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M.Sc. in Food Engineering

UĞUR UĞURLU

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INVESTIGATION OF PHYSICAL AND BIOCHEMICAL CHANGES OF KASHAR CHEESE DURING THE STORAGE PERIOD

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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.

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ABSTRACT

INVESTIGATION OF PHYSICAL AND BIOCHEMICAL CHANGES OF KASHAR CHEESE DURING THE STORAGE PERIOD

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The aim of this study is to investigate the physical and biochemical changes that occur during the storage period of fresh kashar cheese at four different storage temperatures. Kashar cheese samples were stored for 105 days at 5°C, 15°C, 25°C and 37°C after vacuum packaging. During the storage period, effect of storage time and temperature on moisture, pH, protein, water soluble nitrogen, trichloroacetic acid (TCA) soluble nitrogen contents, lipid oxidation and color values of kashar cheese were evaluated. Storage time and temperature significantly changed the moisture content of kashar cheese and also the interaction between temperature and time were significantly affected the moisture content ($P<0.05$). Storage time and temperature significantly affected the pH of kashar cheese ($P<0.05$). Increasing the storage time and temperature increased the protein, water soluble nitrogen and TCA soluble nitrogen contents of kashar cheese samples ($P<0.05$). The storage time significantly changed the amount of malondialdeyde (MDA) values of kashar cheese ($P<0.05$) whereas temperature did not effect ($P>0.05$). Storage at low temperatures had a positive effect on color values, but high temperatures caused deterioration in shape and color. Considering all the results, kashar cheese is physically and biochemically affected by the temperature and time during the storage process.

Key Words: Kashar Cheese, Storage, Physical Change, Biochemical Change, Lipid Oxidation

ÖZET

KAŞAR PEYNİRİNİN DEPOLAMA SÜRECİNDE FİZİKSEL VE BİYOKİMYASAL DEĞİŞİMLERİN İNCELENMESİ

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Bu çalışmanın amacı, taze kaşar peynirinin dört farklı depolama sıcaklığında depolama boyunca meydana gelen fiziksel ve biyokimyasal değişimleri araştırmaktır. Kaşar peyniri örnekleri vakumlu paketlemeden sonra 5°C, 15°C, 25°C ve 37°C'de 105 gün süreyle depolanmıştır. Depolama döneminde, saklama süresi ve sıcaklığın kaşar peynirinin nem, pH, protein, suda çözünür azot, trikloroasetik asit (TCA) çözünür azot, yağ oksidasyonu ve renk değerlerine etkisi değerlendirilmiştir. Depolama süresi ve sıcaklığı kaşar peynirinin nem içeriğini önemli ölçüde değiştirmiş ve sıcaklık ile süre arasındaki etkileşim de nem içeriğini önemli ölçüde etkilemiştir ($P<0.05$). Depolama süresi ve sıcaklığı kaşar peynirinin pH'sını önemli ölçüde etkilemiştir ($P<0.05$). Depolama süresi ve sıcaklığının artması kaşar peyniri örneklerinin protein, suda çözünür azot ve TCA çözünür azot içeriğini artırmıştır ($P<0.05$). Depolama süresi, kaşar peynirinin malondialdehide (MDA) miktarını önemli ölçüde değiştirirken ($P<0.05$), sıcaklıktan etkilenmemiştir ($P>0.05$). Düşük sıcaklıklarda depolamanın renk değerleri üzerinde olumlu etkisi olurken, yüksek sıcaklıklar şekil ve renkte bozulmalara neden olmuştur. Tüm sonuçlar göz önüne alındığında, kaşar peyniri depolama sürecinde depolama sıcaklığı ve süresinden fiziksel ve biyokimyasal olarak etkilenmektedir.

Anahtar Kelimeler: Kaşar Peyniri, Depolama, Fiziksel Değişim, Biyokimyasal Değişim, Yağ Oksidasyonu

"Dedicated to my family"

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LIST OF SYMBOLS

α	Alfa
β	Beta
L*	Lightness Coordinate
a*	Red/Green Coordinate
b*	Yellow/Blue Coordinate

LIST OF ABBREVIATIONS

$AgNO_3$	Silver Nitrate
ANOVA	Analysis of Variance
AFB1	Aflatoxin B1
$CuSO_4$	Copper Sulfate
HCl	Hydrochloric Acid
H_2SO_4	Perchloric Acid
K_2SO_4	Potassium Sulfate
LAB	Lactic Acid Bacteria
MC	Moisture Content
MDA	Malondialdeyde
NaCl	Sodium Chlorür
NSLAB	Non Starter Lactic Acid Bacteria
SPSS	Statistical Package for the Social Sciences
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
TS	Standard of Turkish
WSN	Water Soluble Nitrogen
µl	Mikro Liter

CHAPTER I

INTRODUCTION

The milk and the dairy products have great significance in order to get healthy and balanced diet. The milk and dairy products are the matters that have almost all of the food compounds and beautiful smell and taste to continue of life [1]. There are different sources of milk and they have different properties of sensation such as smell and taste [2]. At the same time the taste sensation of people may change according to culture, country, climate changes and a lot of diversity. Because of this, there are very different kinds of cheeses on the world [1].

There are approximately over than 4000 variety of dairy products on the world and therefore, cheeses take the first rank among all dairy products [3]. In Turkey, the White cheese is widely consumed and takes nearly 60-80 % of the production of total cheeses. Kashar cheese is the second most consumed cheese after the White cheese [4]. According to Turkish Statistical Institue, in 2018, total cheese production of Turkey was 753230 tonnes, and semi-hard cheese production was 241613 tones for the production of kashar cheese [5].

The kashar cheese is the very important fermented dairy product which has been consumed on the world because of its very good taste, flavor and sight [6]. The kashar cheese is a food that has middle hardness, smooth surface, White-yellow color and no pore or have one or two pores, and there is normal flexible structure, lightly salty Turkish traditional cheese [7]. The kashar cheese is very common in all over the world and in especially Balkan countries with in this case, it has similarities with other type of cheeses, that are known kashkaval in Bulgaria, kasseri in Greek, kackavalj in Yugoslavia, caşcaval in Romania, kaşkaval in Hungary, rumi in Egypt and kashar (kaşar) cheese in Turkey [3].

According to Turkish standards, the kashar cheese is divided into two groups, one of them is the fresh kashar cheese, that is the end product of the process and the other is

old or mature kashar cheese which is necessary to be ripened in particular time [8]. According to the standards of the TS 3272 kashar cheese is a semi-hard food, it is made from cow, sheep, goat milk or their mixtures with or without the addition of starter culture after the pasteurization [6]. The raw materials play very critical role and they cause changes in biochemical, compositional, and textural quality all of cheeses, and also in the production of cheese the milk sources and variety of milk are the most effective on quality parameters because of the physical and bio-chemical factors, for instance the temperature, pH, and microflora and dimension of cheese [9]. The kashar cheese firstly is the fresh and after, in order to be old kashar, it should gain its characteristic flavor and taste for this, the time is needed at least 3 month and for more ripening it can be 3, 6 or 12 month in particular conditions [6].

In the ripening period of kashar cheese, there may be some significant spoilage problems, all of this may be effected as negative to criterias of quality of kashar cheese in the maturation process. The amino acids are very important actors in improvement of flavor, because the units structure of protein is the supplier of carbon, nitrogen and energy sources for microbial cells. Therefore, this cells can produce unsuitable flavour because the free amino acid concentrations can be raised in the unavailable physical conditions such as pH and temperature [8].

During the ripening process, the all surface of kashar cheese is plated with thin layer of mould which is reported as dangerous for health and not good the advacement of kashar cheese. Therefore the hygenic conditions is important in order to prevent the physical and biochemical problems and the growth of undesired microorganisms in development of kashar cheese. Some microbial deteriorations can cause the proteolytic and lipolytic effects of some microorganisms. The hydrolytic and enzymatic hydrolyzing of lipids release to fatty acids such as caprylic acid and especially butyric acid, and also some of the *Pseudomonas* species have heat-resistant lipase enzymes producing free fatty acid causing rancidity and unwanted flavour [10,11].

There are a lot of factors that affect on the ripening process of kashar cheese. One of this is the calf rennet which is responsible for the formation of charecteristic taste and aroma in the natural cheeses. In the world, there is important increase in the production and consumption of cheeses, due to this case, the use of calf rennet is

decreased because of the cutting of calves in some countries and lack of number of calves due to this events, different coagulation enzymes is producted. These rennets may have to high proteolitic activity because of the different type of chymosin than the original calve rennet. These can cause to low yields, bitter taste and bad smell in the ripening of kashar cheese [12].

To the best of our knowledge, there are a lot of researches studying of the kashar cheese properties, these studies related to the period of ripening and storage of fresh and mature kashar cheese. However, the aim of this study to investigate the physical and biochemical changes of kashar cheese that have different temperatures (5°C, 15°C, 25°C and 37°C) and 105 day during storage period.

CHAPTER II

LITERATURE REVIEW

2.1. Kashar Cheese

The cheese is a milk product derived from milk which is produced by the coagulation of milk protein casein or proteolytic enzymes or harmless organic acids with or without addition material in different physical and chemical conditions [7, 13].

The kashar (in Turkish kaşar) cheese is a group of pasta-filata, semi-hard and can sliced fermented dairy product. The type of pasta-filata cheeses have elastic structure and dilatable form [14]. The representative of fresh and old kashar cheese samples is shown in Figure 2.1.

Figure 2.1 The kashar cheese samples

The kashar is a type of cheese, this is imperforate and matured with bacteria, and this type of cheeses is obtained, boiled in hot water and kneading after the pecific curd is acidified in particular level on the basis. According to standard of TS 3272/T1 kasar cheese is made from sheep, cow, and goat milk or the mixture of these in suitable process conditions. In the process, kashar pass from some steps such as pasteurization and than some additive matters can add or no to this process after the process kashar cheese can consume fresh or mature after the ripening [7].

In general the best quality kashar cheese is making from sheep's milk however the using of milk can change from some region to another section. For the ripening of

kashar cheese should be cool and humid atmospheric area because of this in some region is more available such as Kars and the south of Marmara. Usually is produced as the range of 6 or 10-12 kg weights in 27-30 cm diameter and 10-13 cm height [8,15].

2.1.1 The Properties of Kashar Cheese

The good quality kashar cheese should have to below properties [16].

i. The external aspect should be mixture of white-yellow color, smooth and the structure normal hard in middle level.
ii. The internal apperance should be similar color with outside and should not be porous and it can be one pore at least.
iii. The structure should be flexible.
iv. The amounts of fat 45-48% in dry matter.
v. The ratio of dry matter is approximately near 58-60%.
vi. The amount of salt (NaCl) proportion in interval of 3-5%.
vii. The taste very lightly salty and have good smell and taste.

2.1.2. The History of Kashar Cheese

In all of societies, there are cheeses and have big importance because of the high feed value. Therefore, there are very type of cheeses in the world due to this case, the history of cheeses can change according to society but the history of cheeses is estimated to be 4000 years and the production of cheese is going to 6000-7000 B.C. In Indian and Europe languages, the cheese word come from "kwat" (ferment-sour). In Turkish the root of cheese is derivated or come from to "panir" in Persian and "caseus" in English [6,7,17].

There are a lot of ideas about where is the center of the word of kashar. As far as we know, the kashar cheese is squeezed under the pressure in order to remove of whey in the production of kashar cheese, this event mean "coerceo" in Latin because of this, it comes from Latin origin in some sources. If the else sources, some of the people is said about kashar, this word is related with Hebrew language and this is connected with "cacher" (kaşer). In judaism that means, when the human eat this cheese, there is not problem for religion. The kashar cheese is made first time by a jewish girl and because of the similar name with cacher, it is referred as kashar. At

the present time, there are similar name of kashar cheese of countries is shown in Table 2.1 [6,7].

Table 2.1 The name of kashar cheese according to countries

Country	Name
Turkish	Kaşar
Bulgaria	Kaşkaval
Yugoslavia	Kackavalj
Romania	Caşcaval
Greek	Kasseri
Hungary	Kaşkaval
Russia	Kavkazskij syr
Egypt	Rumi

2.1.3 The Production of Kashar Cheese

The production process of kashar cheese is shown below and the flow diagram of kashar cheese is shown in Figure 2.2. [16].

1. The milk to be processed into cheese is pasteurized at 72-74°C for 15 second or 65°C for 30 minutes and then cooled to fermentation temperature (32-34°C).
2. Approximately 1% starter culture and 0.01-0.015% (10-15 g / 100 l) calcium chloride are added. The culture to be used; it may consist of bacteria *'Streptococcus thermophilus, Lactobacillus bulgaricus'* or *'Streptococcus thermophiles,Lactobacillus bulgaricus, Lactobacillus casei'* or *'Streptococcus lactis, Lactobacillus casei'*. The milk is pre-matured for 30-35 minutes and when the pH value reaches 6.40-6.45, rennet is added to the extent that the curd is cut maturity in 45 minutes.
3. The formed clot (pH 6.30-6.35) is first cut in 1.5-2.0 cm dimensions; let it rest for 5-10 minutes and then break it until it reaches a lentil-pea size (6-7 mm). It is left alone for about 5 minutes and the clot grains are allowed to collapse.

4. The cendere cloth is spread over the clot and some of the whey (approximately 30% of the milk in the boat) is removed. Or the whey is discharged through the boat's special filter.
5. The clot cheese water remaining in the boat is slowly mixed for 10 minutes. In the meantime, the temperature is gradually raised to 36-38ºC (sometimes 40-42ºC) by giving steam to the steam pipes located between the walls of the boat. After the temperature reaches the specified level, the mixing process is continued for another 15 minutes. During the heating, a temperature increase of 1ºC in 3-4 minutes and this process is applied in 30 minutes. With this process, the walls of the curd grains are hardened, the separation of the whey due to the contraction is facilitated and the acidity increase is encouraged.
6. The curd, which has given enough water and hardened, is transferred to the pressing unit by a suitable pump. Or it is pressed in the boat with its own special press. At the beginning of pressing, the pH is 5.90-6.15. The stainless steel containers to which curd is transferred have a capacity of approximately 20 kg and have a cloth inside. Since acidity develops very rapidly in hot seasons, curd can be washed with cold water before transferring to the pressing unit. Thus, the remaining lactose is removed and excessive acidification can be prevented. In pressing, initially 1 kg of weight is applied for 1 kg curd. Then this value is gradually increased to 15 kg. The temperature of the place where the printing process is done 15-20ºC, total pressing time is 1-2 hours. After printing, the pH value of curd reaches 5.25-5.30.
7. Pressed curd is cut into blocks of 25-30 cm in length and 15-20 cm in width, and covered and left for fermentation at 15-20ºC. When the pH value of curd reaches 5.00-5.05 (~ 60-65ºC), the boiling stage is started.
8. It is checked whether the curd is boiled or not. For this purpose; 'string pull', 'leaf opening' tests and 'acidity' determination are performed.
9. While the boiling and kneading processes are carried out by mechanical methods in enterprises that do not have technological conditions, today these methods have disappeared from the application in technological enterprises.
10. Special equipment developed for boiling and kneading is used in enterprises with a developed technological infrastructure. Systems that cut curd blocks

with a thickness of approximately 0.5 cm, boil them in their own cauldron and acquire plastic properties, knead and mold them and defined as 'aggregate' or 'cashmere machines' are used. The mentioned cashmere machines are of two types, direct system and indirect system.

11. Molded cheeses are rested for 12-24 hours and left to cool. In this process, 5-10 minutes after they are placed in molds, they are turned over and turned inside out. The turning process is repeated 5-6 times within 1-2 hours to make the shape smooth. In the meantime, while the cheeses are still hot, they are pierced with fog from several parts and the gases in the mass are released. swelling is carried out before the cheeses cool, as emphasized; otherwise, the fog traces will not be covered and the possibility of mold will increase.
12. The cheeses that are kept in the ripening room for about 1 day in the molds are taken out of the molds and placed on the shelves in the surface drying room. They are turned over once in the morning and once in the evening. In general, at the end of the 1st week, they are washed with a solution containing potassium sorbate and then kept for 1 day. Finally, they are packaged and transported to cold storage.

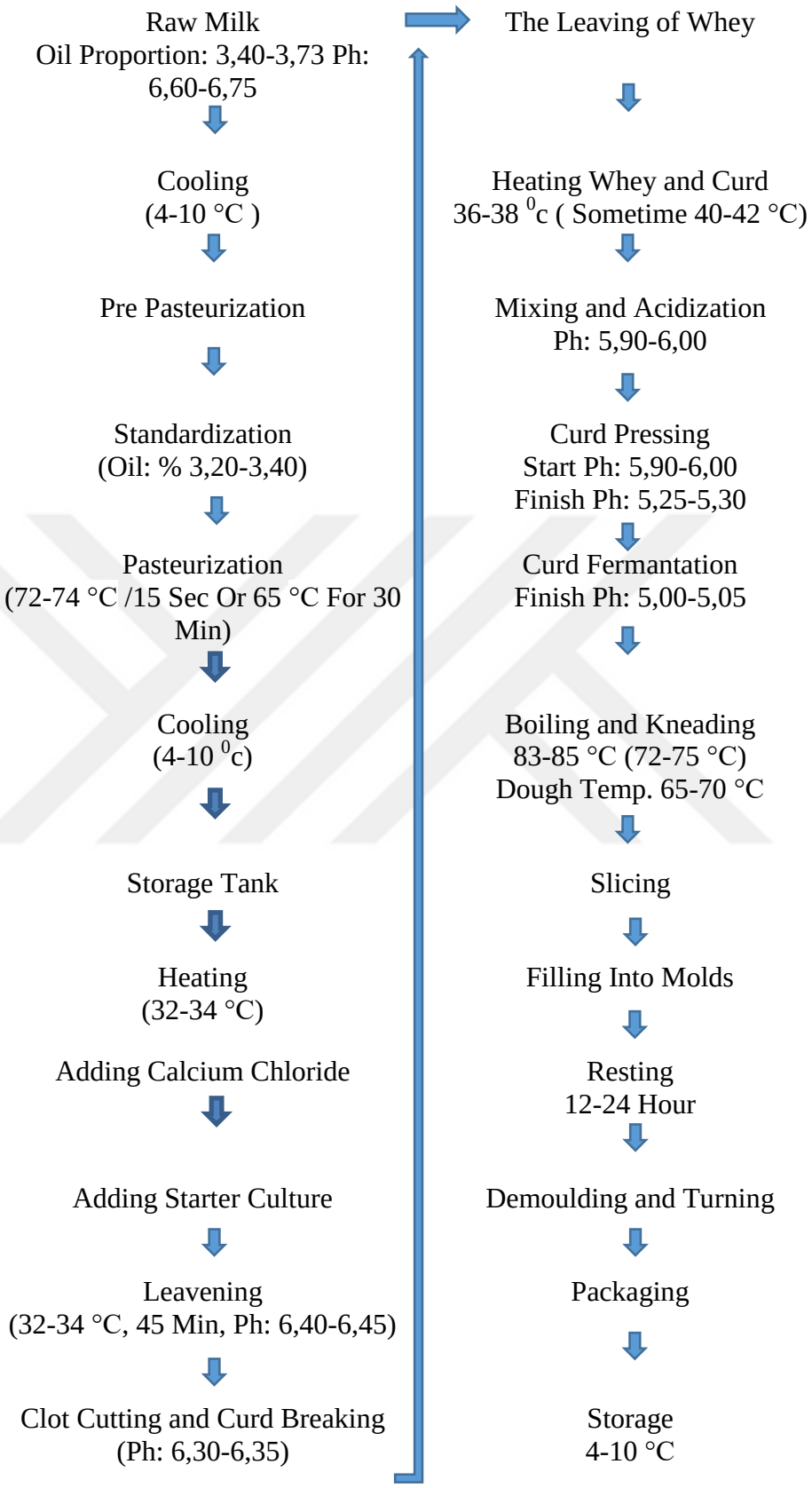

Figure 2.2 The flow diagram of fresh kashar cheese

2.2 Ripening of Cheese

The raw materials used, the applied technique and the ripening conditions play an important role in the cheese varieties to acquire their unique characteristics such as taste, smell, color, consistency, eye and crust. Ripening is the changes that occur with the effect of various enzymes and microorganisms that the cheese undergoes under certain conditions in certain periods [18,19]. Glycolysis, lipolysis and proteolysis are the basic chemical events that occur during the ripening of cheese [20].

