered to be producing milk production.

The primary cheeses manufactured in Turkey as a whole are White Cheese, Kashar, Tulum, Lor and Çökelek. Cow, ewe's and goat's milk are used in the manufacturing of these cheeses. Local cheeses, however, are produced from ewe's milk, such as Ayaş Ovma. It was estimated that 60% of the cheeses generated in Turkey are White

Cheese, 17% Kashar Cheese, 12% Tulum and Mihaliç Cheese, and other local cheeses make up the remaining 11% of the manufacturing (Kamber, 2008).

Cheese is made from the milk of buffalo from which the cream was taken. In the past, the milk cheese of camel was manufactured, especially by nomadic Yörük tribes living in Aydın, Isparta and Antalya. However, when the nomads adopted a settled way of life, cheese made from camel milk ceased production. In reaction to local preferences and needs, most cheeses are still made. Even among these cheeses prevalent to each region, however, each produced has its own distinctive flavor, and texture (Özer et al., 2002).

2.3 Types of Cheese

In Turkey, white cheese, kashar cheese, Mihalic, Tulum and herbed cheese are the most frequently manufactured cheese types. Special cheese from Balkan countries and Turkey with its rich component and lovely taste is kashar cheese that has a significant position among cheese kinds. According to geographical regions are manufactured uniquely different with other regions of the world are consumed in large amounts, more than 50 different of cheeses are manufactured in Turkey. However just three of them are famous, Beyaz (White) cheese, kashar cheese and Tulum cheese. The manufacture of Tulum cheese was 10,000 tons in 2004 (information from the Turkish Statistical Institute, Ankara). Turkey's complete dairy output is 11 million tons per year and 40 percent (4–5 million) of dairy output has been processed as cheese, which is approximately 700–800,000 tons per year (Kamber, 2008). More than 3000 companies, whose daily capacity ranged from several hundred liters to 10–15 tonnes, were discovered to produce and sell milk goods in Turkey. Around 50-150 different cheese are produced in Turkey (Sert, 2007).

2.3.1 Brine Cheese

Brine Cheese, usually supplied in almost every region in the country. No morning meal table would be completed without it and it is also commonly used in cake dishes ("börek," "cake," and "poğaça") and blended green servings (Kamber, 2008).

Brine Cheese's historical background is long and Turks' generation is known to date back to 1400. Until the 1950s, the brine Cheese generation was focused solely on family use; in any event, it was introduced to the metropolitan populations during the

1960s. These days, the cheese is commonly supplied in households, dairies and production locations across the country, under both the crudest and the most current situations. Lately neighborhood in eastern regions start to produce these cheeses and it is lead to be most usable cheese in whole country.

Otlu cheese is a very common variety of brined cheeses in Turkey, and its popularity has gradually increased. The cheese has long been historically produced in the eastern cities, particularly in the province of Van. Otlu cheese is made from herbs that give the cheese a characteristic apperance and aroma / flavour. Such herbs belong to the genus Allium, Thymus, Silene, Ferula and Anthriscus nemorosa whose local names are, respectively, Sirmo, Kekik, Siyabo, Heliz and Mendo. Adding herbs to the cheese is an essential step in the manufacturing process to achieve a characteristic flavor or prolong the cheese's shelf life (Hayaloglu et al., 2008).

It is felt that 8–13% of the nation's milk goes into the manufacture of white Cheese due to the Known by such a significant number of names. White Cheese is additionally made in various ways as indicated by the zone of creation: utilizing cows, water buffalo's, ewes, or goat's milk, or a blend of these and with varying extents of fat and salt. Moreover, variety of bundling materials (tins, barrels, plastic, and so on.) are utilized to store White Cheese, which might be expended dry or in saltwater, without development or develop (Özer et al., 2002).

The physical structure and its appearances of white cheese have its own characteristics lovely taste and softness also; it has hard stability and homogeneous texture, with no eyes or very fine eyes. In addition, it has suitable shape for slicing. The color of white cheese has different colors it depends on the raw material of milk and type of animal milk is used (Kamber, 2008).

2.3.2 Çökelek Cheese

After brine cheese and tulum cheese, çökelek cheese is rarely used in the rural parts of the country. The non-professional society they may mix because they have same appearances. Çökelek Cheese is gotten by leaving entire fat or medium-skimmed milk to stand and Acidified (for example, lemon). Both whey protein and casein are found in this cheese (Kamber, 2008). The dairy products made from yogurt such as yayik butter, tarhana, and Çökelek cheese are very popular products in the Middle

East where they are used to prepare some popular food dishes. Çökelek cheese is made by heating yogurts, then straining them in a special bag of cloth (Şimsek and Sağdıç, 2009).

Lor Cheese is gotten by enriching whey left over from the assembling of any dairy item (regularly margarine from yogurt). Here, since the milk has been recently prepared to make another item, the cheese contains next to no casein and is a "whey protein-rich" cheese. In addition, its fat content is low. The cheeses are unique in relation to one another (Yasar and Guzeler, 2011).

2.3.3 Tulum Cheese

Tulum cheese is semi-hard cheese also; it is fully creamed and fatty cheese. Tulum means goat or sheep's bag in Turkish language. In general, there are two kinds of Tulum cheese, Erzincan Savak Tulum which is delivered principally in the eastern locale of Turkey and Izmir Brined Tulum ("Izmir Salamurali Tulum peyniri" in Turkish) which is delivered in the western district (Kamber, 2008). Erzincan Savak Tulum is famous in the mountains and plateaux of Erzincan, Erzurum, Tunceli, Bingol and Elazig and East Anatolian areas by the Savak clan it is matured at least three months. When individuals state "Tulum cheese" in Turkey, they infer Erzincan Savak Tulum (Ceylan et al., 2007).

The physical appearance of Tulum cheese has its own flavors and aroma; also, it has different shapes and Figure 1. 1size, which upon the animal skin used. The outer surface of cheese is dry creamy-yellow in color. These colors depend on which animal is used. Its body is free from eyes, mushy and lighter in color. The cheese medium-hard cheese in the characters with granules capable of crushing. It has delicious taste up on the regions and the production ways that may took different industries (Kamber, 2008).

2.3.4 Kashar Cheese

Kashar cheese is one of most produced and consumed cheese in Turkey. Similar cheeses are expansively manufactured in Balkan countries and known as kashkaval. The root of kashar cheese is Latin word 'Coercco' meaning squeezing the whey out of cheese under press. After white cheese, kashar cheese is the most popular in the side of commercial. Many years ago when white Cheese production was less

common, there were few cold storage depots and insufficient transportation opportunities, Kashar Cheese was manufactured on elevated mountain pastures. Nowadays, while many large and small companies make it in many areas of the nation, manufacturing in is especially intensive. Edirne, Kars, Kirklareli, Kocaeli, Mus, Trabzon Kadirga, Bayburt, and Tonya are the most renowned Kashar Cheeses (Kamber, 2008).

2.3.5 Physical Structure and Appearances of Kashar Cheese

Kashar Cheese, which belongs to the hard or semi-hard cheese category and is manufactured mostly in the form of a big millstone, has a slightly yellowish color. With a diameter of 30 centimeters, the cheese mold is about 10 centimeters high and weighs about 11–12 kilograms. Fresh Kashar Cheeses weigh 250 g to 2–3 kg while Kashar Cheeses weighs 5–25 kg when matured. The kashar rounds manufactured in Eastern regions such as Kars, Erzurum and Mus weigh about 5–7 kg and have more mould on their exterior surfaces, while Western Anatolian Kashar Cheeses weigh 10–12 kg from locations like Tekirdag, Kirklareli, Edirne and Kocaeli (Kamber, 2008).

Kashar Cheeses manufactured in the Eastern Anatolian area are deeper (10–11 cm) and amber in color, while those manufactured in Western Anatolia are pale yellow in color and flatter, with thicker rind and no mold. Black Sea Kashar cheeses are smaller.

Kashar Cheeses are salty, whole-fat cheeses with their own distinctive, mildly acrid, pungent taste and aroma. They have a granular but not rough texture and, depending on the duration of maturation, they have a tough or soft plastic consistency that does not crumble when sliced, with a smooth, flat, homogenous cross-sectional appearance. Their color varies from white to darker tones through ivory, pale cream and pale yellow. They are wheel-shaped or rectangular depending on the container used and differ in size. They have an exterior rind when they are mature (Kamber, 2008).

2.3.6 Method of Kashar Cheese Production

Kashar cheese process methods it took different steps those connecting one to others. First, fresh milk is standardized for milk fat to 3.5g/100g fat. These stage the milk removing leucocytes and removing foreign materials. After that, it is heat up to 32°C these step is made on traditional cheese. Before pasteurization milk going through a process called homogenization these step is reduced fat molecules, increased the fat surface where casein particles adsorb, increase viscosity, reduce amount of fat in the whey and provided equal distribution of fat milk. Next step is the pasteurization of milk at 72°C for 1-2 minutes, these part of pasteurization usage to reduce all the pathogenic microorganisms in cheese after that starter culture is added (Yasar and Guzeler 2011). These stage warm down 32°C and rennet is added. Coagulation takes for 45 minutes. After that, salt is added. Curd cutting, hold the curd for 10-15minutes, and remove the whey out. Keep draining under pressure 25kg until the end of drain. Drainage or curd it will be hung up a cloth bag until curd ripening. After that slicing and boiling at 72°C for three minutes and knead homogenization after shaping in within the mold. Finally, it will be ripening at 8°C for minimum 90 days' maximum 120 days that it kashar cheese process (Sert, 2007).

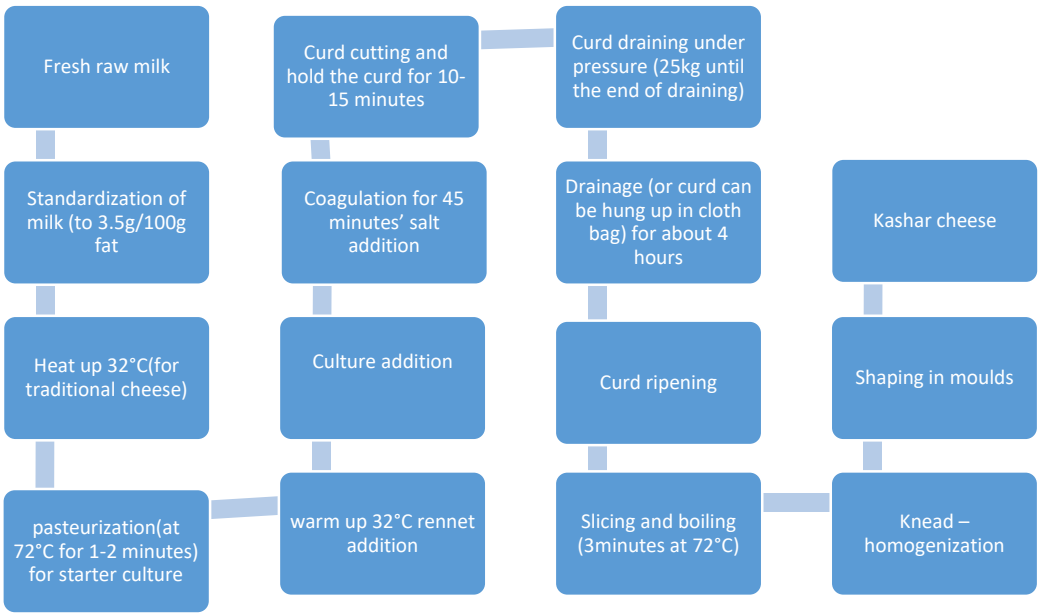

Figure 2.1 Production of kashar cheese (Sert, 2007).

2.3.7 Chemical Properties of Kashar Cheese

As other researchers reported that the protein content of kashar cheese is ranged between 21.62% and 33.95% that means the amount of Nitrogen in milk used for cheese production is high (Kamber, 2008). In the fat of kashar varies in between 7.33% to 34.83% because kashar cheese produced from different milk cows and sheep, goats and their milk have different fat content (Yasar and Guzeler, 2011).

Properties of Kashar Cheese are stated in the Turkish Standard Institute (TSI) TS-3272. As indicated by this, the moisture content in old Kashar (ripened) ought to be max 40%., while it ought to be max. 45% in new kashar (fresh), the salt sum ought to be 7%, the fat sum ought to be min. 45% in entire fat Kashar Cheese, min. 30% in fat Kashar Cheese, and min. 20% in medium-fat Kashar Cheese. At the point of the properties of Kashar Cheese are assessed based on TSI moisture content and salt sums agree to the guidelines and the examples in the examination are in fat and medium-fat classifications (Zehra, 2005).

Table 1 shows the minerals of kashar cheese which is higher also compared to other traditional cheese those are famed in Turkey (Kamber, 2008). It has highest value of calcium, phosphorus, sodium and potassium but magnesium is having low amount.

Table 2. 1 The mineral composition of kashar cheese (mg/100g)

Cheeses	Calcium	phosphorus	Sodium	Potassium	Magnesium
Kashar	1005	568	579	106	38.8
White	908	513	933	178	25.1

2.4 Microbiological Properties

2.4.1 Total Aerobic Mesophilic Bacteria

The aerobic mesophilic bacteria, acquired after adding the starter culture to pasteurized milk, are in the range of 7.00 log cfu/g, values that are the highest in the form premature and ripening time. Due to physical retention of the microorganisms in the coagulum and microbial multiplication during coagulation and whey drainage, this development in counts is a standard occurrence during cheese manufacturing. From that point on, counts decreased to the end of maturation, falling to rates of about 4.29–4.57 log cfu/g. This reduction was due to the rind on the surface of cheeses that prevented oxygen from penetrating the cheese's surface (Sert, 2007).

Based on the outcomes an acquirement it can be concluded that there were generally no significant variations in the counts of total aerobic mesophilic bacteria between cheeses that matured in the two groups of cheese. In traditional and starter culture-added cheese groups, the complete psychotropic bacteria count ranged from 3.58 to 0.30 and from 0.70 to 0.48 log cfu/g during maturation, respectively. The variations in the evolution of the complete psychotropic bacteria discovered between cheese groups can be explained by the thermal treatment of the milk used in the manufacturing of starter-cultivated cheese (Sert, 2007).

Adding starter culture may be influencing the kashar cheese some microbiological status. In addition, it does not develop microbiological like yeast, mold, and coliforms (Ucar and Badem, 2016).

2.4.2 Mold and Yeast

The most important problems faced in dairy products is yeast and molds. The growth of yeast and mold on dairy products, negatively affects the quality and shelf life of cheese the post process contamination during handling and packaging of the product (Yasar and Sahan, 2011). For instance, when yeast and most counts reached 7-8 log cfu/g they cause organoleptic change by hydrolyzing (Sahan et al., 2005).

On the 30th day in traditional cheese, the highest number of molds and yeasts was reached. Inhibition of activities of mold and yeast has been tasted as potential antimicrobial packaging. Alginate polymer can be considered as nontoxic polymer also has special properties such like; biodegradable and good film forming

porperties; good oxygen barrier at low humidity conditions, transparent. Natural antimicrobial agents specially focusing bacteriocins such as nisin, pediocin and entetrocin. Natamycin (E235) is also bacteriocim with GRAS (General recognized as safe) reported that generated by Streptomyces natalensis and it is very effective against mold and yeast even at low concentration for these reasons natamycin prefers to reduce the production of mold and yeast in kashar cheese (Celik et al., 2019).

2.4.3 Lactic Acid Bacteria

Lactic acid bacteria have the ability to inhibit the pathogenic and spoilage microorganisms by producing antimicrobial. Organic acids, hydrogen peroxide diacetyl and bacteriocins are among substance they produce by bacteria that active against other bacteria. (sezer and güven, 2009). The lactic acid bacteria and their metabolites have a preservative impact on foods, especially dairy foods. Organic acids, the main antimicrobial compounds produced by lactic acid bacteria, generate an atmosphere in the dairy food that heavily inhibits the development of pathogenic and spoilage bacteria (Olszewska et al., 2019). LAB naturally found fermented foods or using as the starter culture in some fermented food processing one of them is kashar cheese. Starter culture exhibiting antagonistic activity and pathogenic microorganisms (Olszewska et al., 2019).

2.4.4 Starter Culture

Starter cultures are those microorganisms, which are used inside the manufacturing of cultured dairy merchandise such as yoghurt and cheese. The natural microflora of milk is either inefficient, uncontrollable or unpredictable, or is destroyed altogether via the warmth remedies given to the milk. A starter tradition can provide precise traits in an extra acid from the lactose. Different capabilities of starter cultures may additionally consist of subsequent (Holzapfel, 2002).

- flavor, aroma, and alcohol manufacturing
- proteolytic and lipolytic activities
- inhibition of undesirable organism

There are two organizations of lactic starter cultures:

- simple or defined: single strain, or more than one wherein the range is thought
- combined or compound: more than one pressure every providing its own unique traits

Starter cultures may be classified as mesophilic, as an instance:

- *Lactococcus lactis subsp. cremoris*
- *L. delbrueckii subsp. lactis*
- *L. lactis subsp. lactis biovar diacetylactis*
- *Leuconostoc mesenteroides subsp. cremoris*

- Starter cultures classified in thermophilic:
- *Streptococcus salivarius subsp. thermophilus (S. thermophilus)*
- *Lactobacillus delbrueckii subsp. bulgaricus*
- *L. delbrueckii subsp. lactis*
- *L. casei*
- *L. helveticus*

L. plantarum

combinations of mesophilic and thermophilic microorganisms can also be used as inside the production of some cheeses (Holzapfel, 2002).

2.5 Biogenic Amines in Kashar Cheese

Biogenic Amines in kashar cheese formed by microbial decarboxylation of the corresponding amino acids. In kashar cheese, some factors encourage the formation of biogenic amines like temperature, pH, salt and moisture content and organic acid process of amines. Increasing the level of paraffin content in cheese can be attributed to different microorganisms (Ladero et al., 2010).

These microorganisms may receive from the flora related to the milk or it can build the cheese or by contaminate through cheese manufacturing, storage also it can be happened after starter cultures. Many researchers focusing the production of histamine and its powerful foodborne diseases. Consumption of 100 up to 180mg of biogenic amines it can appear all clinical illness (Ladero et al., 2010).

2.5.1 Chemical Structure of Amino Acid and Biogenic Amines

α-Amino acids are basic protein units and consist of α-carbon atoms covalently attached to the hydrogen atom (H), amino group (NH2), carboxy group (COOH), and side chain R group. Amines are basic nitrogen compounds in which alkyl or aryl groups substitute one, two, or three hydrogen atoms in ammonia. These low molecular nitrogen compounds are formed through four enzymatic reactions: (I) decarboxylation, (ii) transamination, (iii) reductive amination, and (iv) degradation of certain amino compounds of the precursor. The amino acid decarboxylation is the most common way of synthesizing amines in foods. Because those amines are formed by the action of living organisms through the amino acid decarboxylation process, they are called biogenic. Decarboxylation of the amino acid occurs by removing the group α-carboxyl to give the corresponding amine (Özogul et al., 2008).

Most biogenic amine names correlate with the names of their source amino acids; for example, histamine from histidine, tyrosine Tyramine, arginine agmatine, phenylethylalanine β-phenylethylamine, and tryptophan Tryptamine (Figure 2.2). An alternate pathway to form putrescine from arginine through agmatine takes place for plants and some microorganisms. Lysine is decarboxylated to produce Cadaverine by lysine decarboxylase, and it may also be generated by ornithine decarboxylase if the ornithine content is small but the lysine content is high (Özogul et al., 2008).

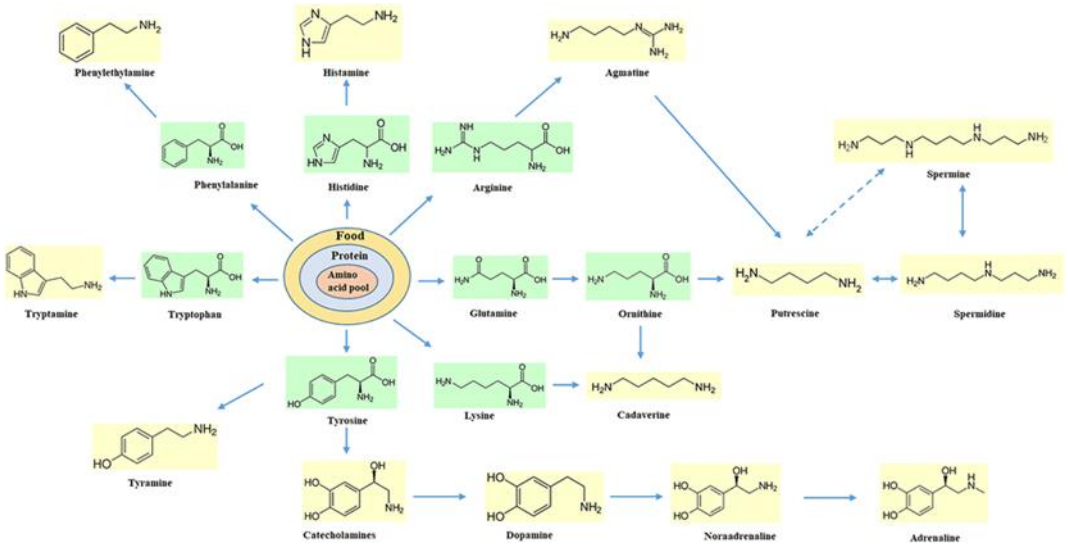

Figure 2.2 Metabolic pathway of biogenic amines formation (Özogul et al., 2008)

Biogenic amines can have three forms of chemical structure: aliphatic (putrescine, Cadaverine, Spermine, and Spermidine); aromatic (Tyramine and phenylethylamine); heterocyclic (histamine, and Tryptamine). These can also be categorized into monoamines (phenylethylamine and Tyramine), diamines (Cadaverine and putrescine), or polyamins (Spermidine and Spermine) based on the number of amine groups. Putrescine, Spermidine, Spermine, and Cadaverine are essential components of living cells, controlling the role of nucleic acid, protein synthesis and membrane stabilization (Özogul et al., 2008).

Dietary BAs are metabolized by acetylation and oxidation reactions enhanced by monoamine oxidase (MAO) or diamine oxidase (DAO) enzymes. Preconditions for the production of biogenic amine by microorganisms are: (I) the availability of free amino acids, (ii) the presence of decarboxylase-positive microorganisms, and (iii) bacterial growth, decarboxylase synthesis, and decarboxylase activity conditions. Proteolysis that occurs either autolytically or bacterially can play an important role in releasing free amino acids from tissue proteins that offer a substrate for decarboxylases reactions. Two reaction mechanisms for amino acid decarboxylation, which is a pyridoxal phosphate-dependent reaction and a non-pyridoxal phosphate-dependent reaction, have been reported (Özogul et al., 2008).

2.5.2 Physiological Role of Amines

BAs play various essential activity in the physiology and the development of eukaryotic cells in their physiological progress. The most active BAs are histamine and Tyramine. Polyamines, for example, putrescine, Spermine and Spermidine likewise assume basic deeds in cell development. Histamine is available in many living tissues as an ordinary constituent of the body and has various impacts in various mammalian and invertebrate creatures. In people, it is found in various fixations in the mind, lung, stomach and digestive organ, uterus and the ureters. It is delivered and put away dominatingly in pole cells, coursing basophiles and neurons. (Ladero et al., 2010).

Histamine adjusts a group of capacities by interfacing with explicit receptors on target cells, in particular H1, H2 and H3, receptors of the G-protein-coupled receptor group. H1 receptors are found in the cerebrum where they are associated with the control of the circadian beat, consideration and cognizance, and in fringe tissues where they intervene vascular and bronchial (Ladero et al., 2010).

Tyramine and β-phenyl ethylamine are incorporated into the gathering of following amines, a group of endogenous mixes with solid basic likenesses to old style monoamine synapses, in spite of the fact that the endogenous dimensions of these mixes are in any event two requests of size beneath that of these neurotransmitters. The impacts of these low physiological fixations have been hard to illustrate, however it has been recommended that they serve to keep up the neuronal movement of monoamine synapses inside characterized physiological points of confinement (Linares et al., 2011). The Interest for their use has expanded in late decades following the revelation of another group of G-protein coupled receptors (GPCR), some of which are explicitly enacted by following amines. This particularity has prompted the proposition of another terminology for these receptors, that of following amine-related receptors (TAARs) (Ladero et al., 2010). Portions of these receptors are situated in veins which may clarify the impact of Tyramine on blood pressure. Tyramine can be changed over into octopamine when taken up in thoughtful nerve terminals, where it uproots norepinephrine (NE) from capacity vesicles. A part of this NE diffuses out of the nerve to respond with receptors, causing hypertension and other sympathomimetic impacts (Ladero et al., 2010).

The physiological deeds of polyamines have been identified with their substance structure. These particles convey a positive charge on their essential and auxiliary amino gatherings at physiological pH. Along these lines, polyamines may go about as ligands at numerous destinations on DNA, RNA, proteins, phospholipids and nucleotide triphosphates. The organic elements of polyamines are basically the guideline of quality articulation by modifying DNA structure and by balancing signal transduction pathways. The ideal working of the phone accordingly requires, the intracellular polyamine substance is carefully controlled at the dimensions of biosynthesis, catabolism, take-up, and efflux. Limited quantities of orally administrated polyamines incite cell development; bigger amounts have no impact or may really repress the development (Linares et al., 2011).

Table 2.2 Physiological and Toxicological effects of Biogenic Amines (Ladero et al., 2010).

BA	Physiological Effect	Toxicological effect
Histamine	Neurotransmitte, local hormone,gastric acid secretion, cell growth and differentiation, regulation of circadian rhythm body temperature, food intake, learning and memory immune response allergic reactions.	Headaches, sweating burning nasal secretion,facial flushing bright red rashes, dizziness ,itching rashes , oedema(eyelids),urticarial ,difficulty in swallowing , diarrhea, respiratory distress bronchospasm , increase cardiac output, tachycardia extra systoles, blood pressure disorders.
Tyramine	Neurotransmitter,peripheral vasoconstriction , increase cardiac output ,increase respiration, elevate blood glucose , release of norepinephrine	Headaches, migraine, neurological disorders , nausea, vomiting , respiratory disorders, hypertension
Putrescine	Regulation of gene expression, maturation of intestine, cell growth and differentiation.	Increased cardiac output, tachycardia, hypotension, carcinogenic effects

2.5.3 Producing Biogenic Amines by Microorganisms

The existence of bacterial strains with the ability to decarboxylate amino acids is an important factor in the creation of BAs in foods. This capacity was defined in various bacteria genera, species and strains, both Gram positive and Gram negative. BAs synthesis in bacteria can be linked to energy production and help safeguard against

acid stress. Additional roles have been suggested in DNA regulation and as free radical scavengers (Ladero et al., 2010).

Fish and fish products are among the ingredients with the largest levels of BA (primarily histamine) as well as Pseudomonas. Because BA manufacturers mostly contaminate Gram-negative bacteria, many writers proposed that BAs could be used as an indicator of bad quality or bad manufacturing procedures following. However, lactic acid bacteria (LAB) are the major manufacturers of BA in fermented products. These can be discovered in the raw materials, be part of the culture of the starter, or contaminate the item during its production. A number of writers proposed the capacity to generate BAs as an adverse feature when choosing a starting, secondary or adjunct crop for use in the production of fermented products (Ladero et al., 2010).

Although *Oenococcus oeni* was originally regarded as, the primary bacterial species responsible for the accumulation of histamine in wine, latest studies have shown that *Lactobacillus hilgardii* and *Pediococcus parvulus* strains may actually be the culprits. Tyramine is the most abundant and frequently identified BA in cheese and fermented meat products, where the primary manufacturers are LAB *Enterococcus* strains and *Lactobacillus*. Initially, Putrescine synthesis was connected with Gram negative bacteria, especially *Enterobacteriacea* members (Til et al., 1997).

Recent reports, however, quote the capacity of some LAB strains from Lb. Wine brevis secluded and that is *Lb. Curvatus, E. Fecalis* isolated from cheese (unpublished results) to produce putrescine by agmatine deamination rather than ornithine decarboxylation; therefore, their contribution to the production of putrescine in food cannot be excluded. In reality, LAB is the primary bacteria responsible for the manufacturing of putrescine in wine. (Til et al., 1997).

Current knowledge of microorganisms producing BA varies by species or strain. The genes encoding BA-producing enzymes (decarboxylation and transporter) were sequenced in some instances and regulatory studies were conducted. Data were collected in others after producer microorganism isolation using decarboxylated media (Ladero et al., 2010).

Table 2.3 Food producer microorganism (Ladero et al., 2010).

Food	BA	Producer microorganisms
Fish	Histamine	*Morganella morganii, Klebsiella pneumonia, Hafnia alvei, Proteus vulgaris, Proteus mirabilis, Enterobacter cloacae, enterobacter aerogenes, photobacterium subsp.*
Cheese	Histamine	*Lactobacillus buchneri*
	Tyramine	*Enterococcus faecalis , Enterococcus faecium, Enterococcus durans, Enterococcus hirae, Lactobacillus brevis,*
	Putrescine	*Entrobacteriaceae(Enterobacter,serratia,Eshcherichia, Salmonella , Hafnia, cetrobacter, Klebsiella , Lactobacillus brevis).*
Wine	Histamine	*Oenococcus oeni, pediococcus parvulus*
	Tyramine	*Lactobacillus brevis, Lactobacillus hirgardii,*
	Putrescine	*Entrobacteriaceae(Enterobacter,serratia,Eshcherichia, Salmonella , Hafnia, cetrobacter, Klebsiella , Lactobacillus brevis).*
	Cadaverine	*Entrobacteriaceae*
Meat	Histamine	*Entrobacteriaceae, Staphylococcus capitis*
	Tyramine	*Staphylococcus carnosus, Staphylococcus Xylosus, Staphylococcus epidermidis, Lactobacillus brevis, Lactobacillus sakei, cyanobacterium.*
	Putrescine	*Entrobacteriaceae ,M.morganii, S.liquefaciens, pseudomonas ,Enterococcus , LB carvatus*
	Cadaverine	*Entrobacteriaceae*

CHAPTER III
MATERIAL AND METHOD

3.1 Material

Kashar cheese sample was kindly produced by Kahkecioğluğlu factory in Gaziantep city, Turkey. The chemical used 1,7-diaminoheptane, sodium bicarbonate, perchloric acid were obtained from (Sigma Aldrich).

Aerobic count agar, potato dextrose agar, MRS, M17 and lactose, raffinose, maltose and fructose was obtained (Sigma Aldrich).

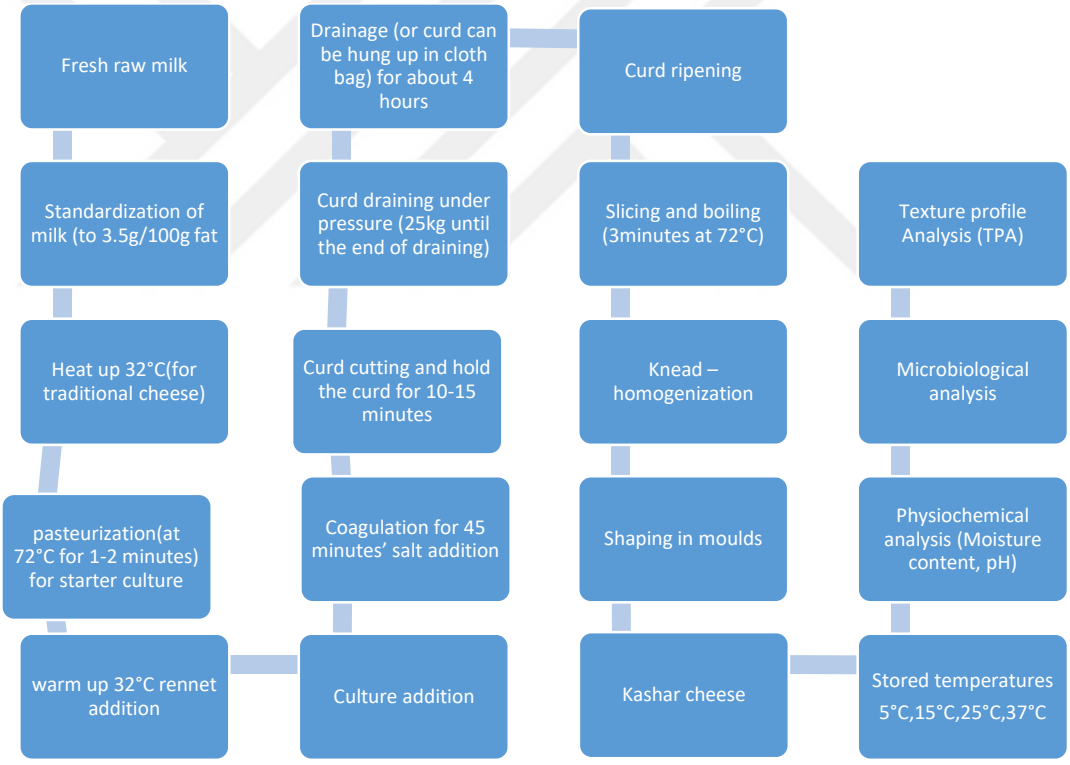

Figure 3.1 Steps of production of kashar cheese (Sert, 2007).