Proteolysis that occurs during cheese ripening; decomposition of proteins and peptides into amino acids as a result of hydrolysis of peptide bonds with the enzyme. It is a very complex and necessary process for aroma, taste and texture transformation. The most important proteolytic element of the maturation process is plasmin and cathepsin D, while others are rennet and similar enzymes (such as pepsin and chymosin) remaining in curd, protease from starter bacteria, non-starter bacteria and secondary starter bacteria (such as fungal acid proteinase) and peptidases [21].

Factors affecting the activities of microorganisms and enzymes more or less affect the ripening and qualities of the cheese. During ripening, especially microorganism-derived intracellular and extracellular enzymes break down the nutrients in raw cheese by glycolysis, proteolysis and lipolysis (is the biochemical event through which triacylglycerols (TAGs) break down via hydrolysis into their constituent molecules: glycerol and free fatty acids (FFAs) by the presence of lipolytic enzymes, also known as bond-breaking hydrolases (lipase and esterase)) to form the distinctive flavor and texture of cheese with the breakdown products they create. The flavor and quality of the cheese depends on the concentrations and proportions of these reaction products. While glycolysis is completed within a few days to a few weeks after cheese production, proteolysis and lipolysis continue during maturation [22].

During the maturation phase, caseins are hydrolyzed in several stages and the taste and aroma and textural structure of the cheese occur. During the production phase, κ-casein and caseino-macropeptide are decomposed by the effect of rennet enzyme and whey is filtered [21,23-24].

Organized in a sequence, enzymes play a role in the ripening of cheese and therefore in the development of flavor. In this process, the activity of rennet (especially chymosin), milk proteinases, starter culture, secondary microorganisms and non-starter lactic acid bacteria is essential. The importance of each of these varies according to the type of cheese. Substrates for these enzymes are lactose, lipids, proteins or compounds produced from them. The sub-products that emerge as a result of the breakdown of casein in various ways and in different steps by the effect of enzymes, play a role in gaining the characteristics of the type of cheeses [25].

During the ripening process of cheeses, hundreds of volatile substances are formed by microorganism activities and chemical reactions, and cheeses have their own unique taste and smell. However, some of these volatile substances have no effect on the aroma of the cheese, some of them give the cheese its characteristic aroma. Flavoring substances in cheeses are formed by the breakdown of proteins, fats and carbohydrates by various factors (aldehydes, ketones, lactones, pyrazines, sulfur compounds, etc.) or the reaction of breakdown products with each other (such as Strecker aldehydes) [21].

Glycolysis contributes to the aroma formation of cheese by producing lactic acid and further breaking it down .Since the concentration of free fatty acids formed by lipolysis is low, it contributes little to the aroma of cheese. However, the free fatty acids can also impart soapy flavor to the cheese flavor. Peptides and free amino acids made up of proteins by proteolysis make the greatest contribution to the aroma harmony of cheese. Besides, bitterness elements caused by hydrophobic peptides negatively affect the aroma of cheese . With proteolysis, free amino acids are broken down at an advanced stage and aroma harmony is enriched [26].

Texture is the most important quality feature that determines consumer taste and quality of cheese. The more the fat rate in cheese is reduced, the more tissue defects increase. Also, the casein / fat ratio of milk is a critical value that affects cheese texture. The casein / fat ratio of milks used in the production of low- fat cheese is higher. While the increase in fat and water content weakens the protein structure, the decrease causes hardening in cheeses [21].

2.3 Factors Affecting Quality Characteristics of Kashar Cheese

2.3.1 Milk and Other Transactions

There are various factors that affect the quality of raw milk. These are the health of the animal from which the milk is obtained, the quality of animal feed, suitable barn and milking conditions, milk collection and transport to the enterprise. Hazards in raw milk can be defined as microbiological (pathogenic microorganisms), chemical (antibiotics, aflatoxin and other chemical substances) and physical (foreign substances) hazards [27].

2.3.2 Microbiological Factors

The most common problem in animals is masticitis. The most common pathogen causing masticitis is *Staphylococcus aureus* [27]. *S. aureus* is especially found in milk from animals with mastitis. The microorganism inactives by pasteurization, but the heat-resistant toxin remains in the milk. *Staphylococcal* poisoning caused by cheese is seen from time to time and studies on this subject show that *S. aureus* develops and produces toxins during the cheese making process. Low acidity resulting from the slow operation of the starter culture added to milk in cheese production prepares the environment for the development of *S. aureus* [28]**.**

Another common pathogen causing masticitis is *Escherichia coli*. Sometimes it causes food-borne infections by producing verotoxin, which is also known as *enterohemorhagic Escherichia coli* (EHEC). Some of the other pathogenic microorganisms that can be transferred from raw milk to the finished product and cause food poisoning are*: Mycobacterium, Brucella melitensis or Brucella abortus, Salmonella typhimurium, Listeria monocytogenes, Campylobacter jejuni, Bacillus cereus, Yersinia enterocolitica* [27].

Salmonella is a high-risk pathogen for the cheese industry [29]**.** With some exceptions, it is easily killed by pasteurization. However, *Salmonella* is perhaps the most resistant of all enteric pathogens to drying, freezing, low pH, cold and dry conditions. For this reason, as a result of inadequate pasteurization and sanitation practices, the microorganism can survive for a long time by growing during cheese making. In cheese, pH and starter activity have a significant effect on the survival time of *Salmonella* [28].

2.3.3 Chemical Factors

Antibiotics are widely used to protect dairy animals from disease and increase productivity. The antibiotics used pass into the milk. The presence of antibiotics in milk poses a problem in two different ways. For the health; cause allergic reactions in sensitive people [30].

Antibiotic residues are the most important chemical risk. The presence of antibiotics slows down or even stops the starter culture activity and directly or indirectly affects the quality of the finished product [27].

Another chemical that can be found in raw milk is aflatoxin. Aflatoxins are produced by molds of some other *Aspergillus, Penicillium* and *Rhizopus* species, especially *Aspergillus flavus* and *Aspergillus parasiticus.* Among the aflatoxins, aflatoxin B1 (AFB1) is known to have the highest toxic effect. Animals fed with AFB1-containing feed expel AFB1 as aflatoxin M1 (AFM1) with milk **[**31**].**

2.3.4 Physical Factors

The physical foreign substances that contaminate the milk from the outside in all stages from milking to the operations applied in the enterprise [16].

Researcher was reported, in her compilation study, although homogenization is a process performed on milk fat, some physical properties of proteins, especially casein and milk, are affected. It was reported that the water holding capacity of the cheese obtained as a result of homogenizing the milk to be processed into cheese increased, the amount of dry matter and fat transferred to the whey decreased and a better maturation was achieved [32].

Researchers reported that homogenization increased the amount of moisture in the cheese and improved the structure, texture and taste of the cheese in their study on Cheddar cheese obtained by using creams homogenized by applying pressure of 0/0 MPa, 3.5/3.5 MPa, 6.9/3.5 MPa and 10.4/3.5 MPa . It was determined that the free fat content of cheeses kept for 1 week was the lowest in cheeses with 6.9/3.5 MPa pressure and the highest in cheeses with 3.5/3.5 MPa pressure. Proteolysis was measured in similar amounts in all treatments as acid-soluble nitrogen and total nitrogen [33].

Researcher analyzed Kasar cheese samples produced from raw and pasteurized milk in terms of physical, chemical and microbiological aspects on the 1st, 30th, 60th and 90th days. While starter culture was added to pasteurized milk, it was not added to raw milk. Use of starter culture effect the dry matter, salt, salt in dry matter, pH, acidity, water-soluble protein, maturation degree, ash and fat values and statistically significant effects on *Lactococci, Lactobacilli, Coliform* and yeast-mold. The effect on the fat and protein values in dry matter and the total number of aerobic mesophilic bacteria is insignificant. The maturation time had a significant impact on the chemical properties of dry matter, oil in dry matter, salt in dry matter, salt in dry matter, pH, acidity, protein, water-soluble nitrogen, oil and ash values, and total aerobic mesophilic bacteria, lactic acid bacteria, *Lactococci, Coliform* bacteria and yeast-mold. During the ripening period, it was determined that there was a regular decrease in the L value, that is, the color darkened. No *Coliform* bacteria were found in any of the samples during maturation [34].

2.3.5 Biochemical Factors

It is stated that proteinases can act synergistically during cheese ripening. For example, β-casein degradation products formed by plasmin are converted into further degradation products by starter proteinase-peptidases. In a review study on lipolysis in milk and its products, lipolysis; it has been stated that it increases with the effect of processes such as shaking and homogenization. It has been reported that there is a significant increase in the free fatty acid content in parallel with the pressure value of the applied homogenization process [32].

In a study carried out to extend the shelf life of Kashar cheese, one group of Kashar cheeses was packed with 18% Polyamide and Polyethylene vacuum packaging, and the other group was treated with 5-10% potassium sorbate solution and covered with Paliamide and Polyethylene vacuum packaging, and another group was covered with a parafin layer treated and untreated with 10 % potassium sorbate. As a result of the study, it was reported that paraffin material was effective in lipolysis and reduced the rate of lipolysis during storage [35].

Researcher examined the effects of air and light transmittance, light intensity and wavelength of light on oxidation of packaging materials in their review study in which they comprehensively discussed the effects of packaging materials used in

cheese, exposure time to light and light intensity on oxidation occurring in cheese. The results obtained in this study show that even 0.5 % packaging gap, which is a very reasonable level in the industry, can negatively affect oxidative stability [36].

It was reported that especially polyunsaturated fatty acids are exposed to oxidation in milk and dairy products, and thus a large number of unsaturated aldehydes, called oxidative rancidity, have negative effects on the taste and aroma of the product. Lipolysis and the formation of free fatty acids, which are necessary for some cheeses to gain their specific taste-aromas, also pave the way for oxidation; it is stated that fats in the form of triglycerides are more stable against oxidation. In cheeses with high lipolysis, that is, free fatty acids, such as Gruyere and Parmesan, unsaturated fatty acids are more oxidized and as a result straight chain. It is reported that butanal, heptanal and nonanal aldehydes are found at higher rates [37].

As a result of different feeding of cattle, 3 types of milk with different free fatty acid contents were obtained. These milks were stored at 4°C for 4 days and TBA (Thio barbutiric acid) and peroxide measurements were made in these milks. In these measurements, there was no significant difference between the peroxide values of the 3 different milks. It was determined that TBA values increased by being affected by the storage time [38].

Researchers packaged the cheeses with different packaging materials and stored some of them in the dark and some of them by exposing them to 1500 lux light. At the end of storage, it was determined that the formation of secondary oxidation products was affected depending on the storage conditions such as temperature, humidity and light intensity and the light transmittance of the packaging [39].

They found that the formation of secondary oxidation products was affected depending on the permeability. It was determined that secondary oxidation products such as hexanal, heptanal, nonanal and 1-pentanol were formed in the samples exposed to light and the taste and odor of the cheese were affected accordingly. They also reported that the amount of oxygen in the headspace of the packages plays an important role in oxidation [39].

2.3.6 Starter Culture

Lactic acid fermentation, which is a very old invention, is used to improve the storage quality, flavor and nutritional quality of foods such as milk, vegetables, meat, fish, legumes and grain products in many different cultures from different parts of the world [40].

Lactic acid bacteria (LAB), especially in milk and milk products, are widely used as starter cultures for fermentation in the food industries. In generally they used to manufacture products like cheese, yoghurts, etc. The most important feature of these microorganisms increases acidity of milk by producing lactic acid from lactose. The increasing of acidity effects the stability of casein as well as and promotes the activity of rennin. After acidification, milk proteins begin coagulation. In addition, due to the lipolytic and proteolytic activities during cheese ripening, lactic acid bacteria have a significant impact on the formation of characteristic taste, aroma and texture [41].

The main lactic acid bacteria used in the food industry are *Lactobacillus, Lactococcus, Streptococcus, Enterococcus, Pediococcus and Leuconostoc*. The main functions of Lactic Acid Bacteria in the production of cultured dairy products can be listed as (i) conversion of lactose to lactic acid, (ii) production of flavor compounds and (iii) modification of product structure in some products. Lactic acid is the main compound that contributes to the flavor of fermented milks and fresh cheeses produced using co-culture with diacetyl and acetaldehyde. Lactic acid bacteria dominate the microbial flora of ripening cheese. LAB in cheese microflora consists of starter cultures and/or support cultures added during production and non-starter LABs [40].

Non-starter lactic acid bacteria (NSLAB); contains non-starter *Lactobacilli, Leuconostoc, Pedicocci* and *Enterococci* species. Non-starter *Lactobacilli* are microorganisms that make up most of the cheese population during ripening [21].

Non-starter *Lactobacilli* can affect cheese quality both positively and negatively. Non-starter *Lactobacilli* reduce the hardness and bitterness of the cheese while creating the desired flavor development during the ripening of the cheese. It has been reported that the addition of non-starter *Lactobacilli* in cheeses in which starter

culture is used causes an increase in aroma, a more intense flavor and an acceleration of ripening [42].

2.3.7 Use of Enzymes

Enzymes obtained from animal, vegetable and microbial sources are used in enzymatic coagulation of milk in cheese technology. These are all acid proteases. These enzymes not only ensure the coagulation of the milk, but also significantly affect the ripening and quality of the cheese. Rennets used to coagulate milk are selected pure proteinase preparations. Traditionally, rennets are prepared from the stomachs of calves, lambs and young goat stomach. The major proteinase in these rennets is chymosin. Rennet supply was insufficient as the world cheese production increased and the calf stomach decreased. Although many proteinases can coagulate milk, only six are of importance. These; bovine, porcine and chicken pepsin and *Rhizomucor miehei, R. Pusillus* and *Cryphonectria parasitica* proteases. The calf chymosin gene has been cloned into selected prokaryotic and eukaryotic organisms. Thus, it is now possible to supply high quality recombinant microbial enzymes [21].

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Fresh kashar cheeses were produced in the Kahkecioğlu factory in Gaziantep under the desired conditions. The chemicals used in analysis were: 60% perchloric acid (Merc KGaA, Darmstad Germany) 2-thiobarbituric acid ≥ 98 (TBA, Sigma-Aldrich), malondialdeyde bis(dimethyl acetal) (Merc, Germany), buffer solution for calibration of pH meter (Merc), potassium sulfate, sulfuric acid, copper sulfate, hydrochloric acid, tris, methyl orange, sodium chloride, potassium chromate and silver nitrate, 12% trichloroacetic acid (TCA, Sigma-Aldrich), sodium hydroxide and boric acid (Sigma-Aldrich) for kjeldahl process.

3.2 The Production of Kashar Cheese

Kashar cheese was produced in the Kahkecioğlu factory. The Commercial microbial rennet (Mucoren 2000) with a strength of 1/150.000 mcu/g was used as an enzyme in cheese production.

3.3 Vacuum Packaging and Storage of Samples

Vacuum packaging evacuates the air inside the package before sealing it. The aim here is (usually) to increase the shelf life of foods by removing the oxygen in the container and to make the contents take up less space with more flexible package molds. Kashar cheese samples were taken after they were produced at the factory and immediately vacuumed in vacuum machine is shown Figure 3.1 into packages weighing approximately 250 g under appropriate and hygienic conditions that shown in Figure 3.2. Fresh kashar cheese were totally stored for 105 days in ovens at the temperatures required for subsequent analyzes (5°C, 15°C, 25°C, 37°C). The first analysis before storage was used as zero day data. Other analyses were performed 8 times in 14-day periods. At each sampling time, 2 samples were taken, and duplicate analyses were done.

Figure 3.1 Vacuum machine

Figure 3.2 Vacuum applied to kashar cheese samples and storage of vacuumed kashar cheese in oven for storage

3.4 Analysis of Kashar During Storage

3.4.1 Determination pH

pH 211 Microprocessor pH meter was used to determine pH values of Kashar cheese samples. First, a sufficient amount of samples was taken and made suitable by crushing in a mortar. Then, 10 g of the sample was weighed on precision scales and placed in the beaker and 90 ml of distilled water was added to it. Afterwards, a magnetic stirrer was placed in the beaker in order to take the correct measurement, and it was placed on the stirrer machine and it was mixed for 10 minutes to make it homogeneous. As a result, the measurement was made with the pH meter and waited until the indicator reached a constant value, and when the value stabilized, the measurement was recorded [32].