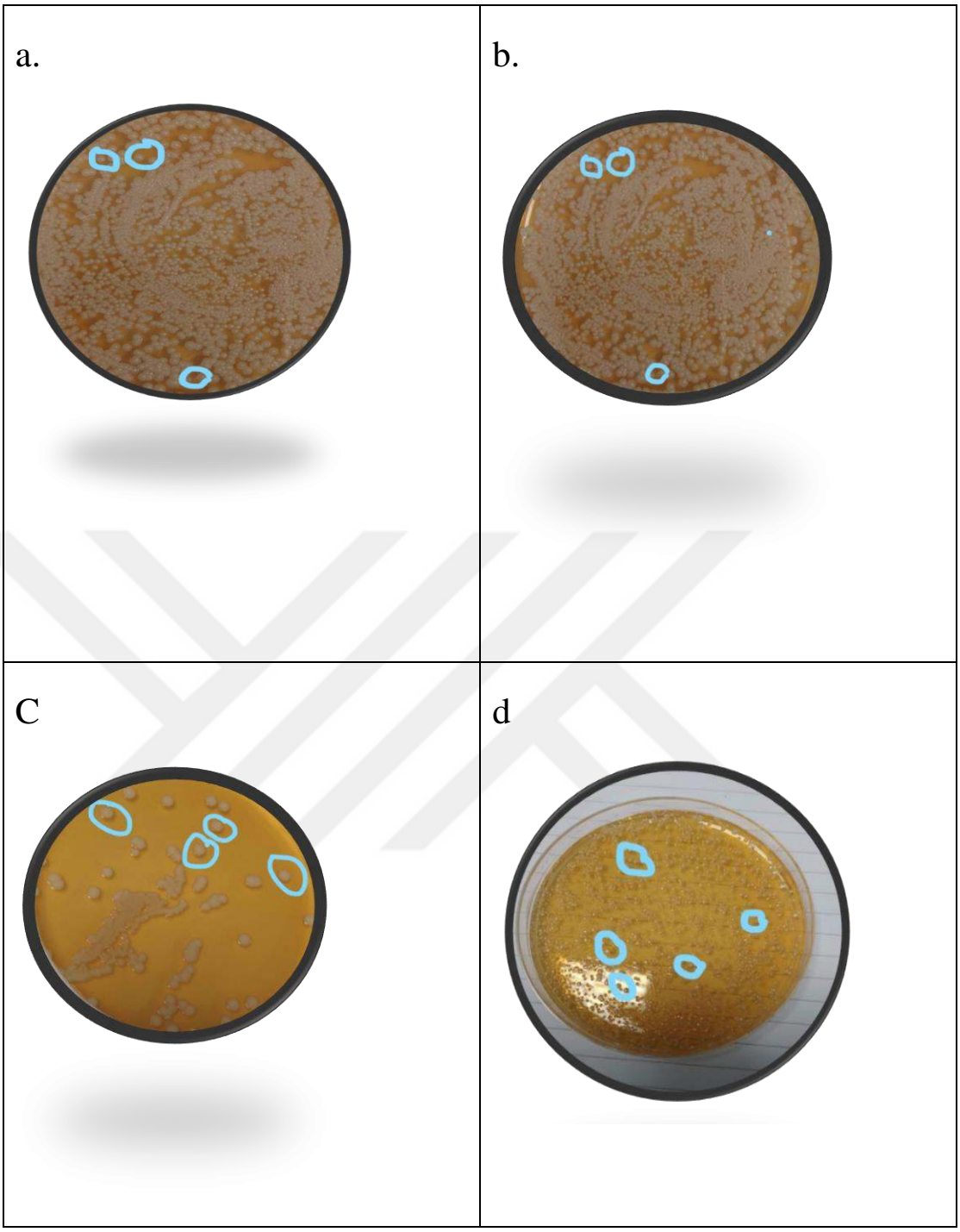

Figure 3.2 These figures Shows *Lactic acid bacteria subsp (a)Lactobacillus acidophilus, (b)S.thermophilus, (c)L.delbrueckii subsp. bulgaricus, (d)Bifidobacterium lactis.*

3.2 Method

3.2.1 Determination of Moisture Content

For determination of moisture content of kashar cheese, oven method was used. To investigate the moisture content of sample; about 5 g of samples were put into oven at 105°C up to constant weight reached. Moisture content of samples was found from the equation that was given below (Erkmen, 2015).

$$M.C\% = (M2 - M3)/(M2 - M1) * 100$$

M1= mass of empty petri dishes.

M2= mass of empty petri dish + weight of Sample.

M3= mass of sample after oven.

3.2.2 Determination of pH

For determination of pH value of kashar cheese, pH meter was used. To investigate the pH of sample; about 10 grams of homogenized sample were mixing with 90ml of distilled water also magnetic sterile was put into mixture and mixed the sample within 5 to 10 minutes. pH of sample was found after it is stabling the sample by pH mater (Mei et al., 2006).

3.2.3 Determination of Texture

The texture of kashar cheese where analyzed kept at four different temperatures at 5°C, 15°C, 25°C and 37°C, were measured to find the suitable storage temperature of kashar cheese that can effects shelf life of kashar cheese for the hardness, springiness, cohesiveness, chewiness, gumminess and resilience. For texture profile analysis (TPA), samples were stored at room temperature at least 2 hours before analyzed so that cuts (3cm × 3cm × 2cm) before and seized for equilibration to room temperature (˜20°C). TPA tests were performed using a TA×T2 texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). TPA tests was preformed especially requirement in the system like, pretest speed as 1.00 mm/sec, test speed as 1.00mm/sec, target mode strain, time 25.00%, duration time 5sec, target type auto force and trigger as 0.005kg and tare mode auto (Mei et al., 2006).

3.2.4 Determination of Biogenic Amines

The biogenic amines were determined by using the chromatographic method (Eerolla et al., 1993). The HPLC consisted of a Shimadzu gradient pump (Shimadzu LC 20AB, Shimadzu Solvent Delivery Module, Kyoto, Japan), a Shimadzu auto-injection unit (Shimadzu SIL20AHT, Kyoto, Japan), a Shimadzu UV detector (Shimadzu SPD 20A, Kyoto, Japan), and a RP-18 guard. Spherisorb ODS2, 200 μm and 4.6 mm 200 mm were the column of the HPLC. A solution of ammonium format (0.4 M) prepared from ultra-pure water (Millipore Elix 10UV and Milli-Q, Millipore S.A.S. 67120 Molsheim, France) and acetonitrile were filtered through a filter of 0.45 μm millipore (Billerica, MA). Forms of ammonium and acetonitrile have been used.

Using a Waring blender, two grams of sample was homogenized with 0.4 M perchloric acid in 10 mL. The sample was filtered and centrifuged at 1790 g for 10 min. The extraction was repeated with another 10 mL solution of 0.4 M perchloric acid, and the supernatants were mixed and formulated with 0.4 M perchloric acid up to 25 mL. One milliliter of extract was piped into a glass stopped test tube, adding 200 μL of 2 N NaOH and 300 μL of saturated sodium bicarbonate solutions. Each sample was added two milliliters of dance chloride (10 mg / mL) solution and incubated at 40 C for 45 minutes. Residual dance chloride has been removed by adding 25 percent ammonia to 100 μL. After 30 minutes. The solution was adjusted to 5 mL with acetonitrile after 30 min, centrifuged at 1790 g for 5 min, filtered the supernatant (0.45 μm) and then injected 20 μL onto the HPLC. To give concentrations from 0.5 to 10 μg / mL, the standard solution of the danced derivatives was diluted to 1 mL with 0.4 M perchloric acide (Şahin Ercan, Soysal, and Bozkurt, 2019).

3.3 Microbiology Analysis

3.3.1 Reagents and Media

All the materials were freshly prepared. Preparation of 225ml peptone water was done by dissolving 5.73g of peptone powder in one litter. Then peptone water was autoclaved at 121°C for 15 minutes (Tabasco et al., 2007).

3.3.2 Preparing MRS with Fructose

For *L. delbrueckii* subsp *bulgaricus* count, applicable dilution was inoculated by pour-plate technique into MRS agar which was prepared by dissolving 61.5g of agar in to 800 ml of distilled water enriched with 1% of fructose then the sample and agar was mixing well and autoclaved at 121°C for 15 minutes (Tabasco et al., 2007)

3.3.3 Preparing M17 with Lactose

For account of *S.thermophilus* count applicable technique was the pour-plate technique into 55g M-17 agar which was prepared by dissolving 55g in to 800ml of distilled water enriched 1% lactose then the sample and agar was mixing well and autoclaved at 121°C for 15 minutes (Tabasco et al., 2007)

3.3.4 Preparing MRS with Maltose

Lactobacillus acidophilus was counted by use of MRS agar which was prepared by dissolving 61.5g in to 800ml of distilled water supplementing with 1% maltose. The technique was poured –plate technique. Then the sample and agar were mixed well and autoclaved at 121°C for 15 minutes (Tabasco et al., 2007)

3.3.5 Preparing MRS with Raffinose

Bifidobacter lactis number was enumerated by means of MRS agar which was prepared by dissolving 61.5g in to 800ml of distilled water enriched with 1% of Raffinose. This technique was pour-plate technique. After then, the agar and the sample together autoclaved at 121°C for 15 minutes (Tabasco et al., 2007).

3.3.6 Plate Count Agar

For Total Aerobic Mesophilic Bacteria (TAMB) count applicable technique was the pour-plate technique which was prepared by dissolving 20.5g agar in to 800ml. After then, the agar and the sample together autoclaved at 121°C for 15 minutes (Çetinkaya and Soyutemiz, 2006).

3.3.7 Potatoes Dextrose Agar

For account of mold and yeast was enumerated by means of which Potatoes Dextrose Agar (PDA). Which was prepared by dissolving 39g of potatoes Dextrose Agar in to 800ml of distilled water. After then, the agar and the sample together autoclaved at 121°C for 15 minutes (Çetinkaya and Soyutemiz, 2006).

3.4 Microbiological Analysis

The determination of bacterial count as a log cfu/ml. The enumeration of bacterial counts in kashar cheese were made at 5°C,15°C,25°C and 37°C corresponding to storage time. All samples were serially with of peptone water (0.1% v/v) pour plate was applied all of the bacterial counts, then plates were incubated. All operations were done as duplicated on petri dishes. Dave and Shah (1998a) reported that enumeration of *L. acidophilus* was completed by spreading to MRS agar with the addition of maltose 1% containing adequate dilution inoculation. Then, petri dishes were incubated at 37°C after 3 days the colonies were counted, *Streptococcus thermophilus:* suitable dilutes were inoculated formed by the pour-plate technique into M-17 agar enriched 1% lactose (M17-lactose) to count *S.thermophilus* and parties were incubated anaerobically with 5% CO2 at 30°C for 72hrs.

Lactobacillus bulgaricus: For count of *L. delbrueckii* subsp. *bulgaricus*, applicable dilutes were inoculated by pour-plated into MRS agar enriched with 1% of fructose and were incubated with anaerobic of 5% CO2 at 30°C for 72hrs.

Bifidobacterium lactis: For counting of *Bifidobacterium lactis* MRS agar that was enriched with 1% of raffinose were used with pour-plated technique anaerobically with 5%CO2 at 30°C for 72hrs.

Lactobacillus acidophilus: *Lactobacillus acidophilus* counts were enumerated with MRS agar supplemented with 1% of maltose which were inoculated spread technique for anaerobically 5% CO2 at 30°C for 72hrs.

Total aerobic mesophilic bacteria*:* were enumerated with PCA in pour-plate technique at 37°C for 48hrs.

Mold and yeast*:* mold and yeast counts were enumerated with Potatoes Dextrose Agar by pour-plate technique at 37°C for 48hrs.

3.5 Statistical Analysis

The results were analyzed by statistical analysis SPSS version 14.0 for Windows (SPSS Inc, Chicago, IL, USA). The one-way analysis of variance (ANOVA test) and Duncan's multiple range test were performed.

CHAPTER IV
RESULTS AND DISCUSSIONS

4.1 Determination of Moisture Content in Kashar Cheese

Moisture content of kashar cheese was recorded during 105 days of storage kept at four different temperatures (5°C, 15°C, 25°C and 37°C). Their results are shown in (Figure 4.1). The statistical analysis was applied to find how the temperature and storage time are affecting the moisture content of kashar cheese. The ANOVA results shows that increasing the storage time and temperatures increased ($P<0.05$) the moisture content (Table 4.1). Moisture content of samples stored at 5°C up to 45 days of storage did not changed significantly ($P<0.05$) compared to other storage time and temperatures. This means that during fresh stage, moisture content of kashar cheese was lower ($P<0.05$) than that of the ripening stage. Mainly because cheese in the fresh stage, contains high salt content which decreases the moisture content. (Çetinkaya and Soyutemiz, 2006), found that salt is the only factor that effects the moisture content during ripening process, also they reported that moisture content of kashar cheese increased during ripening period. Our results supported that report as moisture content was observed at 60, 75, 90 and 105 days corresponding to all studied temperatures were significant ($P<0.05$). As increasing storage, time moisture content increased. ($P<0.05$) significantly. That means that it had directly proportional with time and temperature, also as the time and temperature increased melting of salt. The results obtained in this study is lower than to (Sert, 2007), study results that the fresh stage of kashar cheese was in between 37.9 - 41.6% while ripening was 43.3 and 46.1% , (Toker et al., 2016), reported that moisture content of kashar cheese is in rage between 45.3% and 55.9%. Also these study agree with (Ucar and Badem, 2016) reported that moisture content of kashar cheese in between 54.93-58.04%.

Table 4.1 Effect of time and temperature in moisture content.

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	48.50±0.07bA	48.50±0.07aA	48.45±0.07bA	48.45±0.07cA
15	42.50±3.53aA	46.00±0.00aA	46.00±1.41aA	44.90±0.57bA
30	41.60±1.89aA	46.15±0.02aA	46.00±0.00aA	43.50±0.70aA
45	49.25±0.35bA	48.00±0.00aA	50.00±0.00cA	49.00±0.00cA
60	51.50±2.12bcA	52.00±2.82bA	53.00±0.00dA	53.00±1.41dA
75	54.50±0.00cdA	54.40±0.70cA	55.00±0.00eA	53.00±0.00dA
90	56.00±0.00dA	56.00±0.00cA	56.00±0.00eA	55.00±0.00eA
105	60.00±0.00eA	56.00±0.00cA	58.00±0.00fA	58.00±0.00fA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in temperature.

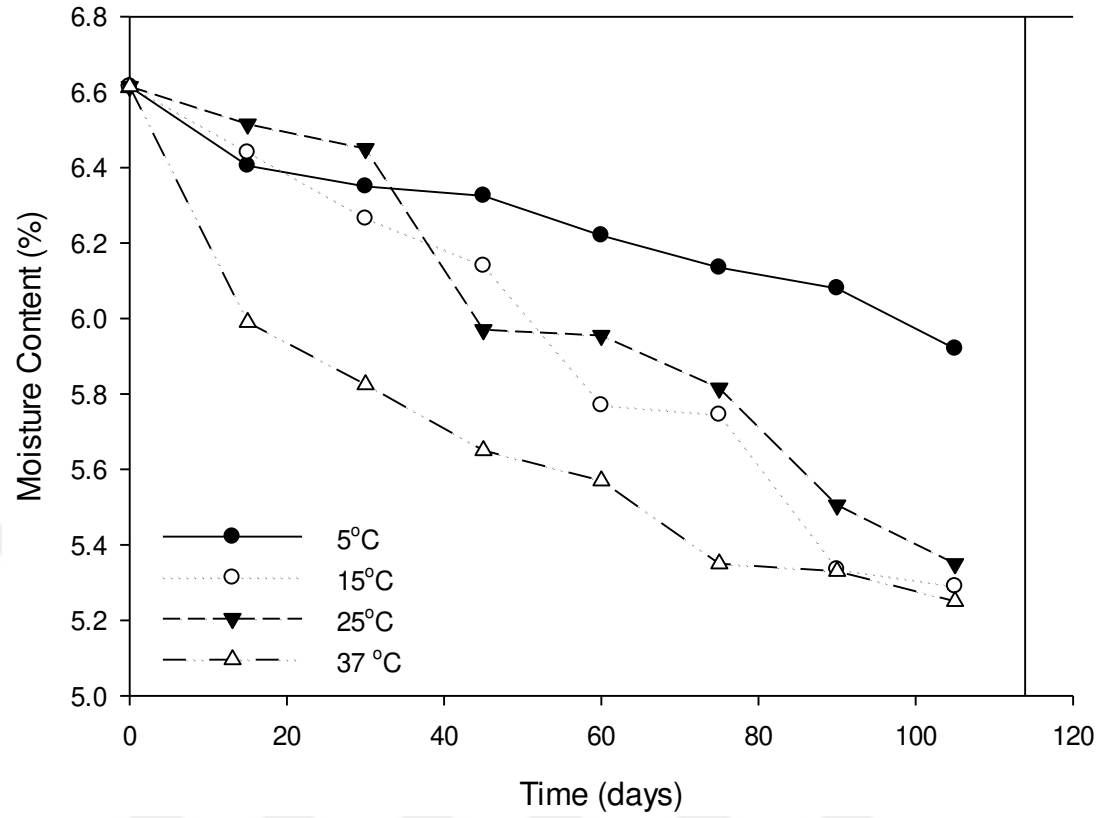

Figure 4.1 Change of moisture content with storage time and temperature.

4.2 Determination of pH Value

pH values of kashar cheese were recorded during 105 days of storage kept at four different temperatures (5°C ,15°C, 25°C and 37°C). The results are shown (Figure 4.2). The statistical analysis was applied to determine effect of the temperature and storage time on the pH value of kashar cheese. The ANOVA results shows that increasing the storage time and temperatures decreased ($P<0.05$) pH values (Figure 4.2). pH value of samples stored at 5°C up to 45 days of storage were not significantly changed ($P>0.05$) compared to other storage time and temperatures. (Elena, 2000) and (Guinee, 2000), reported that, the pH value of kashar cheese slightly increased first 30 days of storage khashar cheese. Our study wasn't agreeing with (Sert, 2007), reported that during 15 days of fresh stage added the sample starter culture of lactic acid bacteria causes reduction in the pH of kashar cheese. As our data showed decreasing, the pH values at all the temperatures up to 105 days of ripening in kashar cheese sample.

pH values of the sample kept at 15°C, 25°C and 37°C for ripening period were significantly affected. Increasing the storage period with increasing temperature, the pH value of the sample decreased. (Temiz, 2010), determined that the packaging treatment on the 60th and 90th of storage with atmosphere conditions the pH value decreased. (Elena, 2000), reported that CO2 is the mechanisms that has been used for inhibition of microbial growth (Riu et al., 2002) determined that the pH of cottage cheese was not affect by packaging with 40% of CO2. The pH value reported in our sample were lower than those found by (Çetinkaya and Soyutemiz, 2006) also similar results by (Öksüztepe et al., 2009) reported that the kashar cheese packaged under Vacuum was almost pH 5.49. Also these study agree the results found by (Karabıyık, 2019) that the pH of kashar cheese from raw milk was 6.60.