3.4.2 Determination of Salt Content

The salt content of Kashar cheese was measured when the product was first produced and in the last period of storage. Mohr method was used to find out the amount of salt in kashar cheese. For this, the kashar cheese was crushed in a mortar or blender and brought to a soluble state. Then, 10 g of this sample was weighed on a precision scale and placed in an Erlenmeyer flask, 90 ml of distilled water was added to it, and a magnetic stirrer was added to make it homogeneous. The sample was then heated with shaking for 10 minutes and filtered after the heating process was finished. 50 ml of the filtered sample was taken and transferred into an Erlenmeyer flask and 2.5 ml of 5% potassium chromate was added on it and then titrated with 0.1 N $AgNO_3$ and when the color became brick red, the value was recorded. The calculation formula is shown below [43].

$$\% \text{ Salt (g)} = \frac{(\ 0.00585 \text{ x V}\ )}{m}\ x\ SF\ X\ 100$$

1 ml 0.1 N $AgNO_3$ = 0.00585 g NaCl

V = Used $AgNO_3$ volume (ml)

N = Adjusted $AgNO_3$ concentration

m = Received amount of sample (g)

SF = Dilution factor (Amount diluted / amount taken)

3.4.2.1 Standardization of $AgNO_3$ Solution

To calculate the actual normality of the titration solution, 0.58443 g sodium chloride was put into the erlenmeyer flask and the erlenmeyer flask was filled up to the 100 ml line with distilled water. 25 ml of the prepared solution was taken and transferred into another Erlenmeyer flask and 1 ml of potassium chromate was added. Then, titration was done with silver nitrate ($AgNO_3$), which was prepared until a red orange color was formed. The normality formula is shown below.

$$\text{N AgNO3} = \frac{\text{N NaCl x V NaCl}}{\text{V AgNO3}}$$

N AgNO3 = Normality of silver nitrate

N NaCl = Normality of sodium chloride 0.1 N

V NaCl = Volume of sodium chloride spent

V AgNO3 = Volume of spent silver nitrate

3.4.3 Determination of Moisture Content

In order to determine the moisture content of Kashar cheese, it was first decided how long the cheese would be kept in an oven. For this, a certain amount of kashar cheese was taken and put in the oven, then it was taken out in certain periods (30 minutes) and kept in a desiccator and weighed. At the end, the time when the moisture content remained constant (2.5 hours) was used for analysis. In addition, they were kept in the desiccator for a certain period of time and then were weighed.

An oven was used to measure the moisture content of the kashar cheese samples. While performing moisture analysis, firstly, the sample was started from the high temperature sample (37°C, 25°C, 15°C, 5°C, respectively) that could loose moisture so that the sample would not lose moisture, and the cheeses were immediately weighed and placed in the oven. Then, 5 g of each sample was weighed on precision scales and placed in petri dishes and kept at 105 °C for 2.5 hours that shown Figure 3.3. It was then taken from the oven and left to cool in the desiccator is shown Figure 3.4 and then weighed again. As a result, the following formula was used to determine the moisture content [44].

$$\%\ \text{moisture content} = \frac{\text{M2} - \text{M3}}{\text{M2} - \text{M1}}\ X\ 100$$

M_1 = Weight of dried empty drying container and lid (g)

M_2 = Analysis sample + weight of drying container and lid (g)

M_3 = Drying container with analysis sample and the weight of the lid after drying (g)

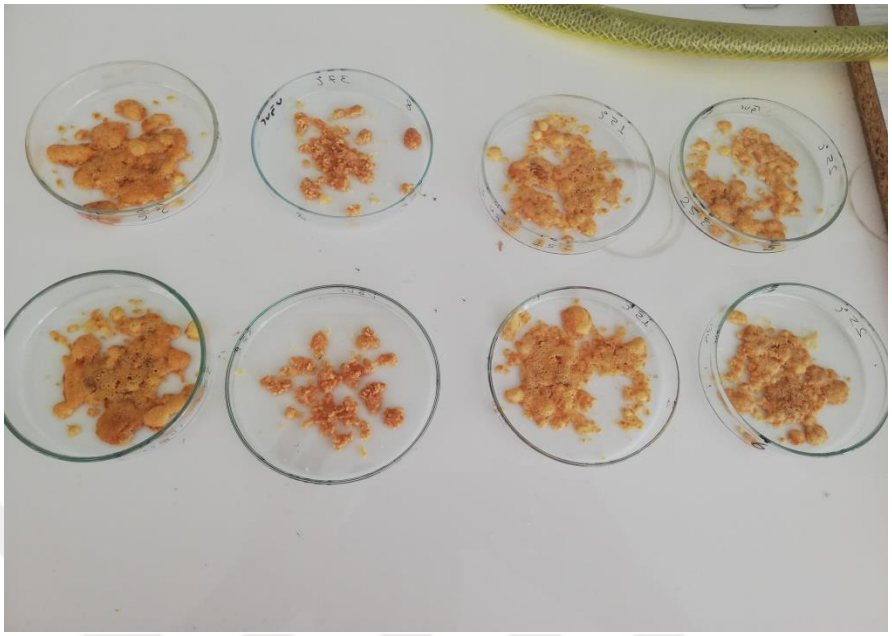

Figure 3.3 Kashar cheese samples after dried for moisture content analysis

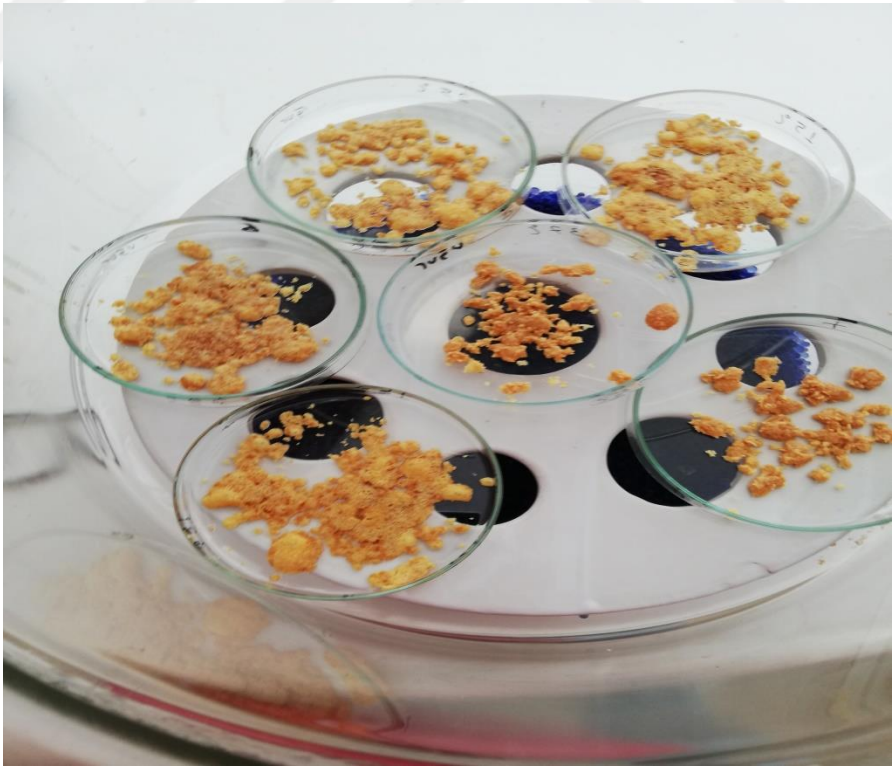

Figure 3.4 Kashar cheese samples cooled in desiccator

3.4.4 Determination of TBARS Analysis

Determination of lipid oxidation is usually achieved by quantification of compounds such as malondialdehyde (MDA) formed by denaturation of radical samples. The reaction of MDA with 2-thiobarbituric acid (TBA) is one of the most widely used methods for the prediction of lipid oxidation [45]. In order to determination of lipid oxidation, amount of MDA formation was determined by using spectrophotometry.

3.4.4.1 TBA Reagent Preparation

1 g Thiobarbituric acid (TBA) was weighed on a precision balance and put into the erlenmeyer flask and then the flask was filled with distilled water up to the 100 ml line. Then it was mixed by keeping it in a hot water bath enough to become homogeneous and dissolve the TBA, and after it was completely dissolved, the reagent was ready [8].

3.4.4.2 Standard Calibration Curve

A standard calibration curve was prepared by malondialdeyde bis(dimethyl acetal). For stock solution 100 µl malondialdeyde bis(dimethyl acetal) solution were taken into a volumetric flask and then completed with 100 ml distilled water. 7 test tubes placed into the stand and 0.1 ml, 0.01 ml, 0.02 ml, 0.03 ml, 0.05 ml, 0.07 ml, and 0 ml were taken from stock solution with the automatic pipette and completed to 10 ml of distilled water. From each dilute solutions, 2 ml of solution were taken and placed into another 7 new tube and completed with 10 ml 0.4 perchloric acid. Then it was shaken and the clear part was transferred to the flask, this process was repeated twice and the flask was completed up to the 25 ml line with 0.4 perchloric acid. 10 ml of the prepared extract was taken and put into test tubes, and then they were centrifuged at 1790 g (7 setting in old devices) for 5 min. After centrifugation, 1 ml extract and 5 ml TBA reagent were put into new test tubes and 1 ml distilled water and 5 ml TBA reagent for blank were added to the test tube. Afterwards, they were placed on the stand and kept in a boiling water bath for 35 minutes, and then they were immediately taken, cooled under the tap, and the absorbance value was measured in a spectrophotometer at 538 nm. 538 nm absorbance value against blank and standard curve were obtained is shown Figure 3.5.

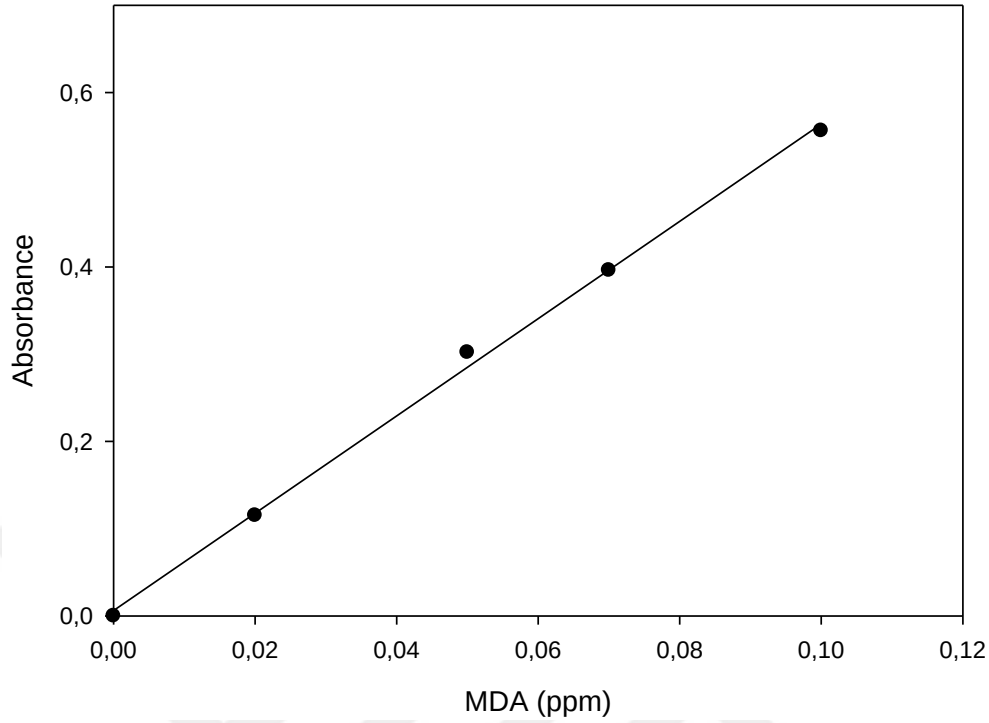

Figure 3.5 Standard calibration curve of MDA concentration (ppm)

3.4.4.3 TBARS Detection of Kashar Cheese

Kashar cheese samples were crushed in a mortar and homogenized. 2 g of the ready-made kashar cheese samples were taken, put into test tubes, and 0.4 M perchloric acid, which was prepared beforehand, was added to dissolve, and the lid was closed and shaken sufficiently (2-5 min). After that, it was rested for a while and after the precipitate formed, the clear part was carefully transferred to the 25 ml balloon jojo, this process was repeated once more. The flask was then filled up to the 25 ml line with 0.4 M perchloric acid. 10 ml of these 25 ml solutions were taken and placed in test tubes and the extraction was completed. The prepared extracts were centrifuged at 1790 g for 5 minutes. After centrifuge, the clear solution in the tubes was transferred to new clean tubes [8].

After preparing TBA reagent and extract,1 ml of each extract was taken and put into test tubes, and 5 ml of TBA reagent was added to them. For the blank, 1 ml of distilled water and 5 ml of TBA reagent were mixed. Afterwards, the prepared solutions were kept in a boiling water bath for 35 minutes and at the end of the time, they were immediately taken and cooled under the tap, and the absorbance values at

538 nm were measured with a spectrophotometer without waiting. The analysis was performed according to the curve obtained from the absorbance versus mda value [8].

3.4.5 Determination of Nitrogen in Kashar Cheese Samples

3.4.5.1 Detection of Total Nitrogen

The kjeldahl method was used to determine the amount of protein in kashar cheese. 1 g of the Kashar cheese sample, which was homogenized by crushing in a mortar, was weighed on a precision scale and put into the digestion tubes. Then, 7 g of potassium sulfate (K_2SO_4), 1 spatula of copper sulfate ($CuSO_4$) and 12 ml of concentrated sulfuric acid were added to it and two small mixing balls were placed in the protein tubes. Then, the combustion process was carried out at 400 ^{0}C for 40 minutes that shown in Figure 3.6. After the combustion process was completed, the samples were sufficiently rested and distillation analysis was performed on the kjeltec 2200 device is shown in Figure 3.7. The sample in the erlenmeyer was then titrated with 0.1 N HCl until the color changed from blue to red. The calculation formula is below [46].

$$\% \text{ Nitrogen} = \frac{\text{V x N x } 0.014}{\text{m}} x100$$

$$\% \text{ Protein} = \% \text{ Nitrogen x F}$$

m = Amount of Kashar cheese (g)

V = Amount of 0.1 N HCl spent (ml)

N = Normality of HCl

F = Dilution factor (6.38)

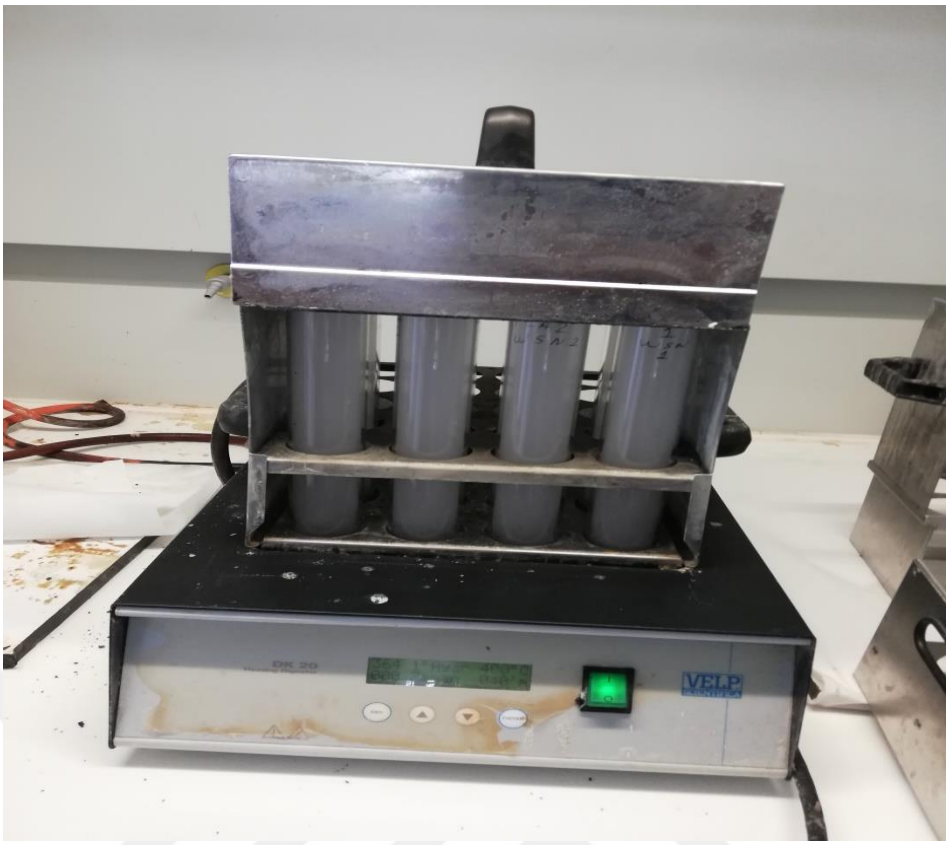

Figure 3.6 Kashar cheese burning process for total nitrogen analysis

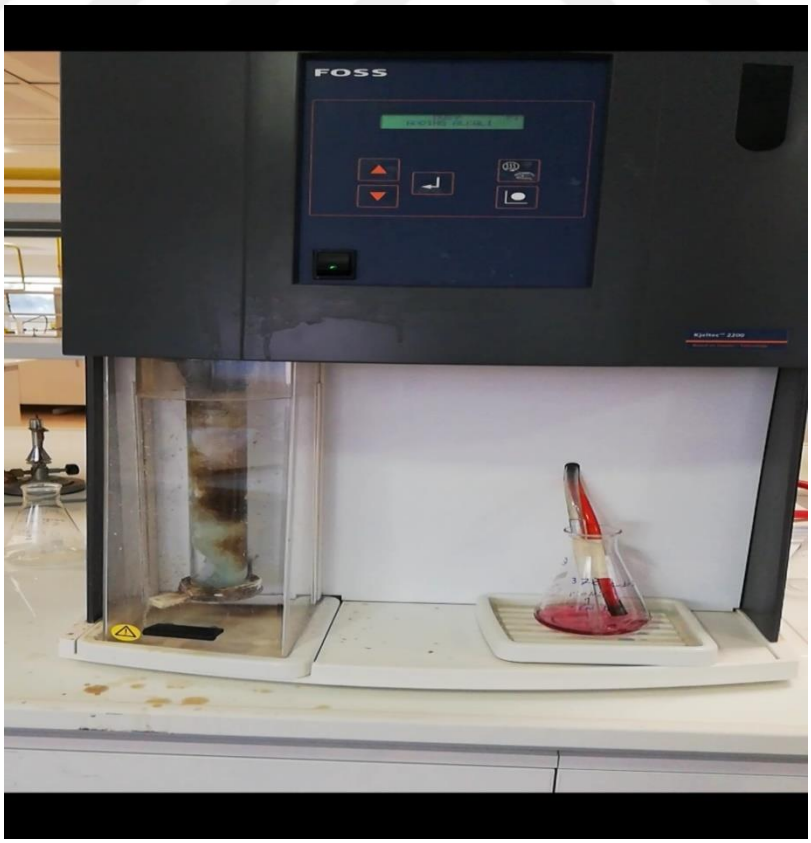

Figure 3.7 Distilation application for total nitrogen

3.4.5.2 Standardization of HCl

The 0.2335 g of tris was diluted with enough distilled water to dissolve and after 3 drops of methyl orange were added.

$$\text{N HCl} = \frac{0.2335 \text{ x } 1000}{\text{V}}$$

V = amount of HCl used in titration (g)

3.4.5.3 Detection of Water Soluble Nitrogen (WSN)

The kjeldahl method was used to determine water soluble nitrogen in kashar cheese. Kashar cheeses were made suitable by crushing in a mortar. Then, 20 g of kashar cheese was weighed on a precision scale and mixed with 100 ml of deionized water. Then the samples were homogenized in a blender for 2 minutes. Then, homogenized analysis samples were kept for 1 hour at 40 degrees Celsius. Then the samples were centrifuged at 3000 g for 30 minutes at 4°C or 20°C and then cooled to 4°C [32].

Then, 3 ml of WSN solution was taken from the samples ready for analysis with an automatic pipette and put into the digestion tubes. Then, 7 g of potassium sulfate (K_2SO_4), 1 spatula of copper sulfate ($CuSO_4$) and 12 ml of concentrated sulfuric acid were added to it and two small mixing balls were placed in the protein tubes. Then, the combustion process was carried out at 400 ^{0}C for 40 minutes. After the combustion process was completed, the samples were sufficiently rested and distillation analysis was performed on the kjeltec 2200 device. The sample in the erlenmeyer was then titrated with 0.1 N HCl until the color changed from blue to red. The calculation is made according to total nitrogen formulate but only dilution factor is different (100/3 is took) and the results are recorded [46].

3.4.5.4 Detection of 12 % Trichloroacetic Acid Soluble Nitrogen (TCA)

25 ml of WSN extract was added to 25 ml of 240g/kg TCA solution. 25 ml of TCA was prepared by dissolving 6 g of TCA in 25 ml of water. Then the suspension was held at room temperature for 2 hours and then filtered through Whatman No. 40 filter papers. Then, 3 ml of TCA solution was taken from the samples ready for analysis with an automatic pipette and put into the digestion tubes.

Then, 7 g of potassium sulfate (K_2SO_4), 1 spatula of copper sulfate ($CuSO_4$) and 12 ml of concentrated sulfuric acid were added to it and two small mixing balls were

placed in the protein tubes. Then, the combustion process was carried out at 400°C for 40 minutes. After the combustion process was completed, the samples were sufficiently rested and distillation analysis was performed on the kjeltec 2200 device. The sample in the erlenmeyer was then titrated with 0.1 N HCl until the color changed from blue to red. The calculation formula is same with the WSN formulate and the results are saved [46].

3.4.6 Determination of Color of Kashar Cheese

Color determinations of the fresh kashar cheseses were carried out using a Hunter Lab Color flex (A-60-1010-615 Model Colorimeter, Hunter Lab, and Reston, VA) and the color schema as shown in Figure 3.8 [7]. The samples were cut and crushed in a mortar acocording to special glasses and placed in such a way that there are no gaps into special glasses then were measured with Hunter LAB Color flex is shown in Figure 3.9. The values (L* = lightness, a* = redness, and b* = yellowness) were recorded.

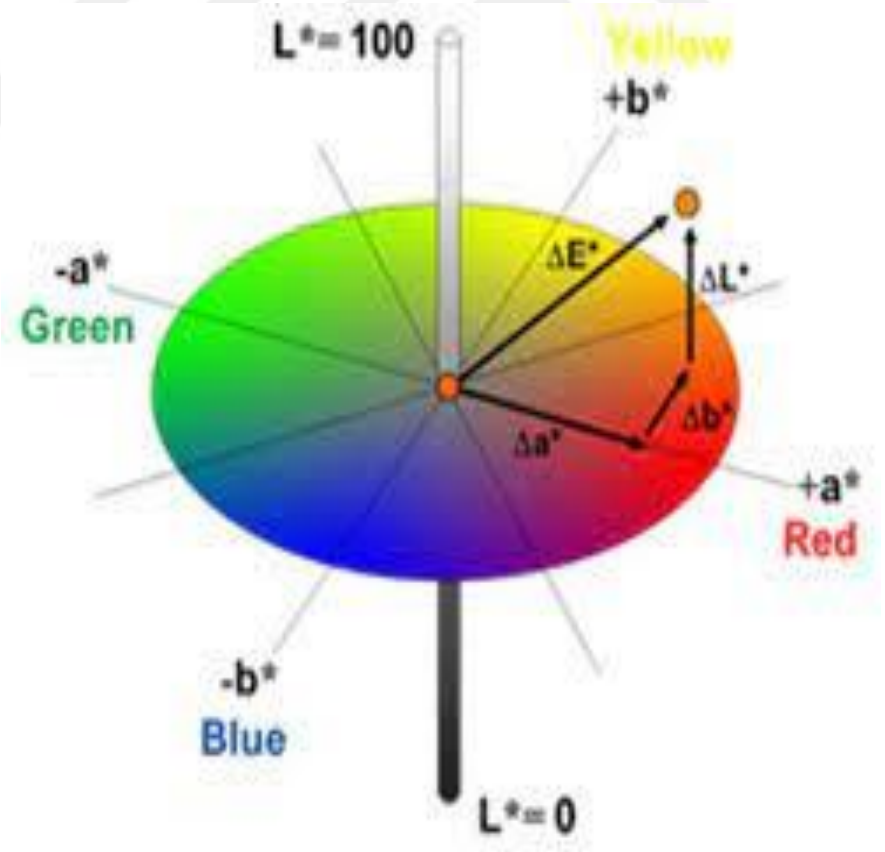

Figure 3.8 L*,a*,b*, Schematic view of color values

• L* – lightness coordinate (L*=0 indicates black and L* =100 is white)

• a* – red/green coordinate, +a* indicates red, – a* indicates green.

• b* – is the yellow/blue coordinate and +b* indicates yellow and -b* indicates blue.

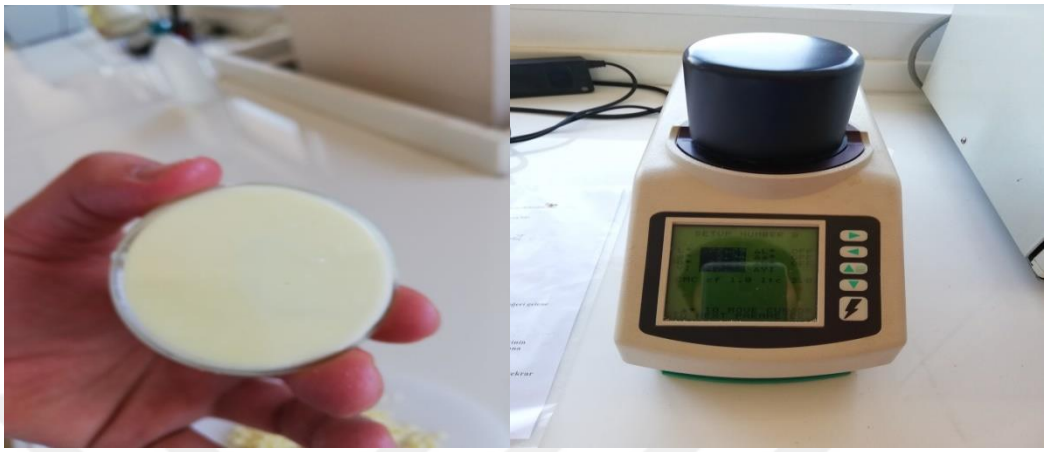

Figure 3.9 Kashar samples cheese was put into special glasses in left and hunter lab color measurement device in right.

3.4.7 Statistical Analysis

Kashar cheese samples stored at different temperatures and storaged samples was analysed and the Statistical analysis was performed to understand how the results of the analysis changed depending on the storage time and different temperatures and whether there was a significant difference by one-way and two way analysis of variance (ANOVA) to test for significant differences. Means of the groups were compared using Duncan multiple range test using an SPSS statistical packet (Version 23). In the differences between the groups were to be significant when $p<0.05$. Also graphs of the results were analysed by the Sigma Plot 14.0 (Systat Software Inc. 2011).

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Changes in Moisture Content of Kashar Cheese During The Storage Period

Moisture content of in Kashar cheese at 4 different temperatures (5°C, 15°C, 25°C and 37°C) during the maturation period of 105 days, 8 times with 2-week periods followed. In the this periods some changes were observed on the samples is shown that Figure 4.1.

One-Way (for time-week) and Two-Way ANOVA (temperature and time-week) Statistical analyzes were applied to investigate the moisture content of kashar cheese at the same storage time and different temperature. The results showing the moisture content changes depending on temperature and time during the storage period of fresh kashar cheese are also given in Table 4.1. According to the ANOVA results, storage time and temperature significantly changed the moisture content of kashar cheese ($P<0.05$) (Figure 4.2). Moisture content values changed in low amount in samples at low temperatures (5°C and 15°C), while a decrease in moisture values was observed in samples at 25°C and 37°C and changed significantly ($P<0.05$). Also the interaction between temperature and time were significantly affected to moisture content values ($P<0.05$).

Moisture content of Kashar cheese varies according to various production methods, but the dry matter of Kashar cheese made with the traditional method was determined in the range of 49.60%-59.70% and the average was 55.30% [47]. The initial moisture content of the kashar cheese it taken from factory are also in the range of the data produced by the traditional method. However, statistical analyzes were made to question the moisture change in kashar cheese storage in vacuum packed at different temperatures is found that have a significance effect ($P<0.05$).

According to the Turkish Food Codex Communique, the moisture content of fresh kashar cheese should be around 45%, but it can change during the ripening period

and should be around 40% at the end of ripening. According to our results, the moisture change of Kashar cheese differs according to the temperatures, no significant change was detected in the Kashar cheese sample left to storage at 5°C, the moisture values were found to be approximately close to each other, this is because the Kashar cheese is at normal storage temperature and in the range of shelf life. But Kashar cheese samples stored at 15°C have lost moisture with the effect of temperature and when the moisture content values are examined, it has been determined that there is a small decrease from 48.93% to 47.90%.

Table 4.1 The effects of storage time and storage temperature on moisture content of kashar cheese

Time (Day)	Moisture Content (%)			
	5°C	**15°C**	**25°C**	**37°C**
0	$48.93\pm0.05^{a\text{-}A}$	$48.93\pm0.05^{a\text{-}A}$	$48.93\pm0.05^{a\text{-}A}$	$48.93\pm0.05^{a\text{-}A}$
15	$49.15\pm0.52^{a\text{-}A}$	$49.12\pm0.05^{a\text{-}A}$	$48.98\pm0.20^{a\text{-}A}$	$45.81\pm0.27^{b\text{-}B}$
30	$48.20\pm0.20^{b\text{-}A}$	$49.03\pm0.21^{a\text{-}B}$	$47.35\pm0.25^{b\text{-}C}$	$43.25\pm0.30^{c\text{-}D}$
45	$49.01\pm0.25^{a\text{-}A}$	$48.93\pm0.28^{a\text{-}A}$	$48.01\pm0.62^{c\text{-}A}$	$45.50\pm0.58^{b\text{-}B}$
60	$48.97\pm0.10^{a\text{-}A}$	$49.03\pm0.38^{a\text{-}A}$	$47.90\pm0.08^{c\text{-}B}$	$42.54\pm0.16^{d\text{-}C}$
75	$47.48\pm0.40^{c\text{-}A}$	$49.20\pm0.26^{a\text{-}B}$	$46.83\pm0.33^{b\text{-}A}$	$42.32\pm0.58^{d\text{-}C}$
90	$49.24\pm0.07^{a\text{-}A}$	$49.40\pm0.13^{a\text{-}A}$	$45.95\pm0.20^{d\text{-}B}$	$40.12\pm0.01^{e\text{-}C}$
105	$48.32\pm0.05^{b\text{-}A}$	$47.90\pm0.12^{b\text{-}B}$	$45.86\pm0.08^{d\text{-}C}$	$40.45\pm0.07^{e\text{-}D}$

Different small letter indicates statistical difference at $\alpha=0.05$ level in time at each column. Different capital letters indicate statistical difference at $\alpha=0.05$ level in temperature at each row.

This situation was more effective for samples left to storage at 25°C and 37°C, and moisture loss increased to a certain extent with the increase intemperature and storage time ($P<0.05$). It is known that vacuum packaging has an effect on this, because moisture loss increases in products without vacuum packaging.

It has been determined in many studies that the moisture loss in cheese is significantly reduced by the use of plastic packaging material in cheese technology. It has been stated that cheese ripening occurs faster at high temperatures (around 13°C), but some gases may occur in vacuum packed cheese packages storaged at 13-16°C, and cheeses should be stored at temperatures below 10°C to prevent these [48]. A certain amount of gas and water accumulation was observed in the vacuum package at temperatures of 25°C and 37°C, but a little at 15°C above 5 °C, which we left to mature. In addition to this, we were defined to amount of water and gas in the samples, according to the volume of accumulated of water in the package and with sight method. This shows that vacuum packaged products trap moisture. This situation allowed water to hold in the pores formed in the kashar cheese during the analysis, and it is estimated in the direction of the analyzes that while the moisture decreases at some values of the analysis, it increases at a point and causes it to decrease again.

Figure 4.1 The gas occured vacuum packeged kashar cheese in storage

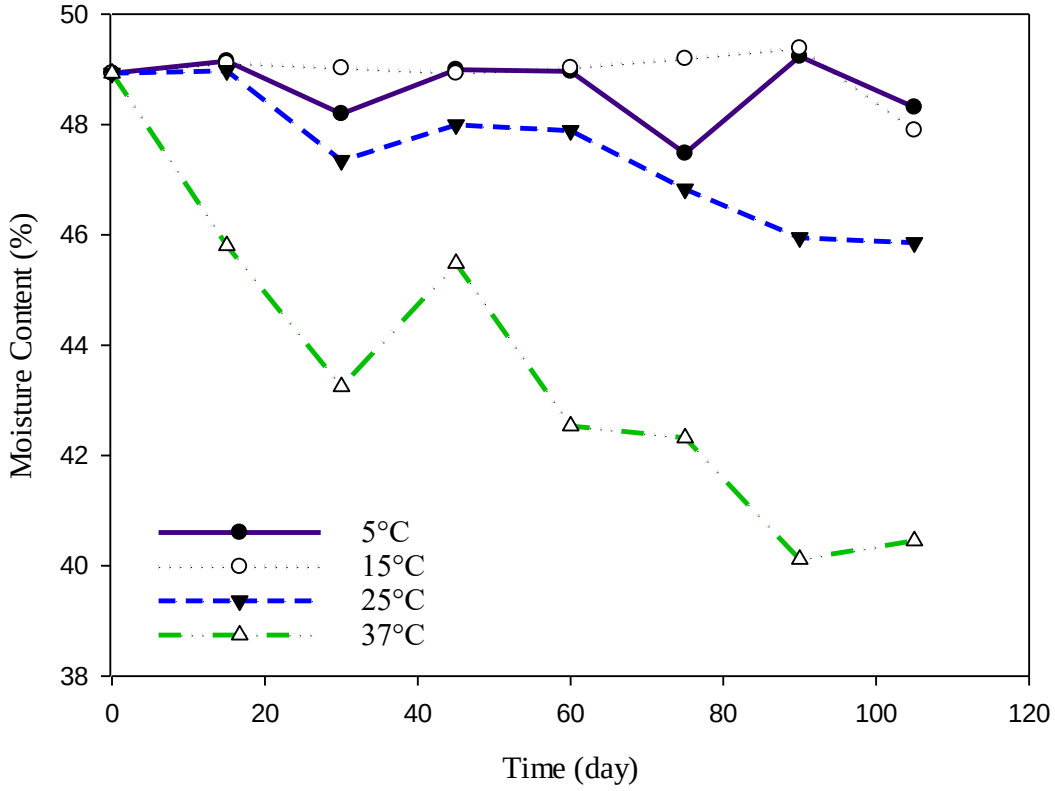

Figure 4.2 Change of moisture content with storage time and temperature

4.2 Changes in pH of Kashar Cheese During The Storage Period

The pH value gives information about the concentration of dissociated free hydrogen ions. pH value, which is a measure of active acidity, is an important factor affecting the quality of fermented product [49-50]. pH value in cheese is important for milk coagulation, proteolysis, texture and aroma [51].

The pH variation of Kashar cheese was investigated over a 105-day storage period at different temperatures (5°C, 15°C, 25°C and 37°C). The results showing the pH changes depending on temperature and time during the storage period of fresh kashar cheese are also given in Table 4.2. One-Way analysis was made in order to investigaate the effects of analysis results by time –week and two way analysis was made for learn how did the analysis results effect by temperature and time-week in the different temperature and same duration of storage on the pH changes of kashar cheese. According to the ANOVA results, storage time and temperature significantly changed the pH of kashar cheese ($P<0.05$) (Figure 4.3).

Table 4.2 The effects of storage time and temperature on pH value

Time (Day)	pH value			
	5°C	**15°C**	**25°C**	**37°C**
0	6.60±0.06^{a-A}	6.60±0.06^{a-A}	6.60±0.06^{a-A}	6.60±0.06^{a-A}
15	6.51±0.01^{b-A}	6.49±0.01^{b-A}	6.18±0.00^{b-B}	5.72±0.02^{b-C}
30	6.43±0.01^{c-A}	6.30±0.01^{c-B}	5.86±0.04^{c-C}	5.84±0.01^{c-C}
45	6.47±0.00$^{b,c-A}$	6.27±0.00^{c-B}	5.83±0.01^{c-C}	6.70±0.02^{d-D}
60	6.43±0.00^{c-A}	5.91±0.02^{d-B}	6.21±0.00^{b-C}	6.22±0.01^{e-C}
75	5.88±0.01^{d-A}	5.70±0.01^{e-B}	6.25±0.01^{b-C}	6.52±0.00^{f-D}
90	5.95±0.03^{e-A}	5.91±0.01^{d-B}	6.21±0.00^{b-C}	6.15±0.01^{g-D}
105	6.04±0.01^{f-A}	5.75±0.01^{e-B}	6.49±0.01^{d-C}	6.43±0.01^{h-D}

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

Also the interaction between temperature and time were significantly affected the pH values ($P<0.05$). According to the pH analyzes made during the storage period, the values of the samples at temperatures of 5°C and 15°C there is little change in the first 45-day period, but decreased as the maturation time and temperature increased ($P<0.05$).

This indicates an increase in acidity of all samples. It is known that the pH value of the product decreases due to the lactic acid produced by the lactic acid bacteria in fermented products [52-53]. Increasing the storage period with increasing temperature, the pH value of the sample decrease [4].

At other temperatures, there was a decrease in pH from 0th day until the 60th day at 25°C, and then an increase was observed until the end of storage, at 37°C it was increased until the 60th day, and then a decrease was generally observed ($P<0.05$).