Table 4.2 Effect of storage time and temperature on pH value.

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	6.61±0.02fA	6.61g±0.02gA	6.61±0.02fA	6.61g±0.02gA
15	6.54±0.05eA	6.44±0.02fA	6.51±0.02eA	5.99±0.00fA
30	6.34±0.01dA	6.23±0.035eA	6.45±0.05eA	5.82e±0.02eA
45	6.30±0.00dA	6.14±0.00dA	5.97±0.01dA	5.65±0.02dA
60	5.22±0.02cA	5.77±0.00cA	5.95±0.03dA	5.67±0.01cA
75	5.12±0.02bA	5.74±0.00cA	5.81±0.02cA	5.35±0.01bA
90	5.12±0.01aA	5.33±0.00bA	5.50b±0.07bA	5.33±0.00bA
105	5.05±0.02aA	5.29±0.00aA	5.37±0.00aA	5.25±0.00aA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

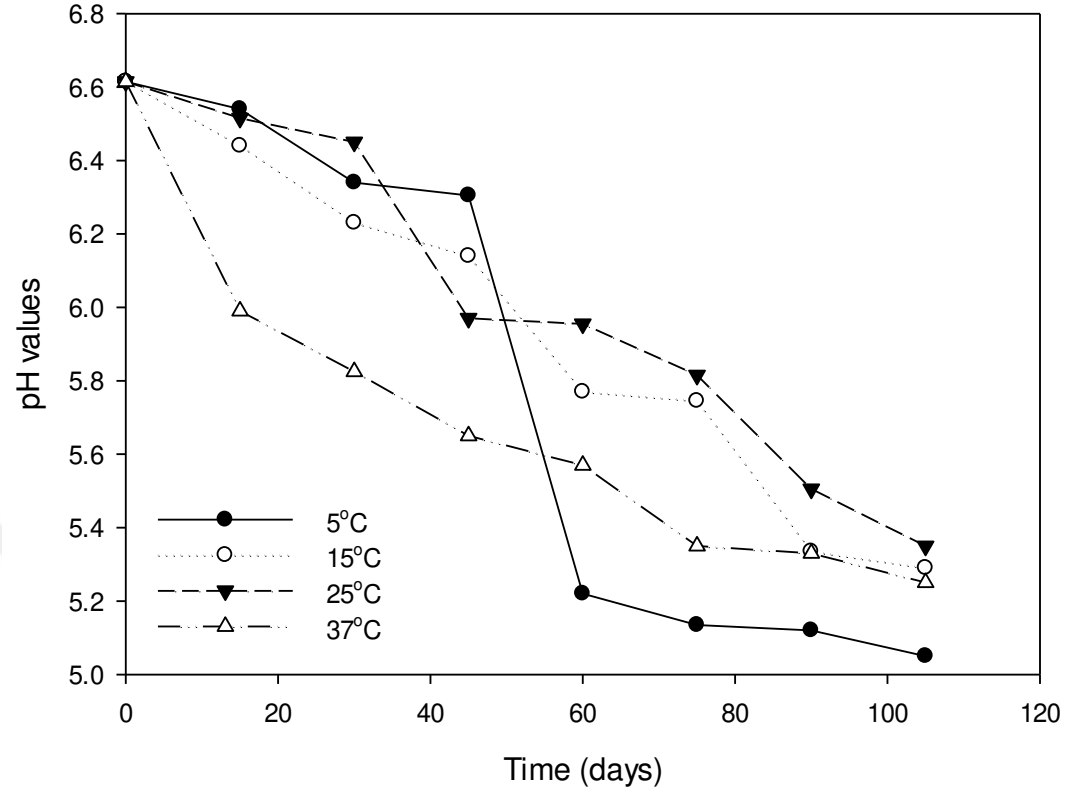

Figure 4.2 Effect of storage time and temperature on pH change.

4.3 Growth of Streptococcus thermophilus

Changes in growth of *Streptococcus thermopilus* in kashar cheese were recorded during 105 days of storage kept at four different temperatures, (5°C ,15°C, 25°C and 37°C). The results are shown in Figure 4.3. The statistical analysis was applied to determine how the temperature and storage time affect the growth of *S. thermophilus*. The ANOVA results showed that increasing the storage time and temperatures increased ($P<0.05$) the growth *of S. thermophilus* (Table 4.3)**.** Storage time of 0,15, 30 and 45 days kept at 5°C reduced growth *of S. thermophilus* significantly ($P<0.05$) compared to other storage time and other stored temperatures. The increase in *S. thermophilus* was observed after 60, 75, 90, 105 days of storage at all the temperatures which were significantly effecting the growth of *S. thermophilus*. The highest *S. thermophilus* was observed by keeping at 37°C for 105 days of storage (Table 4.3). Also these results agree with the results found by (Öksüztepe et al., 2009) increasing LAB after ripening 30 days. the storage time. As (Table 4.3) showed that *S. thermophilus* for first 30 days may be constant or slightly change. (Çetinkaya and Soyutemiz, 2006), reported that the count of *S.thermophilus* was

reported to decrease from 8.24 log cfu/g to 3.10 log cfu/g after 90 days. However, this research showed that Lactic Streptococci increased gradually after 60 days up to 105 days. (Sert, 2007) reported that LAB decreased after 15 days of storage period Table (4.3) showed gradually increasing of *S.thermophilus* after 15 days the results obtained in this study had lower than those found by (Riu et al; 2002) as *S.thermophilus* 6.57-7.40 log cfu/g.

Table 4.3 Effect of temperature and time on the growth of *S. thermophilus*

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	3.52±0.27aA	3.52± 0.27aA	3.52±0.27aA	3.52± 0.27aA
15	4.18±0.83bA	4.33±0.14bA	3.60±0.00bA	4.87±0.01bA
30	4.34±0.59cA	4.50±0.29cA	4.67±0.00cA	5.64±0.01cA
45	4.91±0.21dA	4.63±0.11cA	4.73±0.07dA	5.77±0.00dA
60	4.96±0.14dA	4.86±0.22dA	4.87±0.19eA	5.96±0.01eA
75	5.54±0.01eA	5.55±0.04eA	5.84±0.01fA	6.54±0.01fA
90	5.74±0.03fA	5.57±0.02eA	5.84±0.02fA	6.57±0.00fA
105	5.85±0.02fA	5.84±0.07fA	5.94±0.00gA	6.69±0.01gA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

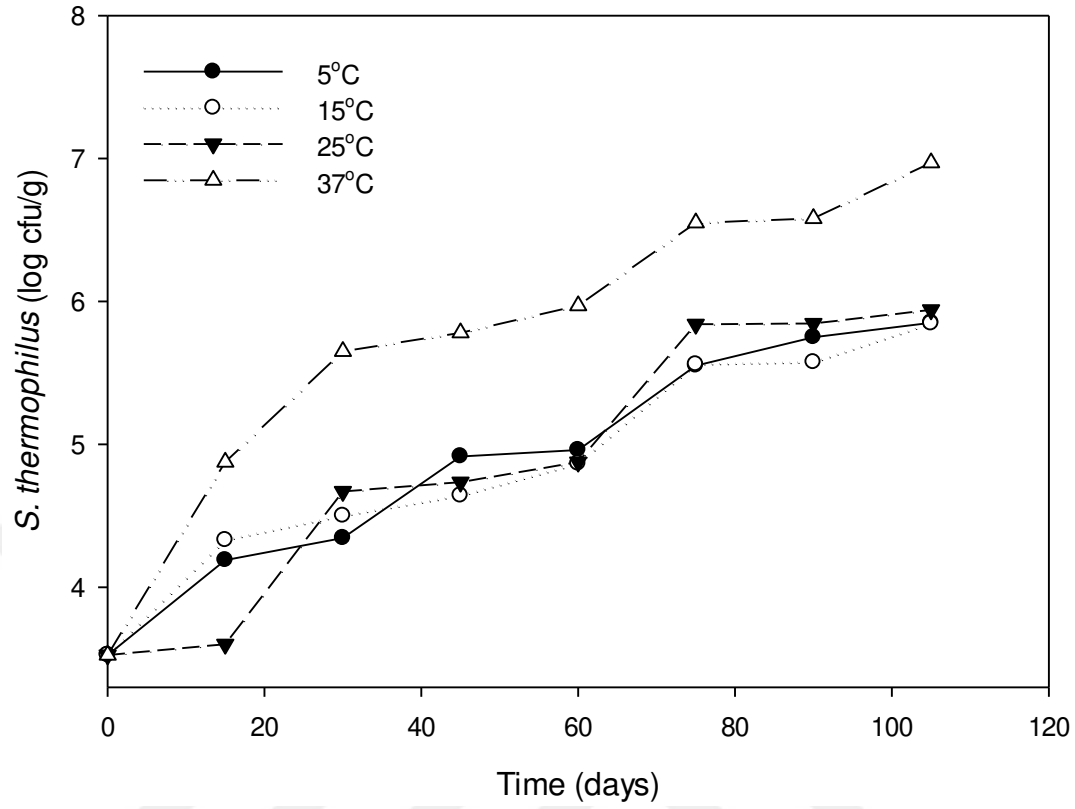

Figure 4.3 Effect of temperature and storage time on the growth of Lactic acid bacteria subsp, *S.thermophilus.*

4.4 Growth of Lactobacillus delbrueckii subsp bulgaricus

Growth of *Lactobacillus delbrueckii* subsp *bulgaricus* in kashar cheese were recorded during 105 days of storage kept at four different temperatures, (5°C, 15°C, 25°C and 37°C). The results are shown Figure 4.4. The ANOVA results showed that increasing the storage time and temperatures increased ($P<0.05$) the growth of *Lactobacillus delbrueckii* subsp *bulgaricus* in (Table 4.4). Growth *Lactobacillus delbrueckii* subsp *bulgaricus* in samples stored at 5°C for 0, 15, 30 and 45 days were not changed significantly ($P<0.05$) compared to other storage time and other stored temperatures. The increase of *Lactobacillus delbrueckii* subsp *bulgaricus* was observed during ripening time of 75, 90 and 105 days. The temperatures had significantly effect on the growth of *Lactobacillus delbrueckii* subsp *bulgaricus.* The highest *Lactobacillus delbrueckii* subsp *bulgaricus* was observed by keeping at 25°C for 105 days of storage (Table 4.4). As the (Halkman, 1991; Çetinkaya and

Soyutemiz, 2006), reported in their studies that LAB count increased after ripening 30 days. This study support *L.bulgaricus* increased with increasing the storage time. As Table (4.4) shows *Lactobacillus delbrueckii subsp bulgaricus* for first 30 days may be constant or slightly changed, (Simov and Ivanov, 2005) reported that the growth of *Lactobacillus delbrueckii* subsp *bulgaricus* during first stage of aging increased.

Table 4.4 Effect of temperature and Storage time on the growth *Lactobacillus delbrueckii* subsp *bulgaricus.*

Time (day)	**Temperature**			
	5°C	**15°C**	**25°C**	**37°C**
0	3.04±0.04aA	3.04±0.04aA	3.04±0.04aA	3.04±0.04aA
15	4.31±0.00bA	4.00±0.92aA	4.15±0.04bA	4.31±0.02bA
30	4.29±0.11bA	4.36±0.00cA	4.60±0.06cA	4.42±0.01cA
45	4.65±0.39bcA	4.48±0.13cA	5.60±0.01dA	4.51±0.01dA
60	4.87±0.29cA	5.61±0.04dA	5.98±0.006eA	5.64±0.05eA
75	4.75±0.00cA	5.67±0.04dA	6.51±0.01fA	5.74±0.02fA
90	5.47d±0.08dA	5.62±0.02dA	6.54±0.01fA	6.47±0.00gA
105	5.45d±0.00dA	6.87±0.01eA	6.94±0.00gA	6.92±0.03hA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

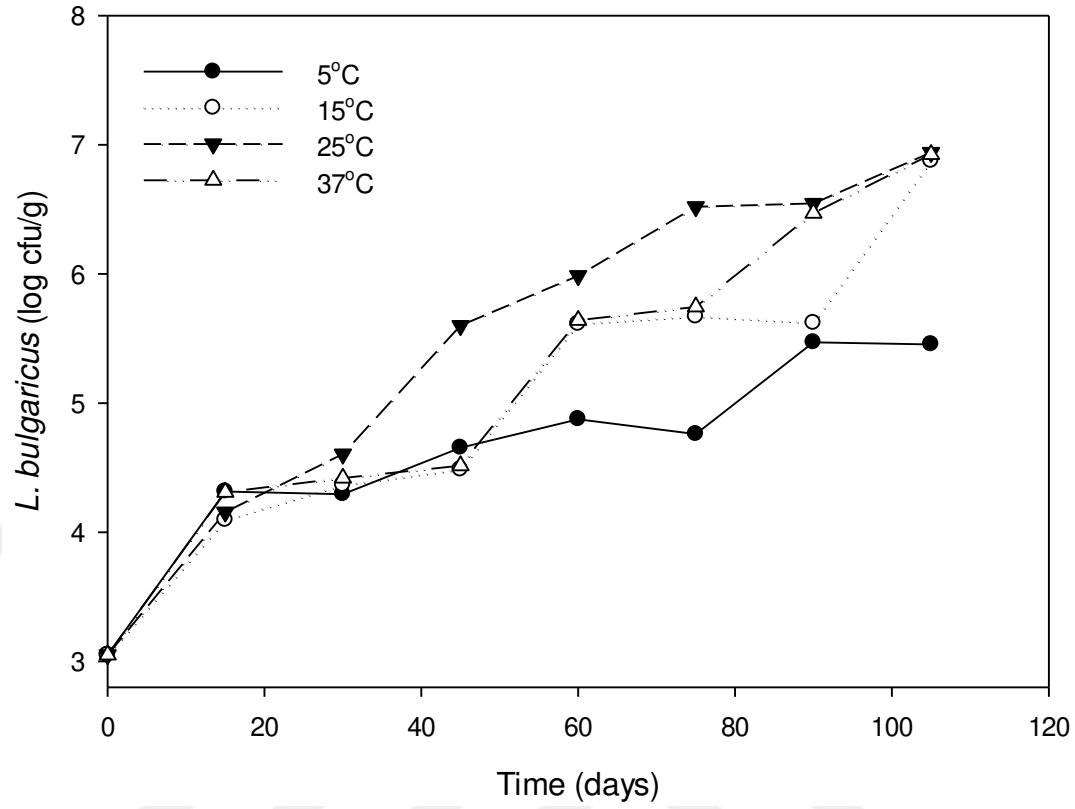

Figure 4.4 Effects of stored temperature and storage period on the growth of *Lactobacillus delbrueckii subsp bulgaricus.*

4.5 Growth of *Bifidobacterium lactis*

Growth of *Bifidobacterium lactis* in kashar cheese were recorded during storage kept at four different temperatures (5°C ,15°C, 25°C and 37°C). Moreover, their results are shown in Figure 4.4. The statistical analysis was applied to find the effects the storage temperature and time on the growth of *Bifidobacterium lactis*. The ANOVA results showed that increasing the storage time and temperatures increased ($P<0.05$) the growth of *Bifidobacterium lactis* (Table 4.5). Results of growth of *Bifidobacterium lactis* during fresh stage were close together. These means that increasing storage time and temperatures were not significantly ($P>0.05$) effecting the growth of *Bifidobacterium lactis.* These results similar to those found by (Gani et al., 2015) and (Armstrong et al., 1969), they reported that LAB in probiotic cheese are responsible to convert milk sugar to lactic acid which increased the moisture content of cheese and decreases the pH of cheese sample.

Table 4.5 Effect of storage temperature and time on the growth *B.Lactis*

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
30	3.79±0.01aA	3.97±0.01aA	4.30±0.00bA	3.72±0.02aA
45	3.72±0.07abA	4.33±0.03bA	4.17±0.05aA	4.22±0.07bA
60	3.81±0.00bA	4.74±0.00eA	4.65±0.03dA	4.18±0.02bA
75	4.51±.00cA	4.67±0.01dA	4.55±0.06cA	4.58±0.04cA
90	4.58±0.00cA	4.56±0.01cA	4.55±0.00cA	4.60±0.00cA
105	5.04±0.00dA	4.97±0.00fA	4.96±0.03eA	5.45±0.01dA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

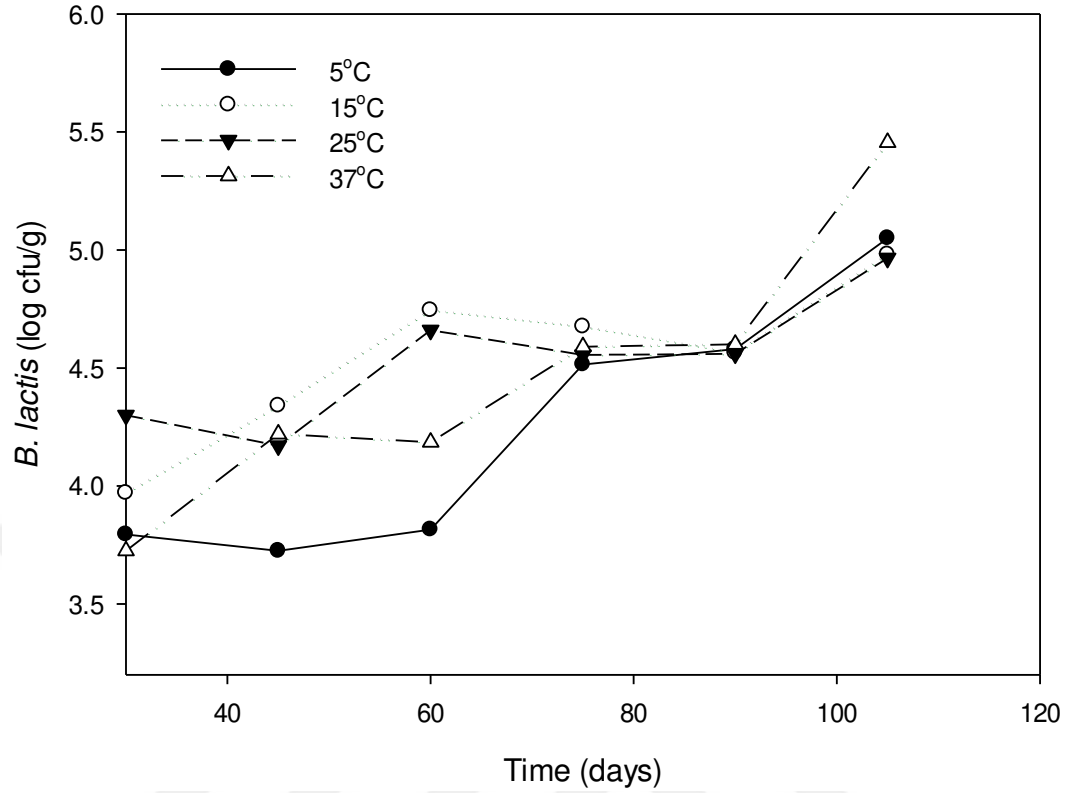

Figure 4.5 Effect of storage time and temperature on the growth of *B. lactis.*

4.6 Growth of Total Aerobic Mesophilic Bacteria

The growth of total aerobic mesophilic bacteria (TAMB) was recorded during 105 days, kept at different degree of temperatures. The results are shown in Figure 4.6. The statistical analysis was applied to determine how the temperature and storage time affect the total aerobic mesophilic bacteria of kashar cheese. The ANOVA results showed that increasing the storage time and temperatures increased ($P<0.05$) the total aerobic mesophilic bacteria (Table 4.5). TAMB count in samples stored at 5°C for 0, 15, 30 and 45 days' change significantly ($P>0.05$) compared to other storage time and other stored temperatures. In this study it was observed that storage time 60,75,90 and 105 days at all the temperatures had significant ($P<0.05$) effect on the growth of TAMB. The increasing storage time and stored temperature caused to decrease total aerobic mesophilic bacteria 4.48-4.18 log cfu/g. (Sert, 2007) reported that total aerobic mesophilic bacteria count increased with increasing ripening time about 4.29-4.57 log cfu/g. Our study confirmed the results of that report. As other

researchers reported that total aerobic mesophilic bacteria increase from poor hygiene manufacturing up to 7.70-8.47 log cfu/g (Sert, 2007). Also the results obtained in study disagree with results found by (Halkman, 1991) and (Uraz and Çitak, 1998), reported that total aerobic mesophilic bacteria count after 120 days is 6.2 log cfu/g.

Table 4.6 Effect of temperature and Storage time on the growth of total aerobic mesophilic bacteria.

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	4.48±0.1bA	4.48±0.1bA	4.48±0.1bA	4.48±0.1dA
15	5.29±0.01dA	5.29±0.02dAA	4.85±0.1cA	4.27±0.03cdA
30	5.45±0.04eA	5.21±0.04dA	4.98±0.02cA	4.22±0.03cA
45	5.20±0.07cdA	4.91±0.01cA	5.60±0.07dA	4.14±0.00bA
60	5.13±0.09cdA	4.27±0.03aA	4.27±0.03a	4.03±0.04ab
75	5.08±0.02cA	4.51±0.02bA	4.51±0.02bA	4.07±0.00abA
90	4.17±0.04aA	4.20±0.00aA	4.20±0.00aA	4.07±0.00abA
105	4.13±0.09aA	4.18±0.06aA	4.18±0.06aA	3.97±0.03aA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

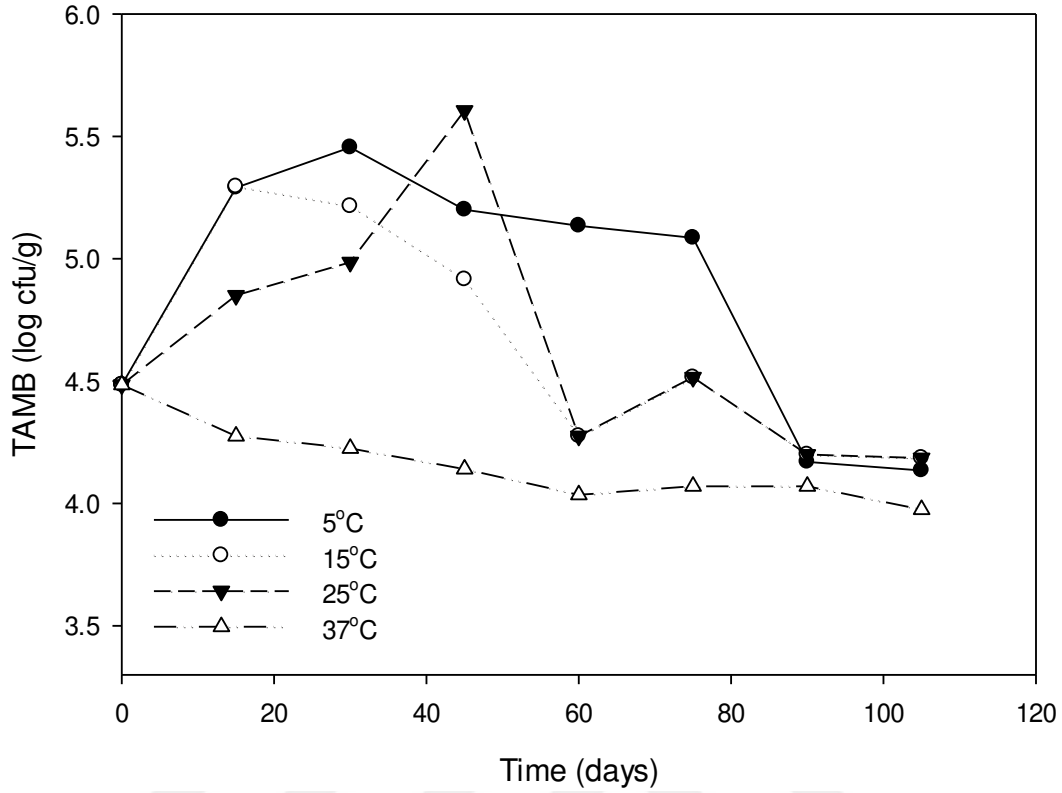

Figure 4.6 Effect of temperature and storage time on total aerobic mesophilic bacteria (TAMB) growth.

4.7 Growth of Mold and Yeast

The growth of mold and yeast was recorded during 105 days kept at different degree of temperatures like, 5°C, 15°C, 25°C and 37°C. The results are shown in Figure 4.7. The statistical analysis was applied to determine the effect of temperature and storage time on the mold and yeast growth of kashar cheese. The ANOVA results showed that increasing the storage time with temperatures increased ($P<0.05$) in the mold and yeast (Table 4.7).

Mold and yeast count of samples stored at 5°C for 0, 15, 30 and 45 days were significantly different ($P<0.05$) compared to other storage time and other stored temperatures. Also mold and yeast count of samples stored at 15°C had significant effect ($P<0.05$). In this study it was observed that storage time 60, 75, 90 and 105 days stored at 25°C and 37°C didn't significantly affect the growth of mold and yeast. It was found in this research that during fresh stage stored low temperature at

5°C ,15°C the growth of mold and yeast increased while gradually starting to decrease during ripening time. These results were lower than those found by (Sezer and Güven, 2009). Also these results were similar to those found by (Gul and Dervisoglu, 2013). This study confirm the result found by (Öksüztepe et al., 2009), the effect of vacuum and N2 or CO2 atmospheres shelf life of creamed or uncreamed cottage of cheese, packaged with aluminum at 5°C reduced yeast and molds and keeps fresh flavor. (Taniwaki et al., 2001), observed the growth of fungi and mycotoxin production in commercial cheddar cheese under modified atmosphere pressure all most eight fungal species incubated under conditions of decreasing concentration of O2 (5% to <0.5%) increased concentration of CO2 (20-40%). They observed that decreasing growth of fungi by 20-40% depends on species and formation of aflatoxins B1, B2 and roquerfortin C, and cyclopiazonic acid were greatly decreased (Sert, 2007), reported that addition of starter culture in kashar inhibits yeast and mould growth of the surface of the cheese. (Aljewic and Cichos, 2015) found that decreasing mold and yeast population in experimental of cheese increases the concentration of acetic acid and diacetyl which are produce lactose, lactate and citrate metabolism.by large population starter lactic acid bacteria (SLAB) (8-9 cfu/g) and *Lactobacillus subsp.*

Table 4.7 Effect of temperature and storage time on the growth of mold and yeast.

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	4.15±0.14bA	4.15±0.14gA	4.15±0.14dA	4.15±0.14eA
15	4.42±0.00cA	4.38±0.01fA	4.41±0.01eA	4.06±0.01eA
30	4.48±0.00cdA	4.42±0.02fA	4.67±0.01fA	4.32±0.02fA
45	4.59±0.00dA	4.57±0.01eA	4.78±0.00fA	4.36±0.01fA
60	4.24±0.04bA	3.78±0.00dA	3.83±0.01cA	3.78±0.01dA
75	4.24±0.01bA	3.58±0.04cA	3.54±0.01bA	3.56±0.01cA
90	4.16±0.02bA	3.38±0.00bA	3.62±0.02bA	3.30±0.03bA
105	3.09±0.00aA	3.10±0.00aA	2.45±0.01aA	2.92±0.03aA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

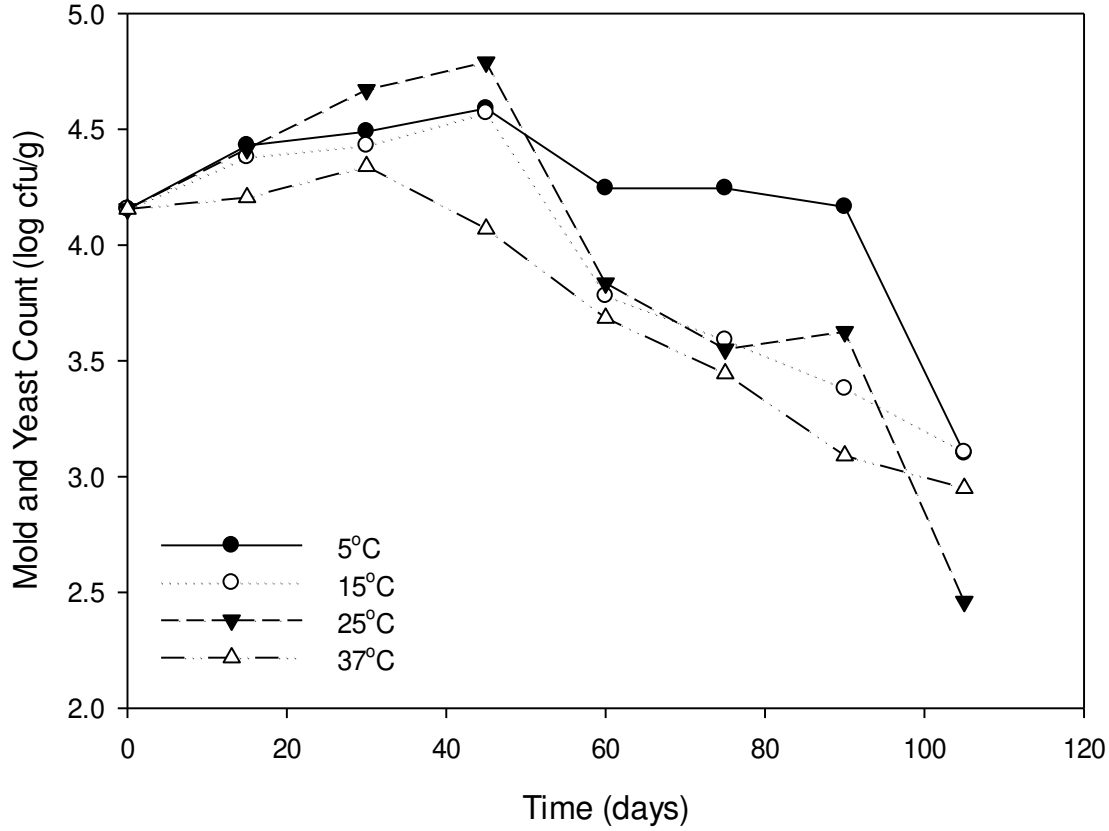

Figure 4.7 Effects of storage time and temperature on the growth of mold and yeast.

4.8 Texture Profile Analysis

For the determination of textural attributes of kashar cheese during 105 days of storage were kept at four different temperatures (5°C, 15°C, 25°C, 37°C). Hardness, cohesiveness, gumminess, springiness and chewiness values were followed. The analysis was carried out as triplication. The results are shown in Figure 4.8. The statistical analysis was applied to find effect of temperature and storage time on texture profile of kashar cheese. The ANOVA results showed that increasing the storage time with temperatures increased ($P<0.05$) in the hardness (Table 4.8). The hardness value of kashar cheese kept at 5°C for fresh stage 0, 15, 30 and 45 days was slightly different ($P<0.05$), comparing with other storage time and stored temperatures. The hardness of kashar cheese decreased during ripening time 60,75, 90 and 105 days, at all temperatures It means that increasing storage time and temperature, the hardness of kashar cheese was softer than those kept at 5°C for

0,15,30, and 45 days. Hardness value the amount of force to compress of the kashar cheese continuously decreased during the ripening time. This can be due to the breaking down of *αs1-casein* in to lower molecular weight peptides in kashar cheese and hydration of the protein matrix. Akalın, (2002), reported that deceasing the hardness of kashar cheese could due to proteolysis of the protein matrix. Results of this study confirmed that hardness of kashar cheese decreased with increasing the storage time (Lawrence, 1987; Guinee, 2000; and Zisu and Shah, 2005). Results obtained in this study was lower than those found by (Eroglu et al., 2016) as the hardness of cheese sample at beginning of the ripening period was in the range of 7223-8679g.

Table 4.8 Effect of temperature and Storage time on Hardness value (g)

Time (day)	**Temperature**			
	5°C	**15°C**	**25°C**	**37°C**
0	6204±288.43cA	6204±288.43cA	6204±288.43cA	6204±288.43cA
15	5692±43.58bA	5071±27.26eA	5790±19.76eA	5391±72.91eA
30	5606±63.94bA	4510±422dA	4510±422dA	4406±241dA
45	5316±100bA	3425±38.12cA	3628±231cA	3366±268.60cA
60	5455±397bA	3287±430bcA	2287±430bA	2608±334bA
75	5340±233bA	2364±269aA	1497±124aA	1444±193aA
90	4117±60aA	2417±347aA	1391±373aA	1339±329aA
105	3953±200aA	2892±128bA	1681±243aA	1402±327aA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

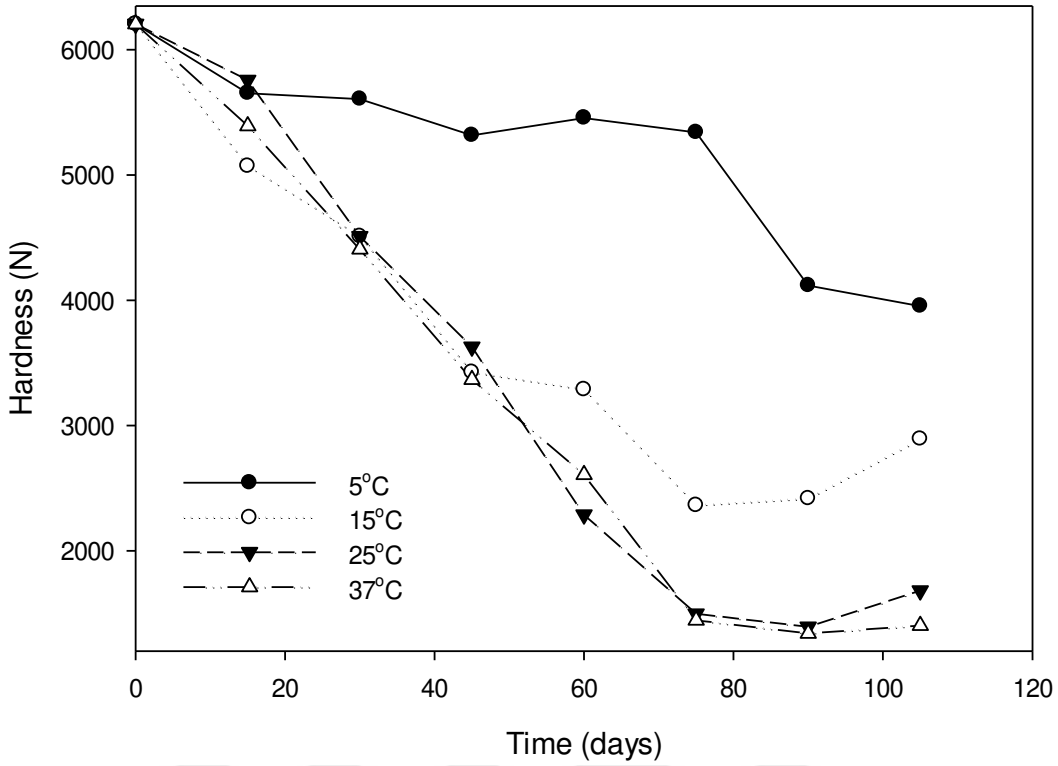

Figure 4.8 Changes of hardness value during storing 105 days

4.9 Springiness

Springiness is degree of elasticity of change the shape of sample after deforming force is removed. The analysis was worked as triplicate. The results are shown in Figure 4.9. The statistical analysis was applied how the temperature and storage time were effecting springiness of kashar cheese. The ANOVA results showed that increasing the storage time with temperatures ($P<0.05$) decreased the springiness (Table 4.9). The fresh stage of kashar cheese kept at 5°C the springiness wasn't significant ($p<0.05$) comparing with other storage time and stored temperatures. Increasing the ripening of kashar cheese with increasing stored temperature had significant effect on the springiness of kashar cheese. Springiness the amount of force to compress of the kashar cheese continuously decreased within the ripening time. Results of springiness of the kashar cheese were agreement with those found by Kahyaoglu et al. (2005) for Gaziantep cheese.