Metin et al., (1991), Kurultay (1993), Koca (2002), in their research on Kashar cheese, reported that the pH values of cheeses decreased until the 30th day of ripening and then increased [54-56]. Investigator reported that pH decreases depending on the type of cheese as a result of the breakdown of lactose remaining in the curd during production, while the pH of cheese increases with the breakdown of lactic acid into other products and/or the formation of alkaline nitrogenous compounds during ripening [57].

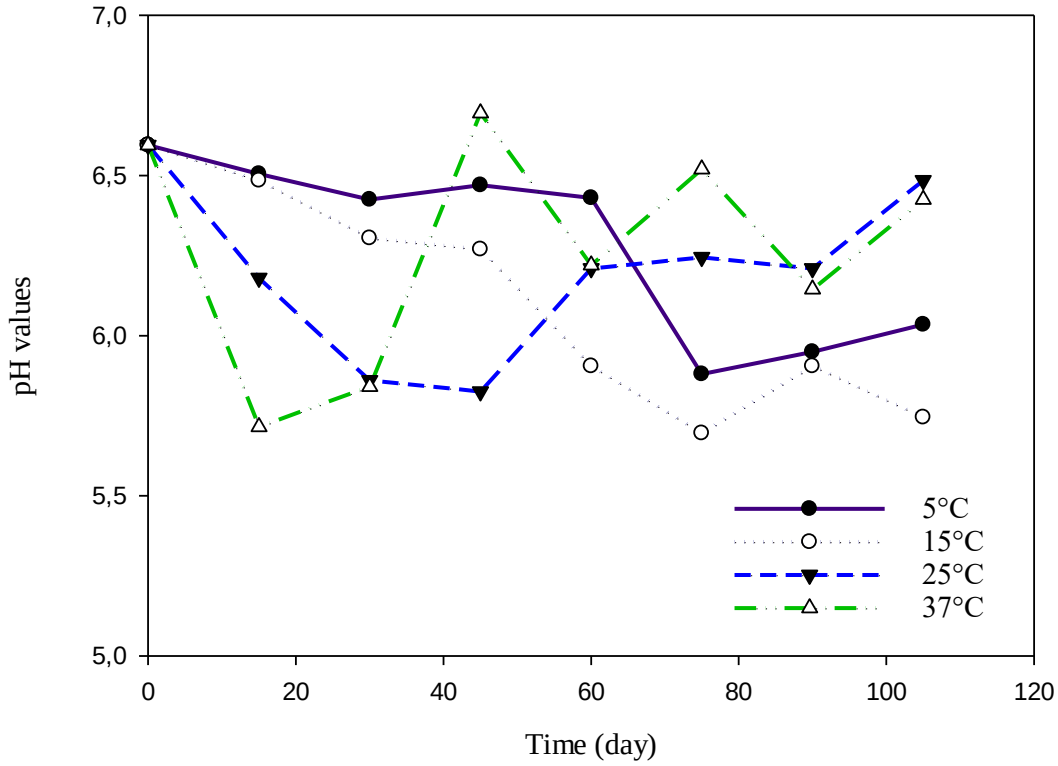

Figure 4.3 Change of pH value of kashar cheese during storage period

Yalçın (2017) noticed that Acidity increased during the storage period, but the expected decreases in pH were not observed. Because amino acids that make up proteins contain both amino (-NH_2) and carboxyl groups, and these components can buffer their environment [58].

Low pH and high salt value in water were obtained in the sample of full-fat fresh kashar cheese. The reason why the pH value is lower in full-fat cheese shows that the starter culture activity is higher. In addition, the protein concentration is higher in reduced fat cheeses. This has a buffering effect [59]. According to our results, the reasons for the increase and decrease in the pH value of 25°C and 37°C Kashar

cheeses, the time and temperature factor and the gas and water accumulation in the environment may have led to a decrease in the salt value and pH decrease in the dry matter or vice versa ($P<0.05$). There may be an increase in pH due to microbial growth with increase of storage time and high temperature.

4.3 Changes in Amount of Salt of Kashar Cheese During The Storage Period

Salting process in cheese production is mainly done to prevent deterioration of cheese, to extend its shelf life and to add flavor to cheese. It has important effects on the pH value, water content and the activities of the enzymes it contains. Therefore, salt significantly affects the quality of cheese [20, 60].

Salt changes that may occur during the storage period of Kashar cheese stored at different temperatures (5°C, 15°C, 25°C and 37°C) were examined in the first and last days of storage. Since all samples had the same temperature of 5°C on the first day of kashar cheese storage, a result was obtained on condition that it was duplicated. According to the Turkish Food Codex Communique, the amount of salt (NaCl) in fresh kashar cheese should be 3% at most. According to this result, the salt value in the first day was 2.075%. When looking at the last day of storage 2.05%, no significant change was detected in the salt content of Kashar cheese samples at 25°C, but little changes were observed at other temperatures.

As seen in Table 4.3**,** the salt ratios of the cheeses were close to each other. It was determined that the similar situation continued in all stages of maturation. As a result of the statistical analysis, no difference was found between the salt ratios of the cheeses ($P<0.05$).

Table 4.3 Change of salt value on kashar cheese during storage period

	Salt value (%)			
Time day	5°C	15°C	25°C	37°C
0	2.075	2.075	2.075	2.075
105	1.97	1.88	2.05	1.93

In many studies on Kashar cheese, researchers reported that the salt content of cheeses increased due to dry matter changes during ripening [61-66]. Our study does not participate in these studies. According to our results, no increase was observed in the salt values of Kashar cheese samples, which we left to mature as vacuum packed, but a decrease was determined in some of them. It is understood that there is a decrease in the moisture values of those who have this decrease during storage and it is known that there is a moisture accumulation in the packages, this can be explained by the accumulation of moisture in the vacuum packaged kashar cheeses by evaporation and as a result, the salt may have passed into this puddle as much as the reduced salt amount. Researcher reported that this is due to the fact that the curd in water boiled kashar cheese absorbs less salt into its body due to the fact that it is boiled in less salty water [47].

4.4 Changes of Nitrogen Content in Kashar Cheese Samples

4.4.1 Changes of Total Nitrogen

Substances in protein structure can not be determined exactly as proteins because they are compounds with high molecular complex structure. For this reason, nitrogen, which is a common component of all proteins and can be determined precisely, is determined. The amount of protein is calculated by multiplying this value with a factor that is different for all proteinaceous substances [4].

Changes in protein content were recorded during the 105-day storage time of kashar cheese samples stored at different temperatures (5°C, 15°C, 25°C and 37°C). The results given show to variations, how time and temperature affect amount of protein of fresh Kashar cheese during the storage period (Table 4.4).

One-Way analysis was made in order to investigaate the effects of analysis results by time –week and univariate ANOVA analysis was made for learn how did the analysis results effect by temperature and time-week on the amount of protein value of kashar cheese. The ANOVA results show that as the storage time and temperature increased, the Total nitrogen of kashar cheese increased ($P<0.05$). According to the ANOVA results, storage time and temperature significantly changed the amount of protein value of kashar cheese ($P<0.05$) (Figure 4.4). Also the interaction between temperature and time were significantly effected to the protein values of kashar

cheese (P<0.05). It was reported that the protein content of Kashar cheese increased during ripening [67-69].

Table 4.4 The Effects of storage time and temperature on amount of protein

Time (Day)	Protein Content (%)			
	5°C	**15°C**	**25°C**	**37°C**
0	$19.37\pm0.28^{a\text{-}A}$	$19.37\pm0.28^{a\text{-}A}$	$19.37\pm0.28^{a\text{-}A}$	$19.37\pm0.28^{a\text{-}A}$
15	$19.02\pm0.43^{a\text{-}A}$	$19.70\pm0.11^{a\text{-}A,B}$	$19.53\pm0.16^{a\text{-}A,B}$	$20.17\pm0.32^{a\text{-}B}$
30	$24.97\pm0.26^{b\text{-}A}$	$23.97\pm0.03^{b,c,d\text{-}A}$	$24.80\pm0.73^{b\text{-}A}$	$25.11\pm0.65^{b\text{-}A}$
45	$23.86\pm0.22^{c\text{-}A}$	$22.83\pm0.63^{b\text{-}A}$	$23.66\pm0.04^{c\text{-}A}$	$23.71\pm0.36^{c\text{-}A}$
60	$27.80\pm0.94^{d\text{-}A}$	$24.95\pm0.60^{c,d\text{-}B}$	$25.88\pm0.20^{d\text{-}B,C}$	$27.50\pm0.32^{d\text{-}A,C}$
75	$23.84\pm0.50^{c\text{-}A}$	$23.70\pm1.01^{b,c\text{-}A}$	$25.34\pm0.54^{b,d\text{-}A}$	$28.26\pm0.52^{d,e\text{-}B}$
90	$25.50\pm0.17^{b\text{-}A}$	$26.44\pm0.26^{e\text{-}A}$	$32.18\pm0.51^{e\text{-}B}$	$26.40\pm0.40^{f\text{-}A}$
105	$24.87\pm0.32^{b,c\text{-}A}$	$25.30\pm0.78^{d,e\text{-}A,B}$	$26.12\pm0.13^{d\text{-}B}$	$28.65\pm0.10^{e\text{-}C}$

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

According to the Turkish food codex communique, the protein rate of fresh kashar cheese is around 24.9. According to our results, the protein values of Kashar cheese samples we stored did not change much in the 30-day period, however, fluctuations were observed in the protein values in the following days, when we compare the protein values of kashar cheese we store with the codex values, it is seen that 5°C and 15°C samples are more suitable for storage. As a result, it was determined that the protein values increased over time (P<0.05). According Mutluer (2007), this partial decrease in protein ratio is due to the fact that some low-molecular peptides and amino acids are water-soluble and tend to pass into cheese brine as a result of the breakdown of casein by various enzymes (coagulating enzyme added to cheese milk, enzymes originating from starter and non-starter bacteria) [70].

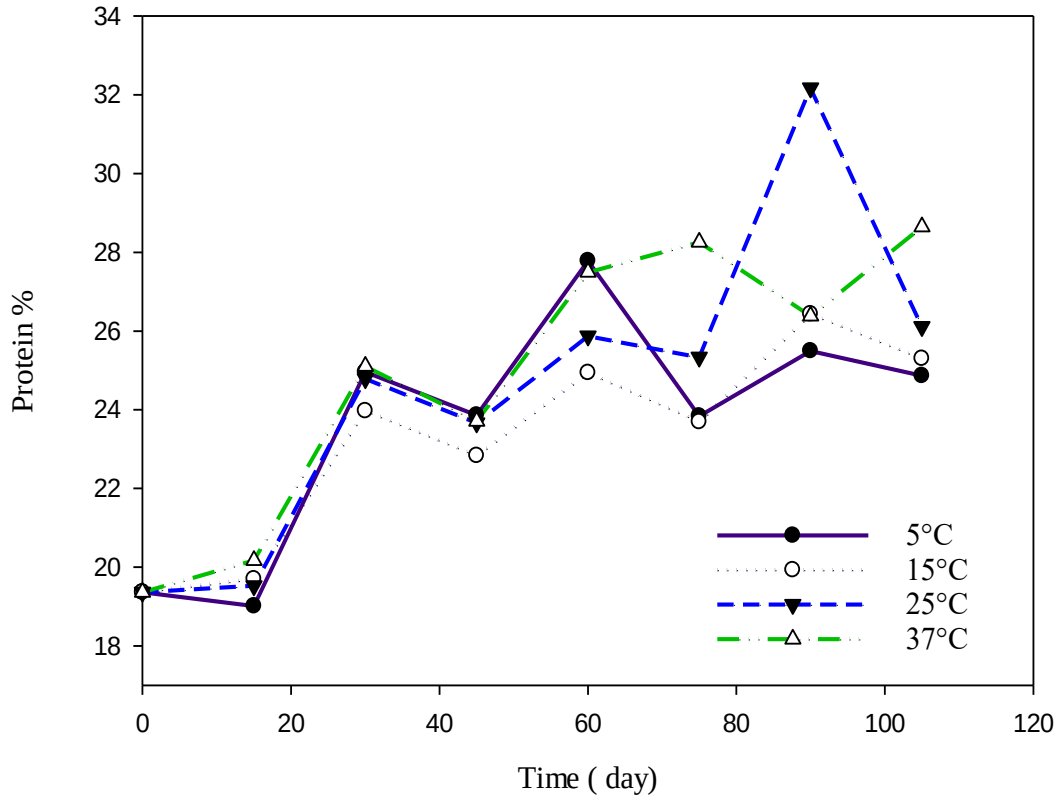

Figure 4.4 Change of protein content of kashar cheese during storage period.

When the protein values of kashar cheese were evaluated from the first storage day to the end of the last storage period, an increase was observed in protein amounts ($P<0.05$). When the results are evaluated, it is understood that the increases in the highest protein amount are 25°C and 37°C.

It is estimated that the biochemical events occurring in the cheese system, especially proteolysis, are affected by the pH value of the cheese medium, and therefore, the protein content of cheeses with different pH values changes. In our study, the presence of different temperatures affects the pH, thus affecting the protein ratios. At the end of the 45th day, Kashar cheese samples are shown in the Figure 4.5.

Figure 4.5 Kashar cheeses in different temperatures at last of the 45. day.

4.4.2 Changes of Water Soluble Nitrogen Content (WSN)

Protein in milk (casein); it is hydrolyzed by the culture and yeast used, and at the end of this hydrolysis, casein is broken down into water-soluble amino acids and peptones. As can be understood, there is a linear relationship between the conversion of nitrogenous components into water-soluble form and the degree of maturation [1].

Water soluble nitrogen (WSN) values of kashar cheese produced in our study were analyzed during maturation periods at different temperatures (5°C ,15°C, 25°C and 37°C). The results given show to changes, how time and temperature affect amount of water soluble nitrogen of fresh Kashar cheese during the storage period (Table 4.5). One-way and Two-way ANOVA Statistical analyzes were applied to learn the WSN of Kashar cheese at conditions the same storage time and different temperature. According to the ANOVA results, storage time and temperature significantly changed the WSN of kashar cheese ($P<0.05$) (Figure 4.6). Also the interaction between temperature and time were significantly effected to the WSN values of kashar cheese ($P<0.05$).

According to our research, the WSN values of kashar cheeses generally increased during the storage period ($P<0.05$). When the WSN values were examined, an

increase was detected until the 60th day of the samples stored at 5°C and 15°C, but a slight decrease was detected after the 60th day. When the samples left to mature at 25°C and 37°C are examined, a regular increase is observed in general ($P<0.05$). In general, the highest WSN values were determined in the last period of maturation versus to first period of storage.

Table 4.5 The effects of storage time and temperature on WSN

Time (Day)	WSN Values (%)			
	5°C	**15°C**	**25°C**	**37°C**
0	$0.24\pm0.01^{a\text{-}A}$	$0.24\pm0.01^{a\text{-}A}$	$0.24\pm0.01^{a\text{-}A}$	$0.24\pm0.01^{a\text{-}A}$
15	$0.78\pm0.03^{b\text{-}A}$	$0.74\pm0.00^{b,c\text{-}A,B}$	$0.67\pm0.01^{b\text{-}B}$	$0.93\pm0.04^{b\text{-}C}$
30	$0.80\pm0.03^{b\text{-}A}$	$0.76\pm0.02^{b,c\text{-}A}$	$0.90\pm0.00^{c\text{-}B}$	$1.38\pm0.03^{c\text{-}C}$
45	$1.03\pm0.15^{b\text{-}A}$	$0.90\pm0.10^{c\text{-}A}$	$1.01\pm0.02^{d\text{-}A}$	$1.76\pm0.03^{d\text{-}B}$
60	$1.08\pm0.18^{b\text{-}A}$	$0.91\pm0.18^{c\text{-}A}$	$1.10\pm0.07^{e\text{-}A}$	$1.98\pm0.08^{e\text{-}B}$
75	$0.96\pm0.13^{b\text{-}A}$	$0.70\pm0.01^{b\text{-}B}$	$1.54\pm0.01^{f\text{-}C}$	$2.02\pm0.02^{e\text{-}D}$
90	$0.93\pm0.17^{b\text{-}A}$	$0.90\pm0.00^{c\text{-}A}$	$1.45\pm0.01^{g\text{-}B}$	$2.14\pm0.03^{f\text{-}C}$
105	$1.00\pm0.06^{b\text{-}A}$	$0.88\pm0.02^{b,c\text{-}B}$	$1.51\pm0.00^{g,f\text{-}C}$	$2.20\pm0.03^{f\text{-}D}$

Different small letter indicates statistical difference at $\alpha=0.05$ level in time at each column. Different capital letters indicate statistical difference at $\alpha=0.05$ level in temperature at each row.

Many researchers have reported that the water-soluble nitrogen ratio of Kashar cheese increases ($P<0.05$) during ripening [59, 64, 69, 71-72]. In addition, it was determined that the differences between cheeses increased as the ripening time progressed, indicating that storage and temperature are effective in the amount of nitrogen dissolved in water, these differences are due to the fact that *Cryphonectria parasitica* protease is more proteolytic than *Rhizomucor miehei* protease, calf rennet, and recombinant chymosin, and hydrolyzes β-casein to a greater extent [73-75]. Coagulating enzymes are more involved in the formation of water-soluble nitrogen fractions (large peptides) [12].

The increase in water-soluble nitrogen values of cheese samples during ripening was found to be statistically significant. It is thought that this increase in the ratio of water-soluble nitrogen in cheeses increases further towards the end of ripening, lysis of the starter bacteria and the increase of proteolytic or peptidolytic enzymes in the cheese mass. The resulting enzymes cause the conversion of large molecule peptides to small molecule peptides or amino acids. It is observed that the amount of nitrogen dissolved in water increases as the hydrophilic properties of the peptides increase with the breakdown [76].