Table 4.9 Effect of storage time and temperature on Springiness (g)

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	0.92±0.04eA	0.92±0.04eA	0.92±0.04eA	0.92±0.04eA
15	0.84±0.04dA	0.86±0.28eA	0.84±0.01fA	0.84±0.00gA
30	0.84±0.00dA	0.72±0.01dA	0.72±0.01eA	0.73±0.43fA
45	0.81±0.01cdA	0.64±0.04cA	0.66±0.02dA	0.62±0.00eA
60	0.80±0.01bcdA	0.64±0.04bA	0.57±0.01cA	0.51±0.02dA
75	0.76±0.11bcA	0.73±0.03dA	0 46±0.04bA	0.34±0.27bA
90	0.72±0.00aA	0.58±0.01abA	0.38±0.01aA	0.34±0.27bA
105	0.76±0.02abA	0.56±0.03aA	0.36±0.03aA	0.26±0.23aA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

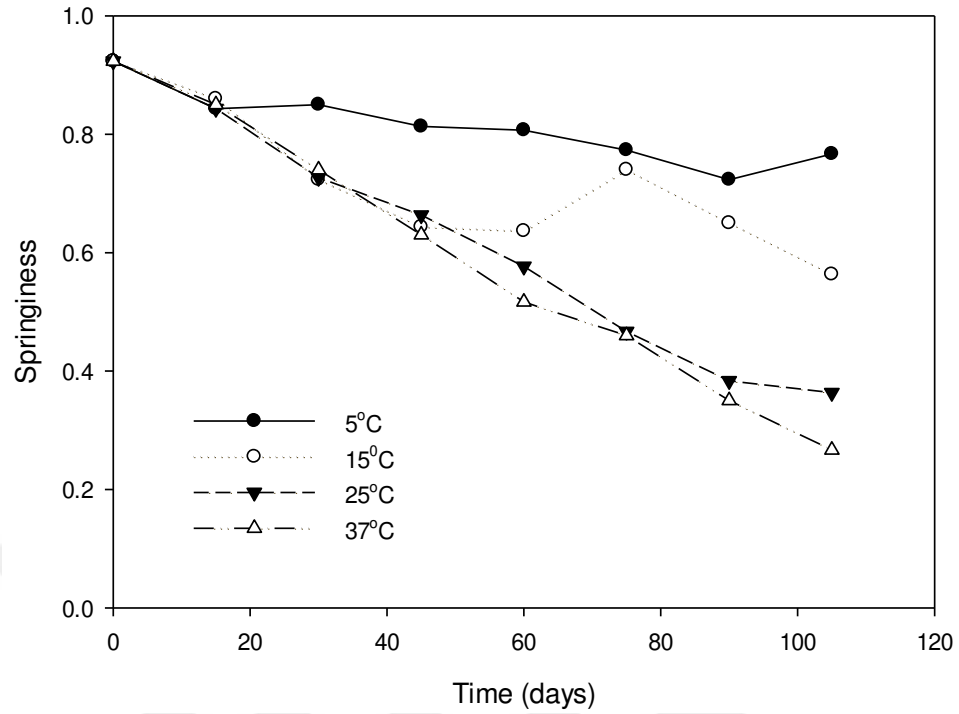

Figure 4.9 Changes of springiness value of kashar cheese during 105 days kept at four different temperatures.

4.10 Cohesiveness Value

Cohesiveness value is defined as the ratio between the positive force area during the second compression (A2) and the first compression force area (A1) (Bourne, 1978). Cohesiveness value of the cheese is associated with the strength of internal bonds of the protein mycelium. Cohesiveness of the kashar cheese decreased ($P<0.05$) throughout the storage period and stored temperature. The results are shown in Figure 4.10. The statistical analysis was applied to find effect of temperature and storage time on the cohesiveness values of kashar cheese. The ANOVA results shows that increasing the storage time with temperatures ($P<0.05$). decreased the cohesiveness values (Table 4.10). Storage time and stored temperature had significant effect on the cohesiveness value of the kashar cheese. The higher cohesiveness of sample means kashar cheese has high protein content. Bryant et al. (1995) reported that increasing temperature causes increase in the cohesiveness. The results obtained in this study disagreed with that report as Increasing storage time and stored temperature caused to decrease in the cohesiveness value.

Table 4.10 Effect of temperature and storage time on Cohesiveness values.

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	0.86±0.01eA	0.86±0.01eA	0.86±0.01dA	0.86±0.01hA
15	0.74±0.02dA	0.74±0.13dA	0.77±0.04cA	0.63±0.24gA
30	0.64±0.00cA	0.64±0.13cA	0.72±0.01bcA	0.63±0.29fA
45	0.56±0.14cA	0.64±0.15abA	0.67±0.01abA	0.63±0.28eA
60	0.56±0.03bA	0.64±0.08abA	0.69±0.08abA	0.62±0.03dA
75	0.54±0.3abA	0.64±0.04abA	0.64±0.04abA	0.67±0.00cA
90	0.54±0.01bA	0.61±0.02abA	0.67±0.02abA	0.53±0.1bA
105	0.51±0.01aA	0.61±0.04aA	0.63±0.04aA	0.54±0.3aA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

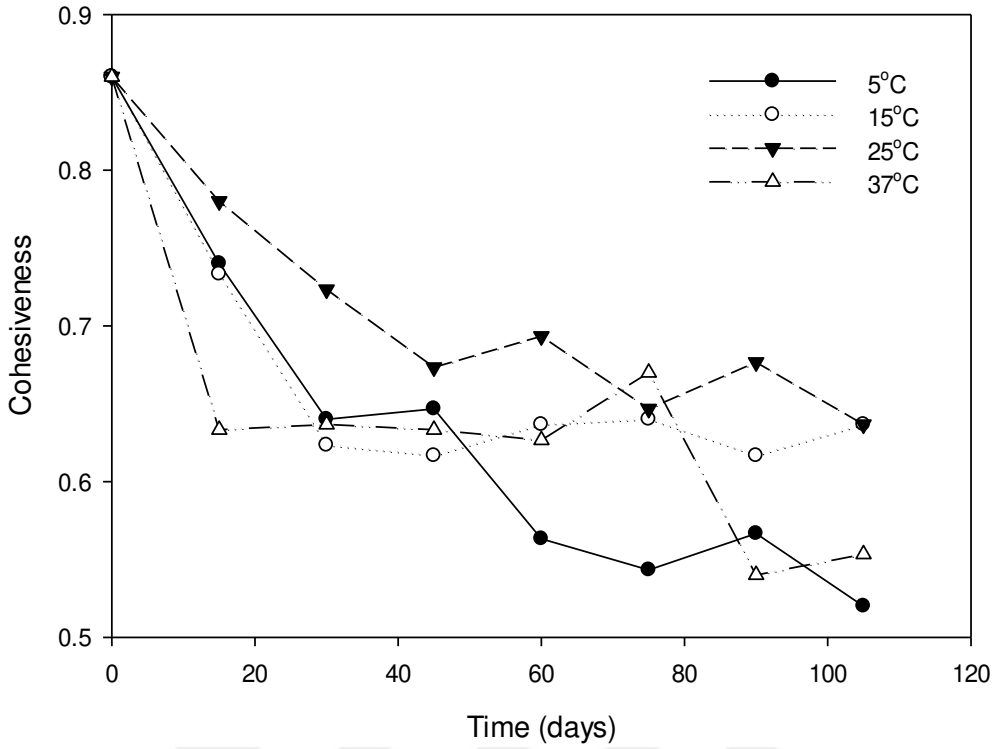

Figure 4.10 Changes of cohesiveness values of kashar cheese during 105 days kept at four different temperatures.

4.11 Gumminess

Gumminess is the measurement of energy required for decomposing a semi solid food products for easily swallowing. Calculation of the gumminess is done by multiplying hardness and cohesiveness value. The results of statistical analysis were applied to find how the temperature and storage time are effecting of gumminess of the kashar cheese. The results are shown Figure 4.11. The ANOVA results shows that increasing the storage time with temperatures decreased ($P<0.05$) gumminess (Table 4.11). The gumminess of kashar cheese kept at 5°C for 0, 15, 30 and 45 days was slightly different comparing with other storage time and stored temperatures. Increasing storage time and temperature had significant effect on the gumminess of kashar cheese. It means that increasing shelf life of kashar cheese and stored temperature the gumminess of kashar cheese will be softer than those kept at 5°C for 0, 15, 30 and 45 days. The results found in this study were similar to those obtained for the hardness of the kashar cheese Kahyaoglu et al. (2005), they reported that increasing temperature significantly affect gumminess value. This results of this

study disagreed with that report Kahyaoglu et al. (2005), Increasing storage time and stored temperature caused decrease the gumminess.

Table 4. 11 Effect of storage time and temperature in Gumminess value

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	5467±409dA	5467±409eA	5467±409fA	5467±409fA
15	4371±507cA	4450±179dA	4175±150eA	4363±238eA
30	3580±511bA	3222±194cA	3222±194dA	3549±318dA
45	3501±263bA	2905±117bcA	2106±161cA	2448±359cA
60	2339±299aA	2343±220aA	1806±264bcA	2278±701bcA
75	2567±197aA	2542±448abA	1185.50±126aA	1422±179aA
90	2597±105aA	2530±177abA	1353±231abA	1500±823abA
105	2375±486aA	2475±315abA	1547±374abA	1342±277aA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

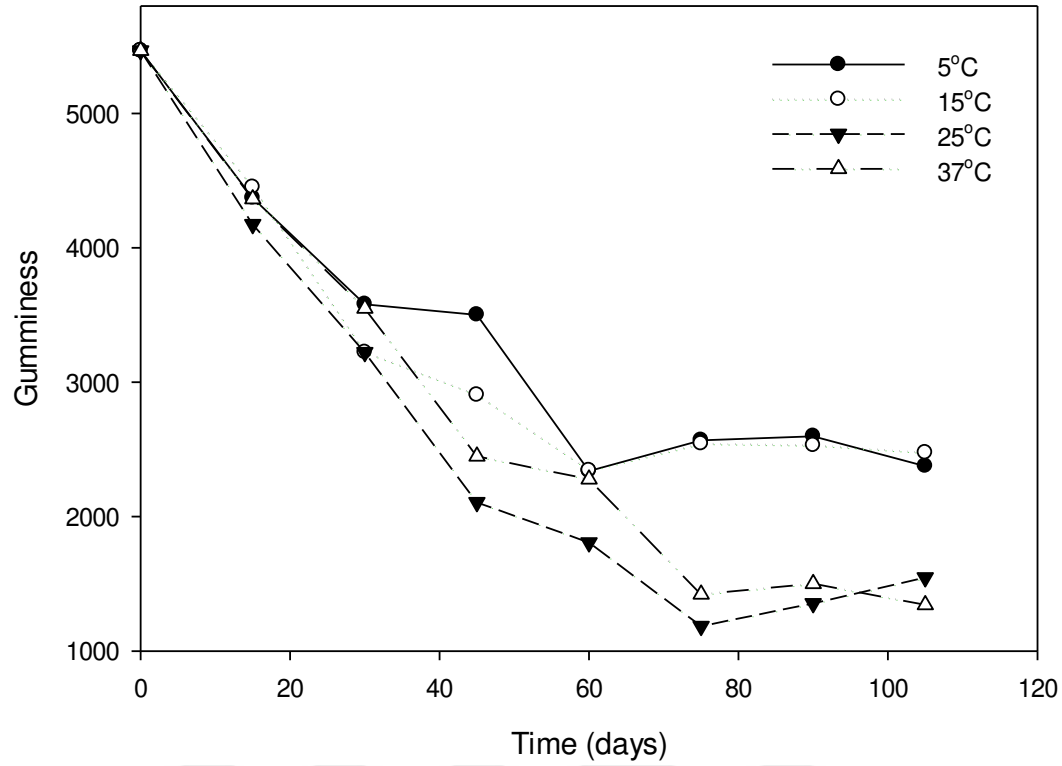

Figure 4.11 Changes of Gumminess value of kashar cheese during 105 days kept at four different temperatures.

4.12 Adhesiveness value

Adhesiveness is the combination of adhesive force and cohesive force (Hoseney and Smewing, 1999). The results of statistical analysis were applied how the temperature and storage time were affecting the adhesiveness of the kashar cheese. The results are shown in Figure 4.12. The ANOVA results showed increasing the storage time with temperatures ($P<0.05$) decreased the adhesiveness value (Table 4.12). The adhesiveness values of kashar cheese kept at 5°C for 0,15, 30, 45, 60 and 75 days were slightly different ($p<0.05$) compared with other storage time and stored temperatures. Increasing temperature and storage time simultaneously affect the adhesiveness of sample. (Eroglu et al., 2016), reported that increasing storage time with increasing ripening the adhesiveness of kashar cheese decrease. Results of this study disagreed this report. Increasing storage temperature with storage time the adhesiveness of kashar cheese increased. Koca and Metin (2004) observed that as the adhesiveness of kashar cheese decreased the increasing the ripening time. Bryat

et al. (1995), reported that fat and moisture content of sample caused to decrease the adhesion value decrease. Akalın (2002), found that adhesiveness decreased depends on the chemical composition of the cheese sample.

Table 4.12 Effect of storage time and temperature an Adhesiveness value

Time (day)	**Temperature**			
	5°C	**15°C**	**25°C**	**37°C**
0	-181.32±6.20aA	-181.32±6.20aA	-181.32±6.20aA	-181.32±6.20a
15	-154.13±0.79bA	-175.11±1.78bA	-170.70±4.18bA	163.12±1.79b
30	-152.11±1.71bA	-154.11±3.05cA	-154.46±2.35cA	-165.36±0.70b
45	-138.47±1.32cA	-122.26±3.81dA	-145.20±3.08dA	-146.12±2.32c
60	-136.93±0.00cA	-121.39±0.48dA	140.39±0.40deA	-140.97±1.95c
75	-121.43±1.15dA	-102.61±3.68eA	-135.57±5.14eA	-126.74±0.44d
90	-79.26±0.99eA	-87.86±1.05fA	-99.98±0.44fA	-102.74±2.06eA
105	-74.97±0.87fA	-76.28±1.60gA	-86.69±0.85gA	-76.11±4.54fA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

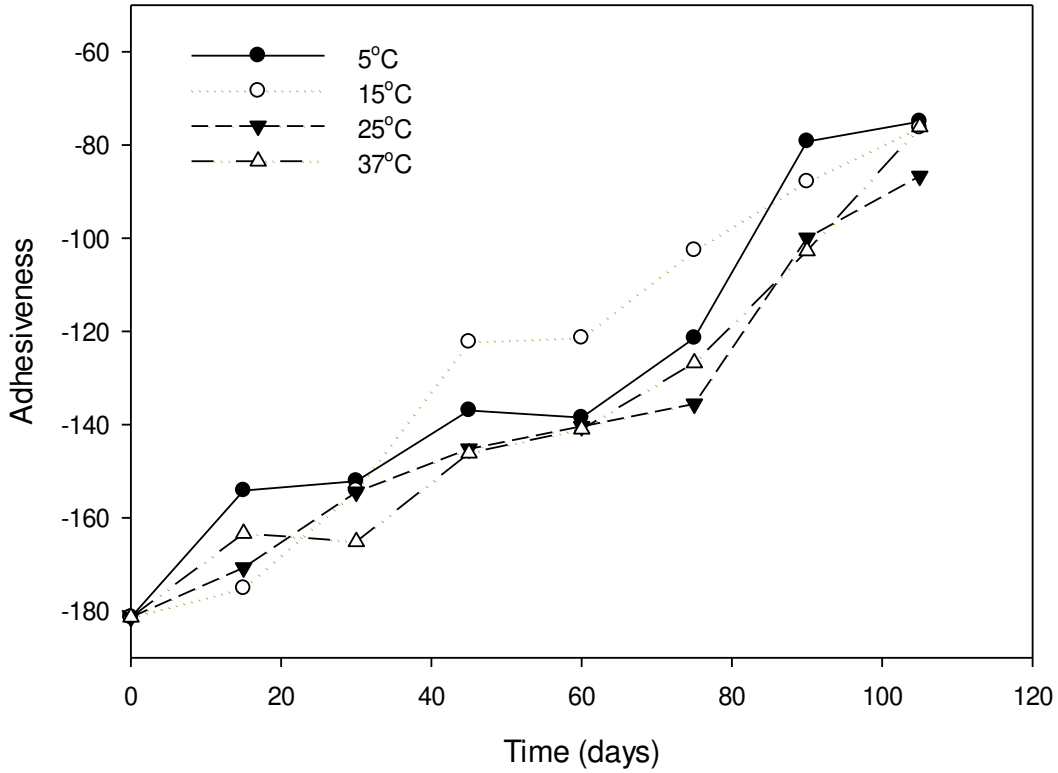

Figure 4.12 Changes of Adhesiveness during 105 days were kept four different temperatures.