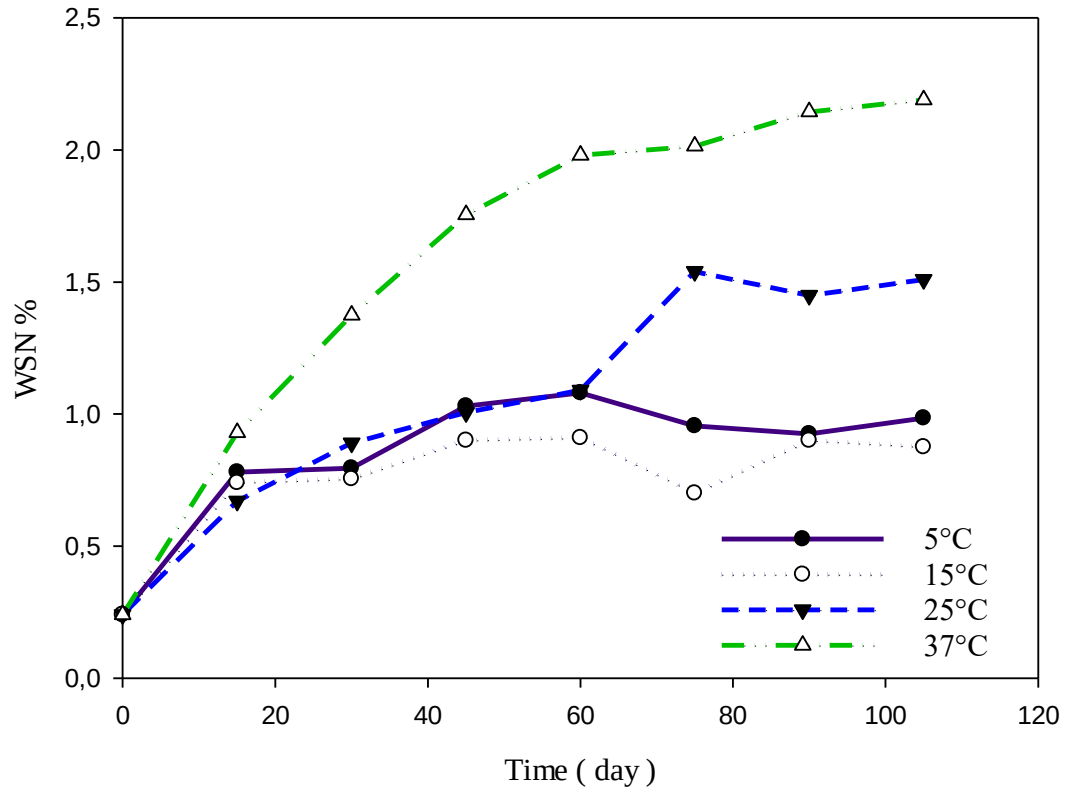

Figure 4.6 Change of WSN (water soluble nitrogen) of kashar cheese

4.4.3 Changes of 12% Trichloroacetic Acid Soluble Nitrogen (TCA-SN)

The ratio of nitrogen soluble in 12% TCA, or in other words non-protein, consists of medium and short chain peptides and amino acids [76].

The nitrogen value dissolved in trichloroacetic acid is a value that indicates the presence of small sized peptides and amino acids. These amino acids and peptides are formed as a result of the degradation of peptides separated from casein as a result

of rennet and plasmin activity by microbial enzymes and are known as the maturity depth index in total [77].

12 % Trichloroacetic acid soluble nitrogen (TCA) values of Kashar cheese samples storaged at different temperatures (5°C, 15°C, 25°C and 37°C) were recorded during 105 days of storage. The results change in the amount of TCA during the storage period of fresh kashar cheese depending on the storage time and temperature conditions is shown in Table 4.6.

Table 4.6 The effects of storage time and temperature on TCA-SN values

Time (Day)	TCA Values (%)			
	5°C	**15°C**	**25°C**	**37°C**
0	$0.25\pm0.05^{a\text{-}A}$	$0.25\pm0.05^{a\text{-}A}$	$0.25\pm0.05^{a\text{-}A}$	$0.25\pm0.05^{a\text{-}A}$
15	$0.12\pm0.11^{b\text{-}A}$	$0.17\pm0.05^{c\text{-}A}$	$0.20\pm0.00^{a\text{-}A}$	$0.37\pm0.03^{a\text{-}B}$
30	$0.18\pm0.21^{a,b\text{-}A}$	$0.20\pm0.01^{a,c\text{-}A}$	$0.41\pm0.06^{b\text{-}B}$	$0.73\pm0.01^{b\text{-}C}$
45	$0.26\pm0.06^{a\text{-}A}$	$0.15\pm0.01^{c\text{-}B}$	$0.44\pm0.02^{b\text{-}C}$	$0.82\pm0.02^{b,c\text{-}D}$
60	$0.11\pm0.00^{b\text{-}A}$	$0.15\pm0.01^{c\text{-}A}$	$0.42\pm0.05^{b\text{-}B}$	$0.94\pm0.12^{c,d\text{-}C}$
75	$0.21\pm0.03^{a\text{-}A}$	$0.38\pm0.00^{d\text{-}B}$	$0.70\pm0.00^{c\text{-}C}$	$1.06\pm0.01^{d\text{-}D}$
90	$0.24\pm0.03^{a\text{-}A}$	$0.25\pm0.01^{a,b\text{-}A}$	$0.65\pm0.02^{c\text{-}B}$	$1.10\pm0.00^{d\text{-}C}$
105	$0.24\pm0.00^{a\text{-}A}$	$0.31\pm0.00^{b\text{-}A}$	$0.73\pm0.01^{c\text{-}B}$	$1.30\pm0.17^{e\text{-}C}$

Different small letter indicates statistical difference at $\alpha=0.05$ level in time at each column. Different capital letters indicate statistical difference at $\alpha=0.05$ level in temperature at each row.

One-way and Two-way ANOVA Statistical analyzes were applied to understand the amount of TCA soluble nitrogen of kashar cheese at conditions the same storage time and different temperature. According to the ANOVA results, the storage time and temperature significantly changed the amount of TCA soluble nitrogen of kashar cheese ($P<0.05$) (Figure 4.7). Also the interaction between temperature and time were significantly effected to the TCA values of kashar cheese ($P<0.05$).

When the results were evaluated, the TCA values were found to be almost the same range of the first and last storage days. In the samples stored at 15°C, a decrease was detected until the 60th day, and then there was a general increase until the end of the maturation period ($P<0.05$).

A regular increase was observed from the first day of storage to the last day in Kashar cheese samples storaged at 25°C and 37°C ($P<0.05$). As a result of the statistical analysis performed to determine the difference between the rates of nitrogen dissolved in 12% TCA of the cheeses, it was determined that the differences were at a significant level ($P<0.05$) in all storage times.

There are some studies in literature which they found similar results with us and they related to due to the fact that *Cryphonectria parasitica* protease is more proteolytic than *Rhizomucor miehei* protease, calf rennet and recombinant chymosin and is more cleaved b-casein [73,75].

Various studies have shown that coagulant enzymes are responsible for the formation of large peptides, and starter cultures are responsible for the formation of small peptides and amino acids [78-80]. The peptides produced by the coagulating enzymes affect the nitrogen rates of cheeses dissolved in 12% TCA [12].

Many researchers have also reported that the TCA soluble nitrogen ratios of kashar cheeses increase in significant level during the ripening period [59,69,72,81-83]. In the presence of factors such as low acidity and high water content, peptides with low molecular weight are formed as a result of the effect of chymosin on αs1-casein [84].

In our study, as a result of storage, the highest TCA-SN value was determined in Kashar cheese samples storage at 25°C and 37°C, which have the lowest acidity values. This shows that some physical changes such as pH affect the biochemical results of cheeses storage at different temperatures as the storage time increases significantly ($P<0.05$).

It is seen that the TCA-SN values given in the literature are generally compatible with the values obtained in our study. It can be said that the differences between the values are due to the milk composition and microbial differences used in the production of cheeses.

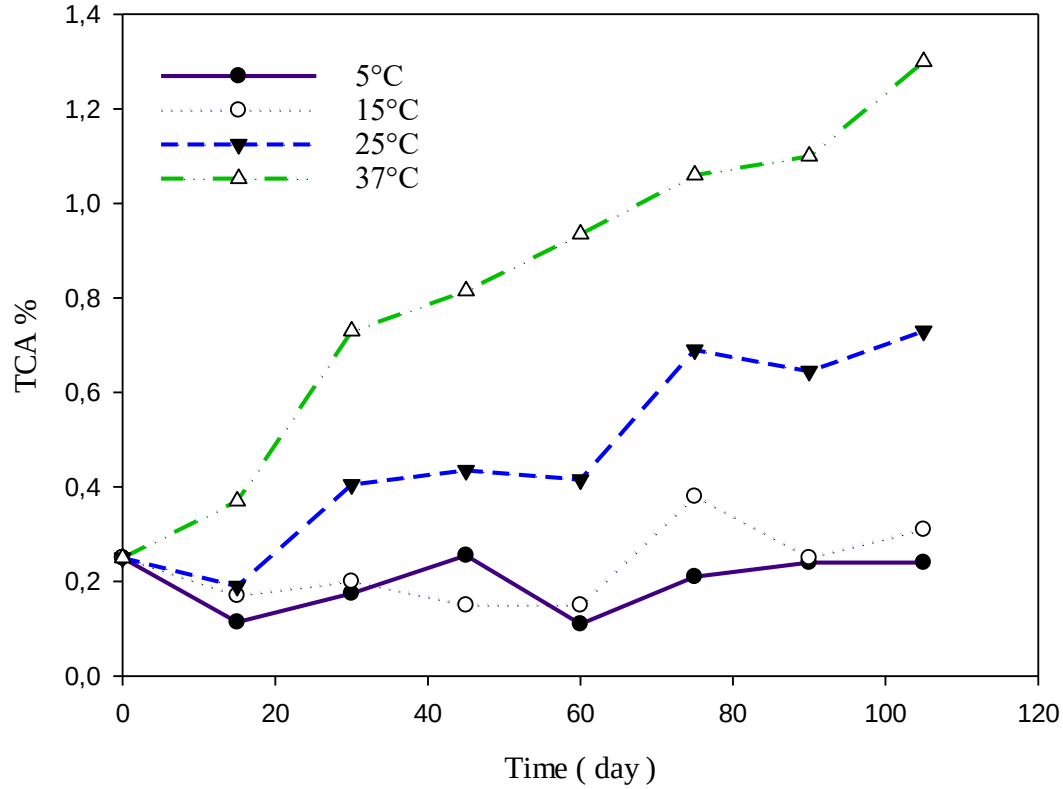

Figure 4.7 Change of TCA value of kashar cheese

4.5 Changes of TBARS (2-Thiobarbituric Acid Reactive Substances) Values

TBARS values is used as a marker of lipid oxidation. Degradation of polyunsaturated fatty acids results in malondialdehyde formation. Lipid oxidation could cause destruction of valuable nutrients, off-flavours and production of toxic compounds [85]. Consequently, TBARS value is a critical parameter especially during storage period of foods that's why it is detected in this study. It could cause adverse sensorial results which affects consumer acceptance [8].

The change of TBARS values of fresh Kashar cheese samples storaged at different temperatures (5°C, 15°C, 25°C and 37°C) all consequents were recorded during 105 days of storage. The results in the measurement of absorbance values in storage period of fresh kashar cheese depending on the storage time and temperature conditions is shown in Table 4.7.

We were compared means with One-Way analysis which was made in order to investigate the effects of analysis results by time–week and univariate ANOVA analysis was made for learn how did the analysis results effect by temperature and

time-week to examine the amount of MDA values of Kashar cheese at the same circumstance storage time and different temperature. According to the Statisticals results, the storage time significantly changed the amount of MDA values of kashar cheese ($P<0.05$) temperature not significantly effected ($P>0.05$) to MDA values of kashar cheese (Figure 4.8). Also the interaction between temperature and time were not significantly effected to the MDA values of kashar cheese ($P>0.05$).

Table 4.7 The Effects of storage time and temprerature on TBARS values

Time Day	TBARS Values			
	5°C	**15°C**	**25°C**	**37°C**
0	$0.0177\pm0.001^{a\text{-}A}$	$0.0177\pm0.001^{a\text{-}A}$	$0.0177\pm0.001^{a\text{-}A}$	$0.0177\pm0.001^{a\text{-}A}$
15	$0.0018\pm0.001^{b,c\text{-}A}$	$0.0023\pm0.004^{b\text{-}A}$	$0.0057\pm0.003^{b,c\text{-}A}$	$0.0025\pm0.001^{b\text{-}A}$
30	$0.0047\pm0.002^{c\text{-}A}$	$0.0039\pm0.002^{b,c\text{-}A}$	$0.0028\pm0.002^{b,c,d\text{-}A}$	$0.0060\pm0.002^{b\text{-}A}$
45	$0.0132\pm0.002^{a\text{-}A}$	$0.0120\pm0.001^{d\text{-}A}$	$0.0103\pm0.000^{e\text{-}A}$	$0.0120\pm0.003^{c\text{-}A}$
60	$0.0014\pm0.005^{b,c\text{-}A}$	$0.0069\pm0.001^{c\text{-}A}$	$0.0066\pm0.002^{c,e\text{-}A}$	$0.0022\pm0.001^{b\text{-}A}$
75	$-0.0014\pm0.001^{b\text{-}A}$	$0.0023\pm0.001^{b\text{-}A}$	$0.0007\pm0.001^{d\text{-}A}$	$0.0048\pm0.010^{b\text{-}A}$
90	$0.0019\pm0.001^{b,c\text{-}A}$	$0.0014\pm0.000^{b\text{-}A}$	$0.0020\pm0.002^{b,d\text{-}A}$	$0.0032\pm0.001^{b\text{-}A}$
105	$0.0024\pm0.002^{b,c\text{-}A}$	$0.0008\pm0.000^{b\text{-}A}$	$0.0017\pm0.001^{b,d\text{-}A}$	$0.0023\pm0.000^{b\text{-}A}$

Different small letter indicates statistical difference at $\alpha=0.05$ level in time at each column. Different capital letters indicate statistical difference at $\alpha=0.05$ level in temperature at each row.

MDA values were effected significantly ($P<0.05$) by storage period of kashar cheese. As the maturation period along, MDA values of all cheese samples decreased, it is estimated that this is due to the fact that the reactive substances are degraded and turned into intermediate products while the kashar is ripening during the storage process. The MDA values fluctuated throughout the maturation period, but the overall trend was to decrease and decreased in all samples.

Researcher reported the lower MDA content of sample containing *L. casei* (at 105 day storage) could probably be because of the environmental conditions at that storage period which might have favored the activity of this specific strain and

resulted in formation of bioactive peptides with high lipid oxidation inhibition activity. Lower values for MDA content in treated samples could be related to their higher antioxidant activity that could be due to bioactive peptides formed by the probiotic bacteria and their capacity to release antioxidant enzymes which enables them to scavenge more free radicals responsible for fat oxidation as compared to the cheese without added probiotics [86]. Analyser was confirmed that intake of dairy products with probiotic bacteria decreases MDA content [87].

Lipid oxidation leads through formation of hydroperoxides to short chain aldehyde, ketones and other oxygenated compounds. They are considered to be responsible for the development of rancidity, cause undesirable flavor and related to heart disease and cancer [88].

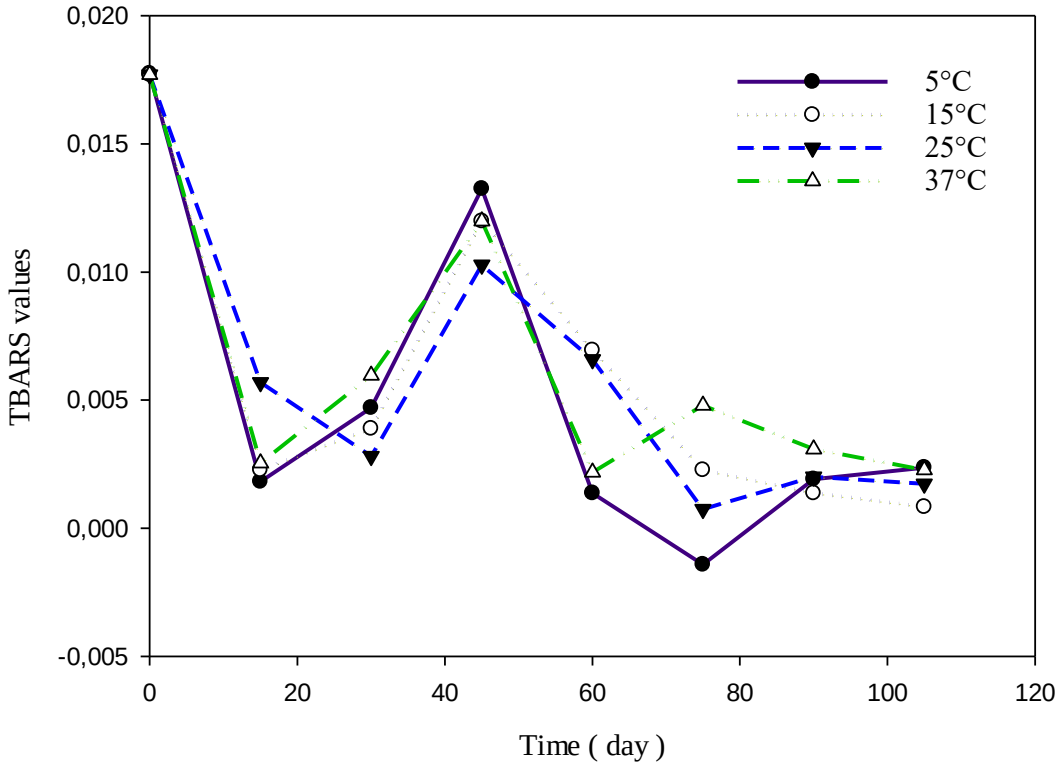

Figure 4.8 Change of TBARS values of kashar cheese

4.6 Changes of Color in Fresh Kashar Cheese During Storage

Color is an important parameter for determining the quality of food and directly related to the storage time and temperature. For this reason, a food should be evaluated in terms of color and measured sensitively. A subjective visual assessment of color measurement can be misleading. The color was measured with Hunter Lab device and each week L*, a*, b* values were recorded. Each letter has a different mean and shows different properties of the food.

4.6.1 L* Value

Fat globules and casein micelles, which make up the colloidal part of milk, play a major role in the white color of cheese because they reflect light in the visible spectrum. L* value is important in terms of expressing the whiteness of cheeses. The amount of fat in the cheese before boiling is effective in making the cheese white, but when the cheese cools after boiling, the serum proteins are separated and the whiteness of the cheese decreases as it cannot reflect light [89].

During the 105-day storage period of Kashar cheeses at different temperatures, changes in L* values, which express brightness or darkness, were recorded. The results given show to variations, how time and temperature affect amount of color L* value of fresh Kashar cheese during the storage period (Table 4.8). One-way and Two-way ANOVA statistical analyzes were applied to investigate the L color value of Kashar cheese at conditions the same storage time and different temperature. According to the ANOVA results, the storage time and temperature significantly changed the amount of L color value of kashar cheese ($P<0.05$). Also the interaction between temperature and time were significantly effected to the TCA values of kashar cheese ($P<0.05$).