4.13 Chewiness value

Chewiness values of kashar cheese were analyzed during 105 days kept at four different temperatures (5°C, 15°C, 25°C and 37°C). The results of statistical analysis were applied to how the temperature and storage time were effecting the chewiness of the kashar cheese. The results are showed in Figure 4.13. The ANOVA results shows that increasing the storage time with temperatures ($P<0.05$). increased chewiness (Table 4.13). The chewiness values of kashar cheese kept at 5°C for 0, 15, 30, 45, 60 and 75 days was slightly significant comparing with other storage time and stored temperatures. Increasing storage period all temperatures affect the chewiness of kashar cheese sample. Increasing storage time and stored temperatures the chewiness of sample increased. This had study has similar results with those found by Koca and Metin (2004), and Akalın (2002).

Table 4.13 Effect of storage time and temperature on Chewiness value

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	5408.86±463.51aA	5408.86±463.51a	5408.86±463.5a	5408.86±463.51a
15	5795.44±120.1bA	5885.01±168.7bA	5670.47±0.00bA	5715.47±128.77b
30	5644.19±49.03bBA	5870.93±33.34bA	5790.64±10.33bcA	5701.14±52.77abA
45	5588.15±0.00abA	5961.18±12.79bcA	5796.61±0.00bcA	5771.03±0.00bcA
60	5588.15±0.00abA	5953.80±0.00bcA	5857.50±0.00bcA	6063.41±0.00Ca
75	5644.19±0.00abA	6441.76d±0.00dA	6209.34±0.00dA	6076.56±0.00da
90	6255.05±0.00cA	6441.76±0.00dA	6209.34±0.00dA	6411.40±0.00dA
105	6455.05±0.00cA	6219.61±0.00cdA	6072.78±0.00cdA	5899.97±0.00bcA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

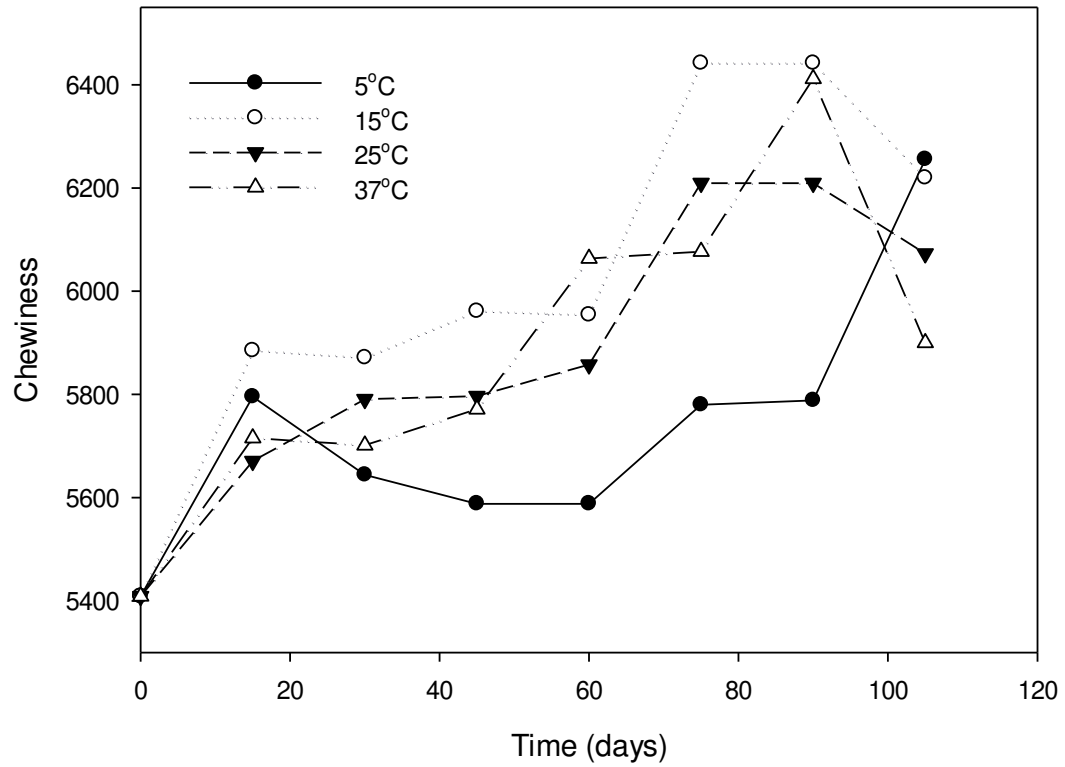

Figure 4.13 Changes of chewiness during 105 days were kept four different temperatures.

4.14 Biogenic Amines in Kashar Cheese

Changes of histamine (HI) in kashar cheese were analyzed 105 days kept four different temperatures (5°C, 15°C, 25°C and 37°C). The results of statistical analysis were applied to how the temperature and storage time were effecting the forming of histamine in the kashar cheese. The results are shown in Figure 4.14. The ANOVA results showed that increasing the storage time and temperature increased ($P<0.05$) histamine concentration (Table 4.14). During storage time histamine concentration value of kashar cheese were kept at 5°C was not changed significantly ($P<0.05$), compared to other storage time and temperatures. HI concentration value of kashar cheese were kept at 15°C, 25°C and 37°C during fresh stage (0, 15, 30 and 45) days were slightly significantly ($P<0.05$). During ripening period (60, 75, 90 and 105) days of kashar cheese were kept at 15°C, 25°C and 37°C the concentration of histamine significantly ($P<0.05$) increased. This study is lower than those found by Ercan et al., (2019), also similar to those found by Innocente and D'Agostin (2002).

Table 4.14 Changes in Histamine concentration (mg/kg)

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	31.68±1.48aA	31.68±0.00aA	31.68±2.8aA	31.68±0.00aA
15	33.74±0.6abA	34.30±0.00bA	35.39±0.5abA	35.40±0.53bA
30	35.34±1.5abA	34.89±0.00abA	35.82±0.2abA	36.81±0.00bcdA
45	33.25±0.6abA	34.99±0.00bcA	35.42±0.3Ba	36.81±1.3cdA
60	35.31±2.2abA	34.38±0.2bcA	36.57±0.4abA	37.86±0.1dA
75	34.31±0.47abA	37.22±2.1bcA	36.27±0.7abA	36.23±0.00bcA
90	34.66±0.00abA	35.23±0.00cdA	36.50±0.6abA	36.69±0.00cA
105	35.77±0.00bA	36.27±0.00dA	36.81±0.00abA	36.28±0.00bcA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

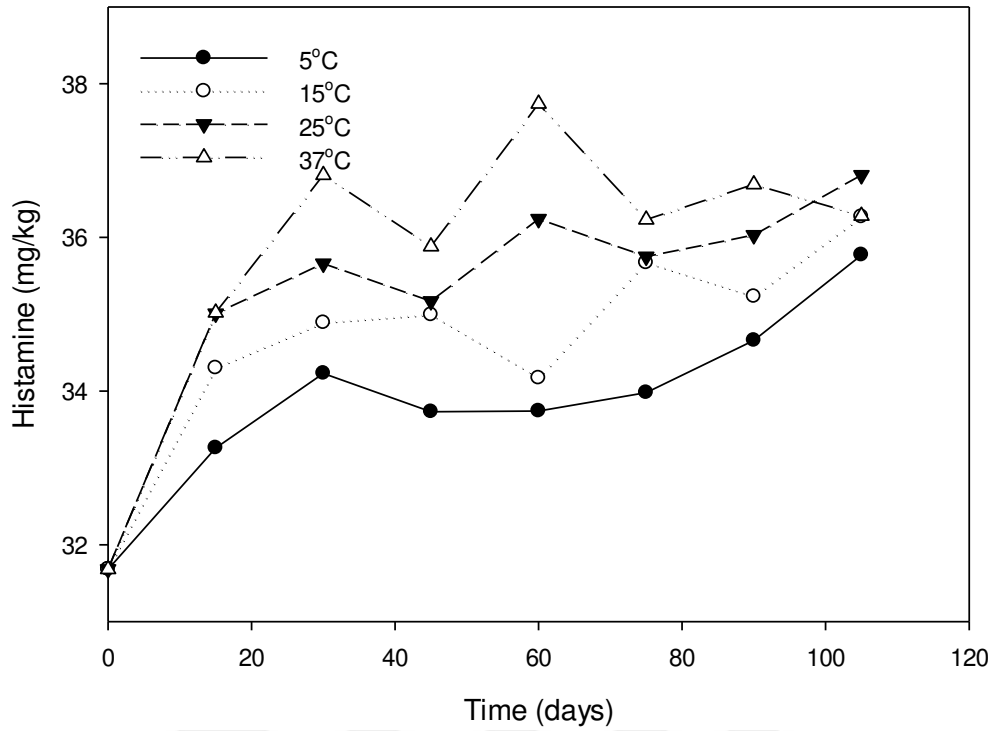

Figure 4.14 Changes of Histamine concentration during 105 days were kept four different temperatures.

4.15 Changes in Spermidine Concentration

Changes of Spermidine in kashar cheese were analyzed 105 days kept four different temperatures. The results of statistical analysis were applied to how the temperature and storage time were effecting the forming of Spermidine in the kashar cheese. The results are shown in Figure 4.15. The ANOVA results showed that increasing the storage time and temperature increased ($P<0.05$) Spermidine concentration (Table 4.15). During storage time Spermidine concentration value of kashar cheese were kept at 5°C and 15°C were not changed significant ($P<0.05$). Spermidine concentration value of kashar cheese were kept at 25°C and 37°C during storage time were significantly ($P<0.05$) increased. This study confirmed those found by (Ercan et al., 2019).

Table 4.15 Change in Spermidine concentration (mg/kg)

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	7.71±0.21bA	7.87±0.01aA	7.87±0.02aA	7.90±0.06aA
15	7.41±0.09aA	8.38±0.10bcA	9.58±0.12bA	13.17±0.07bA
30	8.47±0.09cA	8.60±0.04cA	9.17±0.24bA	27.20±0.07cA
45	8.51±0.02cA	8.61±0.04cA	11.14±0.12cA	29.92±0.07dA
60	9.96±0.02eAB	8.19±0.01abA	12.63±0.05cA	30.20±0.01eA
75	9.40±0.07dAB	8.72±0.04cA	11.92±0.08cA	33.93±0.01gA
90	10.50±0.03fA	9.52±0.33dA	15.94±0.05dA	31.77±0.14fA
105	10.48±0.01fA	11.18±0.22eA	16.36±0.43d	31.66±0.02fA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

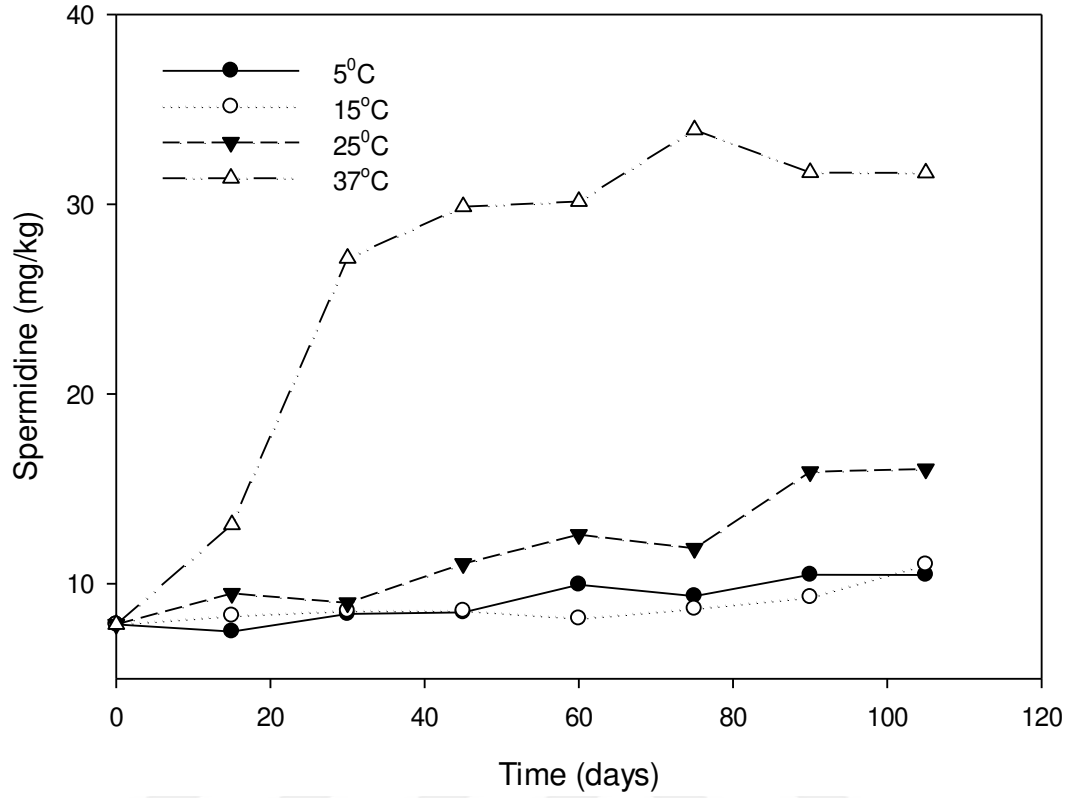

Figure 4.15 Changes of Spermidine concentration during 105 days were kept four different temperatures.

4.16 Changes in Tyramine Concentration

Changes of Tyramine in kashar cheese were analyzed 105 days kept four different temperatures (5°C, 15°C, 25°C and 37°C). The results of statistical analysis were applied to how the temperature and storage time were effecting the forming of Tyramine in the kashar cheese. The results are shown in Figure 4.16. The ANOVA results showed that increasing the storage time and temperature increased ($P<0.05$) Tyramine concentration (Table 4.16). During storage time TYR concentration value of kashar cheese were kept at 5°C was not changed significantly ($P<0.05$), compared to other storage time and temperatures. Tyramine concentration value of kashar cheese were kept at 15°C, 25°C and 37°C during fresh stage (0, 15, 30 and 45) days were slightly changed significantly ($P<0.05$). During ripening period (60, 75, 90 and 105) days of kashar cheese were kept at 15°C, 25°C and 37°C the concentration of Tyramine significantly ($P<0.05$) increased. This study is lower than those found by

Ercan et al., (2019) and Andic et al., (2010) also similar to those found by Innocente and D'Agostin, (2002).

Table 4.16 Change in Tyramine concentration (mg/kg)

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	19.49±0.7aA	19.49±0.7aA	19.49±2.6aA	19.49±2.8aA
15	21.28±0.7aA	19.44±0.7aA	20.13±1.3aA	20.62±0.8aA
30	19.63±0.7aA	19.36±1.3aA	19.52±1.52aA	20.80±4.7bA
45	20.18±0.7aA	20.15±0.4abA	20.01±0.4aA	20.36±3.3abA
60	20.53±0.4aA	20.79±0.8abA	19.46±1.4aA	20.47±2.6abA
75	19.15±1.4aA	21.22±0.5bA	24.25±2.5bA	26.05±2.1bA
90	19.80±1.4aA	19.24±2.0bA	26.49±1.1bA	27.32±1.8bA
105	19.97±0.6aA	21.19±3.9bA	27.79±1.42bA	28.80±1.9bA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

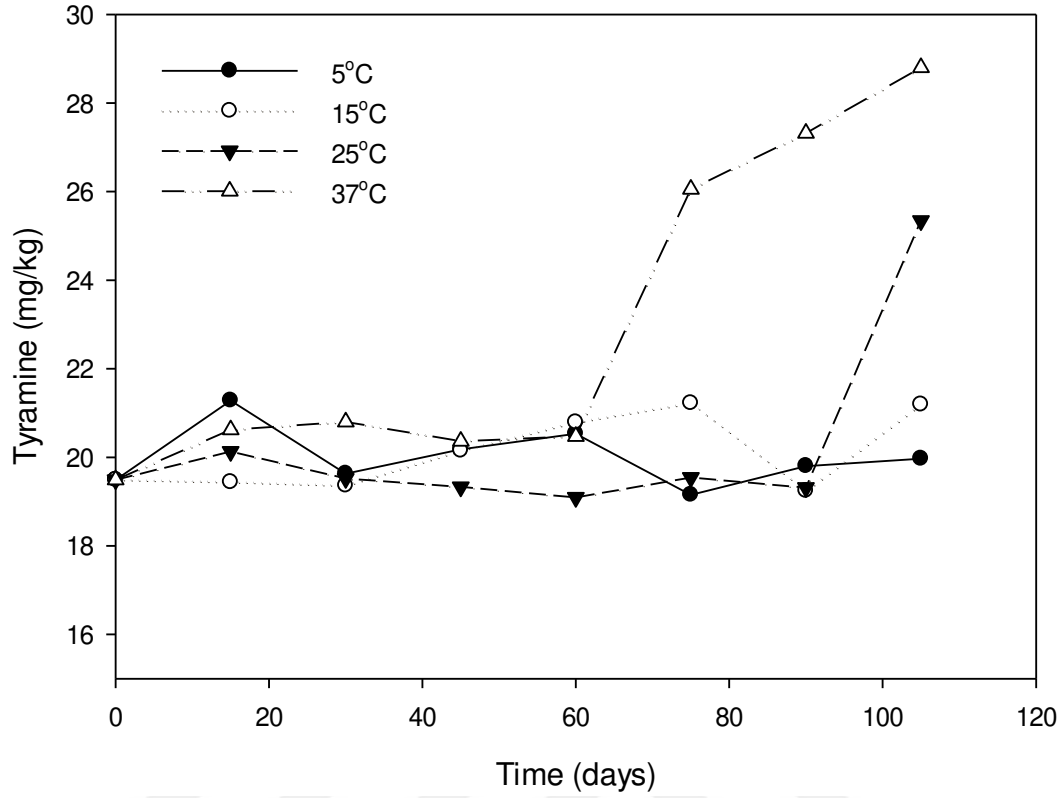

Figure 4.16 Changes of Tyramine concentration during 105 days were kept four different temperatures.

4.17 Change in Tryptamine Concentration

Changes of Tryptamine in kashar cheese were analyzed 105 days kept four different temperatures (5°C, 15°C, 25°C and 37°C). The results of statistical analysis were applied to how the temperature and storage time were effecting the forming of Tryptamine in the kashar cheese. The results are shown in Figure 4.17. The ANOVA results showed that increasing the storage time and temperature increased ($P<0.05$) Tryptamine concentration (Table 4.17). During storage time Tryptamine concentration value of kashar cheese were kept at 5°C was not changed significantly ($P<0.05$), compared to other storage time and temperatures. TRY concentration value of kashar cheese were kept at 15°C, 25°C and 37°C during storage time was significantly ($P<0.05$) increased. This study is lower than those found by Ercan et al., (2019), also similar to those found by Innocente and D'Agostin, (2002).

Table 4.17 Change in Tryptamine concentration (mg/kg)

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	33.44±4.7aA	37.88±11.0aA	32.94±4.01aA	137.88±11.0aA
15	38.82±9.8aA	38.90±11.1aA	38.94±9.4aA	141.14±6.5aA
30	37.59±4.5aA	39.92±6.68abA	58.25±9.2aA	180.73±2.9bA
45	32.73±5.4aA	51.20±6.67abcA	103.89±16.8bA	113.08±17.9aA
60	37.40±2.2aA	51.32±6.3abcA	126.30±6.1cA	237.32±27.5cA
75	38.36±16.2aA	57.14±5.3cdA	134.04±16.4cA	189.58±0.28bA
90	61.56±4.5bA	59.47±1.54cdA	170.96±11.3dA	256.88±15.6cdA
105	67.93±9.6bA	71.41±3.87dA	182.06±11.03dA	285.48±9.31dA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

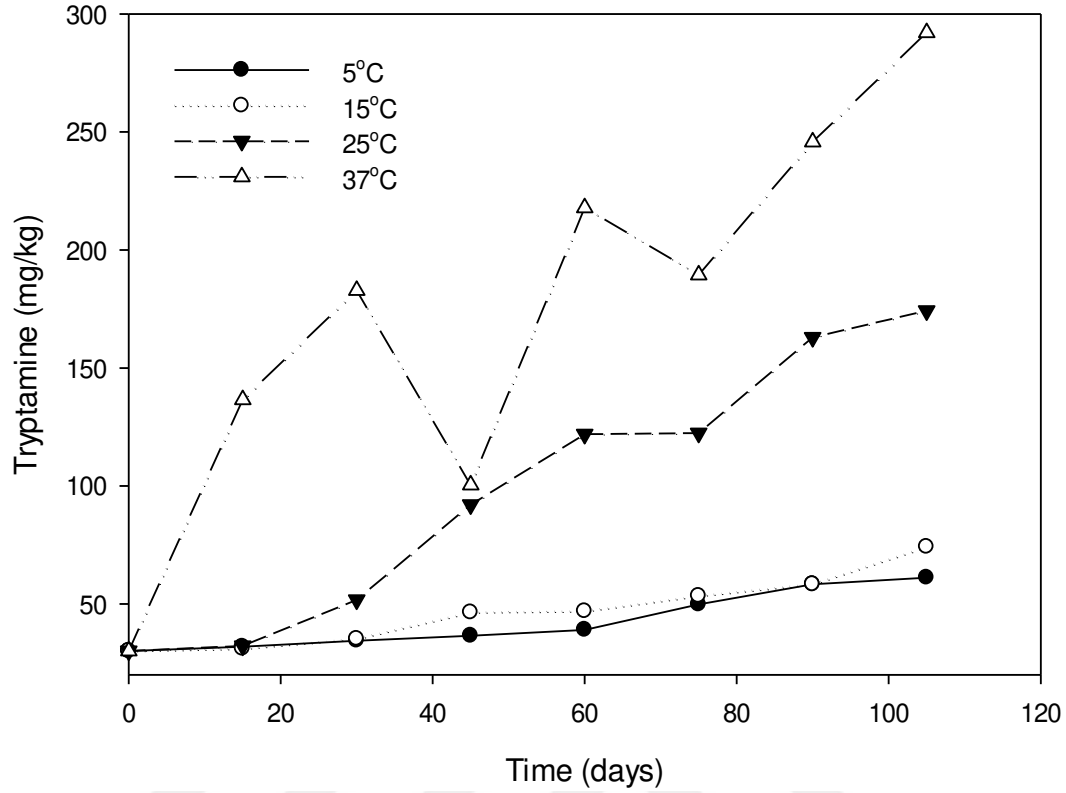

Figure 4. 17 Changes of Tryptamine concentration during 105 days were kept four different temperatures.

4.18 Change in Cadaverine Concentration

Changes of Cadaverine in kashar cheese were analyzed 105 days kept four different temperatures (5°C, 15°C, 25°C and 37°C). The results of statistical analysis were applied to how the temperature and storage time were effecting the forming of Cadaverine in the kashar cheese. The results are shown in Figure 4.18. The ANOVA results showed that increasing the storage time and temperature increased ($P<0.05$) Cadaverine concentration (Table 4.18). During storage time CAD concentration value of kashar cheese were kept at 5°C was not changed significantly ($P<0.05$), compared to other storage time and temperatures. Cadaverine concentration value of kashar cheese were kept at 15°C, 25°C and 37°C during storage time was significantly ($P<0.05$) increased. This study is lower than those found by Ercan et al., (2019), also similar to those found by Innocente and D'Agostin, (2002).

Table 4.18 Change in Cadaverine concentration (mg/kg)

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	22.09±0.35aA	22.09±0.35aA	22.09±0.35abA	22.09±0.35aA
15	21.92±0.05aA	22.54±0.00abA	21.93±0.00aA	22.65±0.45bA
30	22.75±0.31aA	22.96±0.00bA	22.46±0.00bA	24.52±0.21cA
45	22.48±0.54aA	22.95±0.00bA	23.76±0.00cA	25.60±0.00dA
60	22.30±0.00aA	23.64±0.±0.00cA	24.24±0.00dA	25.52±0.00dA
75	22.02±0.00aA	24.45±0.00dA	24.52±0.21dA	25.71±0.00dA
90	21.90±0.14aA	24.74±0.00dA	27.08±0.00eA	28.82±0.00eA
105	22.69±0.14aA	24.27±0.00dA	28.12±0.00fA	27.67±0.00fA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

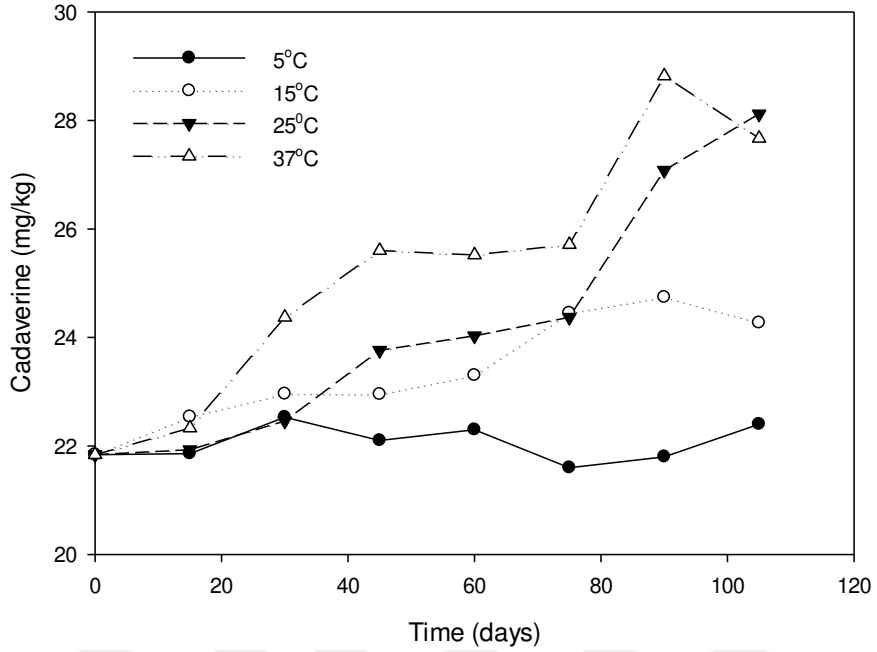

Figure 4.18 Changes of Cadaverine concentration during 105 days were kept four different temperatures.

4.19 Change in Spermine concentration

Changes of Spermine in kashar cheese were analyzed 105 days kept four different temperatures (5°C, 15°C, 25°C and 37°C). The results of statistical analysis were applied to how the temperature and storage time were effecting the forming of Spermine in the kashar cheese. The results are shown in Figures 4.19. The ANOVA results showed that increasing the storage time and temperature increased ($P<0.05$) Spermine concentration (Table 4.19). During storage time Spermine concentration value of kashar cheese were kept at 5°C was not changed significantly ($P<0.05$), compared to other storage time and temperatures. Spermine concentration value of kashar cheese were kept at 15°C, 25°C and 37°C during storage time was significantly ($P<0.05$) increased. This study is lower than those found by Ercan et al., (2019), also similar to those found by Innocente and D'Agostin, (2002).

Table 4.19 Change in Spermine concentration (mg/kg)

Time (day)	Temperature			
	5°C	**15°C**	**25°C**	**37°C**
0	21.79±0.14a,bA	21.79±0.14aA	21.79±0.14aA	21.74±0.07aA
15	21.59±0.11aA	22.83±0.21bA	39.30±0.35bA	45.93±0.07bA
30	21.86±0.07bA	21.51±0.49aA	57.76±0.14cA	47.66±0.07cA
45	21.68±0.07abA	27.38±0.28cA	78.28±0.21dA	82.50±0.14dA
60	27.71±0.07Ca	30.60±0.21dA	95.92±0.07eA	91.88±0.14eA
75	31.86±0.12fA	43.24±0.07eA	141.52±0.21fA	135.82±0.14fA
90	31.47±0.07eA	48.68±0.07fA	142.48±0.14gA	147.66±0.35gA
105	30.78±0.07dA	47.54±0.07gA	149.89±0.07hA	139.87±0.07hA

Different small letter indicates statistical difference at α=0.05 level in each column. Different capital letters indicate statistical difference at α=0.05 level among products in each temperature.

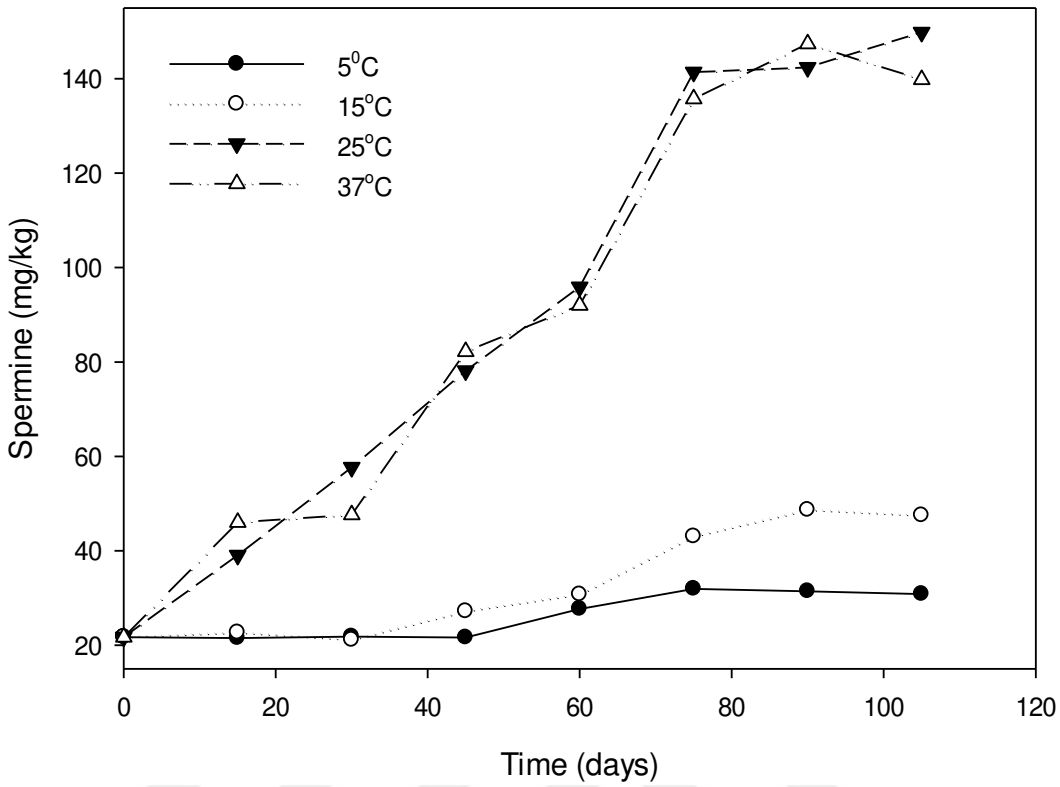

Figure 4.19 Changes of Spermine concentration during 105 days were kept four different temperatures.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study was focused on the effect of the storage time and stored temperature on growth of different microorganisms (LAB, yeast and mould, TAMB), moisture content, pH, and texture of kashar cheese.

In this study the effect of storage time and stored temperature on some properties of kashar cheese were investigated. The entire sample kept at four different temperatures (5°C, 15°C, 25°C, 37°C) up to 105 days.

These following results were obtained.

- In the fresh stage 0, 15, 30 and 45 day of storage); lactic acid bacteria (LAB), total aerobic bactetria (TAMB) and mold and yeast kept at 5°C were not significantly ($P>0.05$) different

- Increasing the storage time and stored temperature significantly ($P <0.05$) effect the growth of Lactic Acid Bacteria (LAB), Total Aerobic Mesophilic Bacteria (TAMB), mold and yeast, moisture content and the pH.
- Increasing the storage period and stored temperature caused to decrease of mold and yeast count.
- During fresh stage kept at 5°C, the moisture contents of kashar cheese were not significantly different ($P>0.05$).

- The pH value of fresh stage kept at 5°C were not significantly ($P>0.05$) different the pH value of sample.
- Increasing storage period and temperature caused to decreas the pH of kashar cheese.

- In the fresh stage (0, 15, 30 and 45 days), hardness, springiness, adhesiveness, gumminess and chewiness kept at 5°C weren't significantly different ($P>0.05$)
- Increasing storage time and temperature caused to decrease the hardness, springiness and gumminess value while adhesiveness and chewiness value were increased.
- During fresh stage of kashar cheese were kept at 5°C and 15°C weren't significantly ($P<0.05$) increasing the concentration of biogenic amines; Histamine, Spermine, Spermidine, Cadaverine and Tyramine, Tryptamine.
- These study observed that increasing storage time and temperature Spermine and Tryptamine concentration increased and research the poisoning point.

5.2 Recommendations

- ❖ Microbiological status of kashar cheese increased during ripening period therefore ripening period have to be controlled.
- ❖ During fresh stage of kashar cheese, the microbial growth can be controlled by salt preservation.
- ❖ Moisture content of kashar cheese increased during ripening period therefore ripening period have to be controlled.
- ❖ pH of kashar cheese decreased during ripening period therefore decreasing pH was good prevention to microbial growth except Lactic acid bacteria at that pH.
- ❖ Ingestion of biogenic amines may result in the food poisoning.
- ❖ Increasing storage time and temperature the Spermine and Tryptamine concentrations increasing speedily, at that point should be aware.

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