According to these results, although there were some decreases and increases in L* values in 5°C, 15°C and 25°C samples, they generally took the same values, but there was a general decrease in the range of 37°C (80.54-74.75) ($P<0.05$). The highest L* values were determined at 25°C. A higher L* value means that the sample has a brighter color. When the cheese groups were compared in terms of L* values, the difference between them was found to be statistically significant at the $P<0.05$ level.

They reported in a study conducted on Gaziantep cheeses, stated that the L value decreased at the beginning of storage; subsequently increased [90]. It was reported that L* values of Kashar cheeses [91-92], Mozzarella cheeses [93] and Cheddar cheeses [94] were decreased during the ripening period ($P<0.05$)

Table 4.8 The effects of storage time and temperature on color L* values

Time (Day)	Color L values			
	5°C	**15°C**	**25°C**	**37°C**
0	$78.70\pm0.13^{a\text{-}A}$	$78.70\pm0.13^{a\text{-}A}$	$78.70\pm0.13^{a\text{-}A}$	$78.70\pm0.13^{a,b\text{-}A}$
15	$80.94\pm0.27^{b\text{-}A,B}$	$78.82\pm0.40^{a,b\text{-}C}$	$81.96\pm0.50^{b,c\text{-}B}$	$80.54\pm0.72^{c\text{-}A}$
30	$79.92\pm0.33^{c\text{-}A}$	$80.50\pm0.21^{c\text{-}A}$	$83.01\pm0.17^{d\text{-}B}$	$79.95\pm0.41^{c,d\text{-}A}$
45	$79.78\pm0.14^{c\text{-}A}$	$79.27\pm0.06^{b\text{-}A}$	$83.82\pm0.56^{f\text{-}B}$	$78.06\pm0.20^{a,e\text{-}C}$
60	$80.26\pm0.37^{b,c\text{-}A,B}$	$81.02\pm0.39^{c,d\text{-}B}$	$82.61\pm0.24^{c,d\text{-}C}$	$79.28\pm0.75^{b,d\text{-}A}$
75	$80.48\pm0.48^{b,c\text{-}A}$	$83.58\pm0.10^{e\text{-}B}$	$81.63\pm0.21^{b\text{-}C}$	$80.72\pm0.33^{c\text{-}A}$
90	$82.90\pm0.22^{e\text{-}A}$	$81.25\pm0.12^{d\text{-}B}$	$83.51\pm0.18^{e,f\text{-}A}$	$77.48\pm0.44^{e\text{-}C}$
105	$80.46\pm0.18^{b,c\text{-}A}$	$82.11\pm0.23^{f\text{-}B}$	$83.66\pm0.08^{e,f\text{-}C}$	$74.75\pm0.43^{f\text{-}D}$

Different small letter indicates statistical difference at $\alpha=0.05$ level in time at each column. Different capital letters indicate statistical difference at $\alpha=0.05$ level in temperature at each row.

4.6.2 a* Value

During the 105-day storage period of Kashar cheeses, the positive and negative coordinates and the changes in a* value, which represents redness and greenness, were investigated. The results given show to changes, how time and temperature affect measurement of color of a* value of fresh Kashar cheese during the act of storing period (Table 4.9). One-way and Two-way ANOVA statistical analyzes were applied to investigate the color a* value of Kashar cheese at conditions the same storage time and different temperature. According to the ANOVA results, storage time and temperature significantly changed the color a* value of kashar cheese ($P<0.05$). Also the interaction between temperature and time were significantly

effected to the color a* values of kashar cheese (P<0.05). Positive values are indicative of red color, negative values are indicative of green color. In the coordinate system, the center is colorless, as the a* values increase and away from the center, the color separation increases [95].

Table 4.9 The effects of different time and temperature on color a* values

Time (Day)	Color a* Values			
	5°C	**15°C**	**25°C**	**37°C**
0	-1.63±0.01$^{a,b\text{-}A}$	-1.63±0.01$^{a\text{-}A}$	-1.63±0.01$^{a\text{-}A}$	-1.63±0.01$^{a\text{-}A}$
15	-1.62±0.53$^{a,b\text{-}A}$	-2.04±0.04$^{b,c\text{-}A}$	-1.65±0.02$^{a\text{-}A}$	0.28±0.07$^{b\text{-}B}$
30	-1.97±0.01$^{a,b\text{-}A,B}$	-2.15±0.17$^{b\text{-}A}$	-1.52±0.27$^{a\text{-}B}$	0.82±0.11$^{c\text{-}C}$
45	-1.86±0.20$^{a,b\text{-}A}$	-1.91±0.01$^{c\text{-}A}$	-1.05±0.13$^{b\text{-}B}$	1.18±0.20$^{c\text{-}C}$
60	-2.10±0.21$^{a\text{-}A}$	-1.96±0.10$^{b,c\text{-}A}$	-0.84±0.00$^{b\text{-}B}$	1.75±0.38$^{d\text{-}C}$
75	-1.67±0.13$^{a,b\text{-}A}$	-1.55±0.11$^{a,d\text{-}A}$	-0.86±0.15$^{b\text{-}B}$	1.78±0.12$^{d\text{-}C}$
90	-1.55±0.05$^{a,b\text{-}A}$	-1.66±0.05$^{a\text{-}A}$	-0.52±0.04$^{c\text{-}B}$	2.98±0.05$^{e\text{-}C}$
105	-1.43±0.01$^{b\text{-}A}$	-1.37±0.08$^{d\text{-}A}$	-0.89±0.15$^{b\text{-}B}$	3.60±0.25$^{f\text{-}C}$

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

It was determined that a* values of all cheese samples except 37°C were on the negative axis, that is, in the color area defined as green. Although the a* values of kashar cheeses stored at 5°C and 15°C during storage had a fluctuating appearance, it was determined that the storage process have a significant effect on the a* values of the cheeses (P<0.05). It was determined that there was a positive increase in a* values of cheese samples stored at 25°C and 37°C. Samples at 25°C showed a decrease from -1.65 to -0.89, and the green color decreased and tended to be colorless. In the samples at 37°C, it showed an increase from 0.28 to 3.60 and showed an increase in the red color value. The change in a* value of Kashar cheeses during the storage period was found to be statistically significant (P<0.05).

4.6.3 b* Value

In color analysis, b* value defines yellow and blue colors with positive and negative coordinates. Positive values indicate yellow color, negative values indicate blue color [96]. In the coordinate system, the center is colorless, as the b* values increase and away from the center, the color separation increases [97].

During the 105-day storage period of Kashar cheeses at different temperatures, the positive and negative coordinates and the changes in b* value, which expresses yellowness and blueness, were recorded every 2 weeks. The results showing the color b* value changes depending on temperature and time during the storage period of fresh kashar cheese are also given in Table 4.10. The One-Way and Two-Way ANOVA statistical analyzes were applied to determine effect of the different temperature and same duration of storage on the color b* value changes of kashar cheese. According to the ANOVA results, storage time and temperature significantly changed the color b* values of kashar cheese ($P<0.05$). Also the interaction between temperature and time were significantly effected to the color b* values ($P<0.05$).

When the results were examined, b* values increased until the 60^{th} day in the samples with 5°C, then decreased until the 90^{th} day, and a small increase was observed until the end of maturation, and the first and last values (18.71-18.03) were found close to each other ($P<0.05$). The b* values recorded as (18.32-16.42) and (18.93-17.50) showed a general decrease for temperatures of 15°C and 25°C, respectively. Unlike these, b* values increased from 18.05 to 23.97 in samples with 37°C. In general, the products were yellow in color and kashar cheeses stored at 37°C had the highest yellow color.

Table 4.10 The effects of different time and temperature on color b* values

Time (Day)	Color b* Values			
	5°C	**15°C**	**25°C**	**37°C**
0	$18.71\pm0.28^{a\text{-}A}$	$18.71\pm0.28^{a\text{-}A}$	$18.71\pm0.28^{a,b\text{-}A}$	$18.71\pm0.28^{a\text{-}A}$
15	$18.80\pm0.30^{a\text{-}A}$	$18.32\pm1.15^{a,b\text{-}A}$	$18.93\pm0.54^{b\text{-}A}$	$18.05\pm0.57^{a\text{-}A}$
30	$19.35\pm0.03^{a,b\text{-}A}$	$18.93\pm0.06^{a\text{-}A,B}$	$17.25\pm0.15^{c\text{-}C}$	$18.68\pm0.28^{a\text{-}B}$
45	$19.86\pm0.25^{b\text{-}A}$	$18.76\pm0.20^{a\text{-}B}$	$17.04\pm0.20^{c\text{-}C}$	$20.01\pm0.03^{b\text{-}A}$
60	$19.24\pm0.53^{a,b\text{-}A}$	$17.28\pm0.04^{b,c\text{-}B}$	$18.15\pm0.03^{a,d\text{-}A,B}$	$21.63\pm1.04^{c\text{-}C}$
75	$17.46\pm0.08^{c,d\text{-}A}$	$17.11\pm0.24^{c\text{-}A}$	$17.26\pm0.48^{c\text{-}A}$	$21.74\pm0.15^{c\text{-}B}$
90	$17.26\pm0.00^{c\text{-}A}$	$17.27\pm0.17^{b,c\text{-}A}$	$17.59\pm0.18^{c,d\text{-}A}$	$23.27\pm0.10^{d\text{-}B}$
105	$18.03\pm0.32^{d\text{-}A}$	$16.42\pm0.16^{c\text{-}B}$	$17.50\pm0.03^{c,d\text{-}C}$	$23.97\pm0.08^{d\text{-}D}$

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

4.6.4 Yellowness Index (YI) Value

The results showing the color yellowness index (YI) values changes depending on temperature and time during the storage period of fresh kashar cheese are also given in Table 4.11. The One-Way and Two-Way ANOVA statistical analyzes were applied to determine effect of the different temperature and same duration of storage on the color YI values changes of kashar cheese. According to the ANOVA results, storage time and temperature significantly changed the color YI values of kashar cheese ($P<0.05$). Also the interaction between temperature and time were significantly effected to the color YI values ($P<0.05$).

Table 4.11 The effects of different time and temperature on color YI values

Time (Day)	Color YI Values			
	5°C	**15°C**	**25°C**	**37°C**
0	$35.27\pm0.44^{a\text{-}A}$	$35.27\pm0.44^{a\text{-}A}$	$35.27\pm0.44^{a\text{-}A}$	$35.27\pm0.44^{a\text{-}A}$
15	$34.41\pm0.30^{a,b\text{-}A}$	$34.15\pm2.04^{a\text{-}A}$	$34.59\pm0.72^{a,b\text{-}A}$	$35.40\pm1.14^{a\text{-}A}$
30	$35.61\pm0.17^{a,c\text{-}A}$	$34.57\pm0.30^{a\text{-}A}$	$31.59\pm0.55^{c,d\text{-}B}$	$37.12\pm0.73^{a\text{-}C}$
45	$36.61\pm0.54^{c\text{-}A}$	$34.90\pm0.32^{a\text{-}B}$	$31.45\pm0.60^{c\text{-}C}$	$40.37\pm0.20^{b\text{-}D}$
60	$35.18\pm1.21^{a\text{-}A}$	$31.79\pm0.08^{b\text{-}A}$	$33.84\pm0.12^{b,e\text{-}A}$	$43.10\pm2.32^{c\text{-}C}$
75	$32.55\pm0.11^{d,e\text{-}A}$	$31.19\pm0.50^{b\text{-}A}$	$32.59\pm0.95^{c,d,e\text{-}A}$	$42.72\pm0.48^{c\text{-}C}$
90	$31.61\pm0.12^{d\text{-}A}$	$32.01\pm0.30^{b\text{-}A}$	$32.93\pm0.30^{d,e\text{-}B}$	$47.66\pm0.40^{d\text{-}C}$
105	$33.74\pm0.58^{b,e\text{-}A}$	$30.58\pm0.30^{b\text{-}B}$	$32.39\pm0.05^{c,d\text{-}C}$	$50.70\pm0.35^{e\text{-}D}$

Different small letter indicates statistical difference at $\alpha=0.05$ level in time at each column. Different capital letters indicate statistical difference at $\alpha=0.05$ level in temperature at each row.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this thesis, the physical and biochemical changes of kashar cheeses storaged at different temperatures (5°C, 15°C, 25°C, 37°C) were investigated during the storage period. In order to investigate these changes, Kashar cheese samples were stored at different temperatures (5°C, 15°C, 25°C, 37°C) for 105 days and the data were collected in 2-week periods.

According to the results, moisture content analyzes of kashar cheeses are of the desired quality in the appropriate storage and time period. In our samples, this was found to be suitable for the 5°C sample, but the samples at 25°C and 37°C, despite being vacuum packed, lost moisture and suffered from some microbial disorders and quality loss in terms of taste and odor.

The samples with pH values of 5°C and 15°C were found to be close to the storage of kashar cheeses under suitable conditions. Because coagulant enzymes and lactic acid bacteria estimated that can have a decreaser effect on pH in samples at 25°C and 37°C.

There was no significant difference in salt content. Protein values are among the important parameters in cheese production. Protein values increased during the maturation period and many studies in the literature supported this. In some samples, the difference was interpreted as the casein breaking down in various ways and becoming water-soluble due to quality materials and unsuitable methods. Similarly, an increase in WSN values was detected during maturation. This showed that storage temperature and time were effective in hydrolysis of rennets and casein.

TBARS values, which are indicators of lipid oxidation, are important for Kashar cheese. Because lipid oxidation causes many unsuitable conditions such as bad taste,

spoilage and nutrient loss in kashar cheese. In our samples, some increases (in the range of 2-4 weeks) and decreases after storage were detected.

Color is an important criterion in kashar cheese as in many products. In our samples, the L* values of kashar cheeses were generally found high and they took a brighter and brighter appearance, and the a* values were generally determined on the green axis, and the positive color, that is, the yellow color, was found to be dominant in the b* values. These results similar to the appropriate color range desired in kashar cheeses.

As a result, the physical and biochemical maturation of Kashar cheeses at different temperatures, the detection of possible damages and deterioration and faulty processes, and the study of producing and consuming quality and safe products shed light on everyone in making production processes and products more beautiful, and by providing benefit in quality and safe food production and makes life easier.

5.2 Recommendations

Although it is generally for all food products, the packaging developed in accordance with the quality standard affects many food product properties physically and biochemically when used in vacuumed foods. The use of vacuum packaged products and the ripening of fresh kashar cheeses at appropriate temperatures and times will positively affect many quality parameters such as pH, moisture and physical and biochemical changes. As general the kashar cheese should be stored at the range of 105-120 days and it should be preserved in the range of 5°C - 10°C and should be natural coagulations enzymes and quality process.

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APPENDICES

Appendix A

One way ANOVA analysis

Table A.1 ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
pH 5°C	Between Groups	1,116	7	,159	245,313	,000
	Within Groups	,005	8	,001		
	Total	1,121	15			
pH 15°C	Between Groups	1,658	7	,237	398,868	,000
	Within Groups	,005	8	,001		
	Total	1,663	15			
pH 25°C	Between Groups	,992	7	,142	188,986	,000
	Within Groups	,006	8	,001		
	Total	,998	15			
pH 37°C	Between Groups	1,769	7	,253	381,385	,000
	Within Groups	,005	8	,001		
	Total	1,774	15			

Table A.1.1 pH 5°C

Duncan[a]

		Subset for alpha = 0.05					
Time Day	N	1	2	3	4	5	6
75	2	5,8800					
90	2		5,9500				
105	2			6,0350			
30	2				6,4250		
60	2				6,4300		
45	2				6,4700	6,4700	
15	2					6,5050	
0	2						6,5950
Sig.		1,000	1,000	1,000	,129	,207	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.1.2 pH 15°C

Duncan[a]

		Subset for alpha = 0.05				
Time Day	N	1	2	3	4	5
75	2	5,6950				
105	2	5,7450				
60	2		5,9050			
90	2		5,9050			
45	2			6,2700		
30	2			6,3050		
15	2				6,4850	
0	2					6,5950
Sig.		,074	1,000	,189	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.1.3 pH 25°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
45	2	5,8250			
30	2	5,8600			
15	2		6,1800		
60	2		6,2100		
90	2		6,2100		
75	2		6,2450		
105	2			6,4850	
0	2				6,5950
Sig.		,237	,057	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.1.4 pH 37°C

Duncan[a]

		Subset for alpha = 0.05							
Time Day	N	1	2	3	4	5	6	7	8
15	2	5,7150							
30	2		5,8400						
90	2			6,1450					
60	2				6,2150				
105	2					6,4250			
75	2						6,5200		
0	2							6,5950	
45	2								6,6950
Sig.		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.2 ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Moisture	Between Groups	5,153	7	,736	10,738	,002
Content 5°C	Within Groups	,548	8	,069		
	Total	5,701	15			
Moisture	Between Groups	2,782	7	,397	8,317	,004
Content	Within Groups	,382	8	,048		
15°C	Total	3,164	15			
Moisture	Between Groups	20,380	7	2,911	35,023	,000
Content	Within Groups	,665	8	,083		
25°C	Total	21,045	15			
Moisture	Between Groups	123,605	7	17,658	158,349	,000
Content	Within Groups	,892	8	,112		
37°C	Total	124,497	15			

Table A.2.1 Moisture content 5°C

Duncan[a]

		Subset for alpha = 0.05		
Time Day	N	1	2	3
75	2	47,4800		
30	2		48,1950	
105	2		48,3150	
0	2			48,9300
60	2			48,9650
45	2			48,9950
15	2			49,1500
90	2			49,2350
Sig.		1,000	,659	,308

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.2.2 Moisture content 15°C

Duncan[a]

		Subset for alpha = 0.05	
Time Day	N	1	2
105	2	47,9000	
0	2		48,9300
45	2		48,9300
30	2		49,0250
60	2		49,0300
15	2		49,1150
75	2		49,1950
90	2		49,3850
Sig.		1,000	,092

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.2.3 Moisture content 25°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
105	2	45,8600			
90	2	45,9500			
75	2		46,8300		
30	2		47,3500	47,3500	
60	2			47,8900	
45	2			47,9950	
0	2				48,9300
15	2				48,9800
Sig.		,763	,109	,064	,867

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.2.4 Moisture content 37°C

Duncan[a]

		Subset for alpha = 0.05				
Time Day	N	1	2	3	4	5
90	2	40,1150				
105	2	40,4500				
75	2		42,3150			
60	2		42,5350	42,5350		
30	2			43,2450		
45	2				45,4850	
15	2				45,8050	
0	2					48,9300
Sig.		,345	,529	,066	,366	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.3 ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Protein 5°C	Between Groups	127,261	7	18,180	87,681	,000
	Within Groups	1,659	8	,207		
	Total	128,920	15			
Protein 15°C	Between Groups	91,634	7	13,091	40,509	,000
	Within Groups	2,585	8	,323		
	Total	94,220	15			
Protein 25°C	Between Groups	231,844	7	33,121	207,636	,000
	Within Groups	1,276	8	,160		
	Total	233,120	15			
Protein 37°C	Between Groups	177,540	7	25,363	157,210	,000
	Within Groups	1,291	8	,161		
	Total	178,831	15			

Table A.3.1 Protein 5°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
15	2	19,0150			
0	2	19,3650			
75	2		23,8350		
45	2		23,8600		
105	2		24,8650	24,8650	
30	2			24,9650	
90	2			25,4950	
60	2				27,7850
Sig.		,464	,062	,221	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.3.2 Protein 15°C

Duncan[a]

		Subset for alpha = 0.05				
Time Day	N	1	2	3	4	5
0	2	19,3650				
15	2	19,7000				
45	2		22,8300			
75	2		23,6900	23,6900		
30	2		23,9700	23,9700	23,9700	
60	2			24,9450	24,9450	
105	2				25,3050	25,3050
90	2					26,4350
Sig.		,572	,091	,067	,054	,082

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.3.3 Protein 25°C

Duncan[a]

		Subset for alpha = 0.05				
Time Day	N	1	2	3	4	5
0	2	19,3650				
15	2	19,5250				
45	2		23,6600			
30	2			24,7900		
75	2			25,3350	25,3350	
60	2				25,8750	
105	2				26,1150	
90	2					32,1750
Sig.		,699	1,000	,210	,098	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.3.4 Protein 37°C

Duncan[a]

		Subset for alpha = 0.05					
Time Day	N	1	2	3	4	5	6
0	2	19,3650					
15	2	20,1700					
45	2		23,7050				
30	2			25,1050			
90	2				26,3850		
60	2					27,4950	
75	2					28,2600	28,2600
105	2						28,6500
Sig.		,080	1,000	1,000	1,000	,093	,360

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.4 ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Water Soluble	Between Groups	1,000	7	,143	10,064	,002
Nitrogen 5°C	Within Groups	,114	8	,014		
	Total	1,114	15			
Water Soluble	Between Groups	,698	7	,100	17,761	,000
Nitrogen 15°C	Within Groups	,045	8	,006		
	Total	,743	15			
Water Soluble	Between Groups	2,883	7	,412	527,149	,000
Nitrogen 25°C	Within Groups	,006	8	,001		
	Total	2,889	15			
Water Soluble	Between Groups	6,663	7	,952	536,223	,000
Nitrogen 37°C	Within Groups	,014	8	,002		
	Total	6,677	15			

Table A.4.1 Water soluble nitrogen 5°C

Duncan[a]

		Subset for alpha = 0.05	
Time Day	N	1	2
0	2	,2400	
15	2		,7800
30	2		,7950
90	2		,9250
75	2		,9550
105	2		,9850
45	2		1,0300
60	2		1,0800
Sig.		1,000	,050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.4.2 Water soluble nitrogen 15°C

Duncan[a]

		Subset for alpha = 0.05		
Time Day	N	1	2	3
0	2	,2400		
75	2		,7000	
15	2		,7400	,7400
30	2		,7550	,7550
105	2		,8750	,8750
45	2			,9000
90	2			,9000
60	2			,9100
Sig.		1,000	,060	,070

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.4.3 Water soluble nitrogen 25°C

Duncan[a]

		Subset for alpha = 0.05						
Time Day	N	1	2	3	4	5	6	7
0	2	,2400						
15	2		,6700					
30	2			,8900				
45	2				1,0050			
60	2					1,0900		
90	2						1,4500	
105	2						1,5100	1,5100
75	2							1,5400
Sig.		1,000	1,000	1,000	1,000	1,000	,064	,314

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.4.4 Water soluble nitrogen 37°C

Duncan[a]

		Subset for alpha = 0.05					
Time Day	N	1	2	3	4	5	6
0	2	,2400					
15	2		,9300				
30	2			1,3750			
45	2				1,7550		
60	2					1,9800	
75	2					2,0150	
90	2						2,1450
105	2						2,1900
Sig.		1,000	1,000	1,000	1,000	,430	,317

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.5 ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TCA 5°C	Between Groups	,050	7	,007	5,810	,012
	Within Groups	,010	8	,001		
	Total	,060	15			
TCA 15°C	Between Groups	,094	7	,013	14,905	,001
	Within Groups	,007	8	,001		
	Total	,101	15			
TCA 25°C	Between Groups	,564	7	,081	59,661	,000
	Within Groups	,011	8	,001		
	Total	,575	15			
TCA 37°C	Between Groups	1,830	7	,261	43,488	,000
	Within Groups	,048	8	,006		
	Total	1,878	15			

Trichloroacetic Acid Soluble Nitrogen (TCA-SN)

Table A.5.1 Trichloroacetic acid soluble nitrogen (TCA-SN) 5°C

Duncan[a]

		Subset for alpha = 0.05	
Time Day	N	1	2
60	2	,1100	
15	2	,1140	
30	2	,1750	,1750
75	2		,2100
90	2		,2400
105	2		,2400
0	2		,2500
45	2		,2550
Sig.		,113	,069

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.5.2 Trichloroacetic acid soluble nitrogen (TCA-SN) 15°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
45	2	,1500			
60	2	,1500			
15	2	,1700			
30	2	,2000	,2000		
0	2		,2500	,2500	
90	2		,2500	,2500	
105	2			,3100	
75	2				,3800
Sig.		,156	,149	,091	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.5.3 Trichloroacetic acid soluble nitrogen (TCA-SN) 25°C

Duncan[a]

		Subset for alpha = 0.05		
Time Day	N	1	2	3
15	2	,1900		
0	2	,2500		
30	2		,4050	
60	2		,4150	
45	2		,4350	
90	2			,6450
75	2			,6900
105	2			,7300
Sig.		,141	,456	,057

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.5.4 Trichloroacetic acid soluble nitrogen (TCA-SN) 37°C

Duncan[a]

		Subset for alpha = 0.05				
Time Day	N	1	2	3	4	5
0	2	,2500				
15	2	,3700				
30	2		,7300			
45	2		,8150	,8150		
60	2			,9350	,9350	
75	2				1,0600	
90	2				1,1000	
105	2					1,3000
Sig.		,160	,305	,160	,075	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.6 ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TBARS 5°C	Between Groups	,001	7	,000	16,342	,000
	Within Groups	,000	8	,000		
	Total	,001	15			
TBARS 15°C	Between Groups	,001	7	,000	24,332	,000
	Within Groups	,000	8	,000		
	Total	,001	15			
TBARS 25°C	Between Groups	,000	7	,000	24,818	,000
	Within Groups	,000	8	,000		
	Total	,000	15			
TBARS 37°C	Between Groups	,000	7	,000	11,822	,001
	Within Groups	,000	8	,000		
	Total	,000	15			

TBARS (2-Thiobarbituric Acid Reactive Substances)

Table A.6.1 TBARS 5°C

Duncan[a]

		Subset for alpha = 0.05		
Time Day	N	1	2	3
75	2	-,0014		
60	2	,0014	,0014	
15	2	,0018	,0018	
90	2	,0019	,0019	
105	2	,0024	,0024	
30	2		,0047	
45	2			,0132
0	2			,0177
Sig.		,170	,220	,090

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.6.2 TBARS 15°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
105	2	,0008			
90	2	,0014			
15	2	,0023			
75	2	,0023			
30	2	,0039	,0039		
60	2		,0069		
45	2			,0120	
0	2				,0177
Sig.		,139	,115	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.6.3 TBARS 25°C

Duncan[a]

		Subset for alpha = 0.05				
Time Day	N	1	2	3	4	5
75	2	,0007				
105	2	,0017	,0017			
90	2	,0020	,0020			
30	2	,0028	,0028	,0028		
15	2		,0057	,0057		
60	2			,0066	,0066	
45	2				,0103	
0	2					,0177
Sig.		,264	,051	,056	,053	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.6.4 TBARS 37°C

Duncan[a]

		Subset for alpha = 0.05		
Time Day	N	1	2	3
60	2	,0022		
105	2	,0023		
15	2	,0025		
90	2	,0031		
75	2	,0048		
30	2	,0060		
45	2		,0120	
0	2			,0177
Sig.		,170	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.7 ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Color L*	Between Groups	20,075	7	2,868	34,123	,000
Value 5°C	Within Groups	,672	8	,084		
	Total	20,747	15			
Color L*	Between Groups	40,659	7	5,808	113,639	,000
Value 15°C	Within Groups	,409	8	,051		
	Total	41,068	15			
Color L*	Between Groups	39,459	7	5,637	63,467	,000
Value 25°C	Within Groups	,711	8	,089		
	Total	40,170	15			
Color L*	Between Groups	53,701	7	7,672	34,002	,000
Value 37°C	Within Groups	1,805	8	,226		
	Total	55,506	15			

Table A.7.1 Color L* value 5°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
0	2	78,7000			
45	2		79,7800		
30	2		79,9250		
60	2		80,2650	80,2650	
105	2		80,4600	80,4600	
75	2		80,4800	80,4800	
15	2			80,9400	
90	2				82,8950
Sig.		1,000	,056	,060	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.7.2 Color L* value 15°C

Duncan[a]

		Subset for alpha = 0.05					
Time Day	N	1	2	3	4	5	6
0	2	78,7000					
15	2	78,8200	78,8200				
45	2		79,2750				
30	2			80,5050			
60	2			81,0250	81,0250		
90	2				81,2550		
105	2					82,1150	
75	2						83,5850
Sig.		,610	,079	,050	,339	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.7.3 Color L* value 25°C

Duncan[a]

		Subset for alpha = 0.05					
Time Day	N	1	2	3	4	5	6
0	2	78,7000					
75	2		81,6350				
15	2		81,9600	81,9600			
60	2			82,6100	82,6100		
30	2				83,0150	83,0150	
90	2					83,5100	83,5100
105	2					83,6650	83,6650
45	2						83,8200
Sig.		1,000	,307	,061	,211	,070	,347

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.7.4 Color L* value 37°C

Duncan[a]

		Subset for alpha = 0.05					
Time Day	N	1	2	3	4	5	6
105	2	74,7550					
90	2		77,4800				
45	2		78,0600	78,0600			
0	2			78,7000	78,7000		
60	2				79,2850	79,2850	
30	2					79,9500	79,9500
15	2						80,5400
75	2						80,7250
Sig.		1,000	,257	,215	,253	,199	,156

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.8 ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Color a* 5°C	Between Groups	,717	7	,102	2,133	,155
	Within Groups	,384	8	,048		
	Total	1,101	15			
Color a* 15°C	Between Groups	1,026	7	,147	18,596	,000
	Within Groups	,063	8	,008		
	Total	1,089	15			
Color a* 25°C	Between Groups	2,539	7	,363	20,564	,000
	Within Groups	,141	8	,018		
	Total	2,680	15			
Color a* 37°C	Between Groups	36,759	7	5,251	153,238	,000
	Within Groups	,274	8	,034		
	Total	37,033	15			

Table A.8.1 Color a* 5°C

Duncan[a]

		Subset for alpha = 0.05	
Time Day	N	1	2
60	2	-2,1000	
30	2	-1,9750	-1,9750
45	2	-1,8650	-1,8650
75	2	-1,6750	-1,6750
0	2	-1,6300	-1,6300
15	2	-1,6250	-1,6250
90	2	-1,5500	-1,5500
105	2		-1,4350
Sig.		,051	,054

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.8.2 Color a* 15°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
30	2	-2,1550			
15	2	-2,0400	-2,0400		
60	2	-1,9650	-1,9650		
45	2		-1,9150		
90	2			-1,6650	
0	2			-1,6300	
75	2			-1,5500	-1,5500
105	2				-1,3750
Sig.		,074	,214	,249	,084

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.8.3 Color a* 25°C

Duncan[a]

		Subset for alpha = 0.05		
Time Day	N	1	2	3
15	2	-1,6550		
0	2	-1,6300		
30	2	-1,5200		
45	2		-1,0550	
105	2		-,8900	
75	2		-,8600	
60	2		-,8400	
90	2			-,5200
Sig.		,358	,166	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.8.4 Color a* 37°C

Duncan[a]

		Subset for alpha = 0.05					
Time Day	N	1	2	3	4	5	6
0	2	-1,6300					
15	2		,2800				
30	2			,8200			
45	2			1,1850			
60	2				1,7500		
75	2				1,7850		
90	2					2,9800	
105	2						3,5950
Sig.		1,000	1,000	,084	,855	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.9 ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Color b* 5°C	Between Groups	12,063	7	1,723	22,705	,000
	Within Groups	,607	8	,076		
	Total	12,671	15			
Color b* 15°C	Between Groups	12,408	7	1,773	9,054	,003
	Within Groups	1,566	8	,196		
	Total	13,974	15			
Color b* 25°C	Between Groups	7,023	7	1,003	11,673	,001
	Within Groups	,688	8	,086		
	Total	7,711	15			
Color b* 37°C	Between Groups	69,512	7	9,930	49,447	,000
	Within Groups	1,607	8	,201		
	Total	71,119	15			

Table A.9.1 Color b* 5°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
90	2	17,2600			
75	2	17,4650	17,4650		
105	2		18,0300		
0	2			18,7100	
15	2			18,7950	
60	2			19,2450	19,2450
30	2			19,3550	19,3550
45	2				19,8600
Sig.		,478	,074	,059	,065

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.9.2 Color b* 15°C

Duncan[a]

		Subset for alpha = 0.05		
Time Day	N	1	2	3
105	2	16,4250		
75	2	17,1100		
90	2	17,2750	17,2750	
60	2	17,2800	17,2800	
15	2		18,3150	18,3150
0	2			18,7100
45	2			18,7650
30	2			18,9350
Sig.		,107	,054	,224

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.9.3 Color b* 25°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
45	2	17,0450			
30	2	17,2550			
75	2	17,2650			
105	2	17,5050	17,5050		
90	2	17,5900	17,5900		
60	2		18,1500	18,1500	
0	2			18,7100	18,7100
15	2				18,9300
Sig.		,122	,068	,093	,474

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.9.4 Color b* 37°C

Duncan[a]

		Subset for alpha = 0.05			
Time Day	N	1	2	3	4
15	2	18,0550			
30	2	18,6800			
0	2	18,7100			
45	2		20,0050		
60	2			21,6350	
75	2			21,7450	
90	2				23,2700
105	2				23,9700
Sig.		,198	1,000	,812	,157

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.10 ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Color YI 5°C	Between Groups	38,736	7	5,534	18,114	,000
	Within Groups	2,444	8	,305		
	Total	41,180	15			
Color YI 15°C	Between Groups	48,260	7	6,894	11,082	,001
	Within Groups	4,977	8	,622		
	Total	53,237	15			
Color YI 25°C	Between Groups	26,537	7	3,791	12,643	,001
	Within Groups	2,399	8	,300		
	Total	28,936	15			
Color YI 37°C	Between Groups	446,184	7	63,741	63,804	,000
	Within Groups	7,992	8	,999		
	Total	454,177	15			

Yellowness Index (YI)

Table A.10.1 Color YI 5°C

Duncan[a]

		Subset for alpha = 0.05				
Tıme Day	N	1	2	3	4	5
90	2	31,6150				
75	2	32,5500	32,5500			
105	2		33,7450	33,7450		
15	2			34,4150	34,4150	
60	2				35,1850	
0	2				35,2750	
30	2				35,6150	35,6150
45	2					36,6150
Sig.		,129	,063	,260	,076	,108

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.10.2 Color YI 15°C

Duncan[a]

		Subset for alpha = 0.05	
Tıme Day	N	1	2
105	2	30,5800	
75	2	31,1950	
60	2	31,7900	
90	2	32,0100	
15	2		34,1550
30	2		34,5750
45	2		34,9000
0	2		35,2750
Sig.		,127	,218

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.10.3 Color YI 25°C

Duncan[a]

		Subset for alpha = 0.05				
Tıme Day	N	1	2	3	4	5
45	2	31,4550				
30	2	31,5900	31,5900			
105	2	32,3900	32,3900			
75	2	32,5950	32,5950	32,5950		
90	2		32,9350	32,9350		
60	2			33,8400	33,8400	
15	2				34,5900	34,5900
0	2					35,2750
Sig.		,086	,050	,061	,208	,246

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Table A.10.4 Color YI 37°C

Duncan[a]

		Subset for alpha = 0.05				
Time Day	N	1	2	3	4	5
0	2	35,2750				
15	2	35,3950				
30	2	37,1250				
45	2		40,3750			
75	2			42,7200		
60	2			43,0950		
90	2				47,6650	
105	2					50,7000
Sig.		,114	1,000	,717	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Appendix B

Univariate ANOVA analysis

Table B.1 Tests of between-subjects effects

Dependent Variable: pH

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	5,831[a]	31	,188	283,252	,000
Intercept	2473,943	1	2473,943	3725467,485	,000
Temperature	,296	3	,099	148,671	,000
Time Day	1,694	7	,242	364,318	,000
Temperature * Time Day	3,841	21	,183	275,455	,000
Error	,021	32	,001		
Total	2479,796	64			
Corrected Total	5,852	63			

a. R Squared = ,996 (Adjusted R Squared = ,993)

Table B.1.1 pH

Duncan[a,b]

Temperature	N	Subset 1	Subset 2	Subset 3
15,00	16	6,1131		
25,00	16		6,2013	
37,00	16			6,2688
5,00	16			6,2863
Sig.		1,000	1,000	,064

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,001.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.1.2 pH

Duncan[a,b]

		Subset					
Time Day	N	1	2	3	4	5	6
90	8	6,0525					
75	8		6,0850				
30	8		6,1075				
105	8			6,1725			
60	8			6,1900			
15	8				6,2213		
45	8					6,3150	
0	8						6,5950
Sig.		1,000	,090	,184	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,001.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.2 Tests of between-subjects effects

Dependent Variable: Moisture Content

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	441,656[a]	31	14,247	183,255	,000
Intercept	142400,570	1	142400,570	1831665,820	,000
Temperature	289,736	3	96,579	1242,268	,000
Time Day	69,462	7	9,923	127,639	,000
Temperature * Time Day	82,458	21	3,927	50,507	,000
Error	2,488	32	,078		
Total	142844,713	64			
Corrected Total	444,143	63			

a. R Squared = ,994 (Adjusted R Squared = ,989)

Table B.2.1 Moisture content

Duncan[a,b]

		Subset			
Temperature	N	1	2	3	4
37,00	16	43,6100			
25,00	16		47,4731		
5,00	16			48,6581	
15,00	16				48,9388
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,078.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.2.2 Moisture content

Duncan[a,b]

		Subset					
Time Day	N	1	2	3	4	5	6
105	8	45,6312					
90	8		46,1713				
75	8		46,4550				
30	8			46,9538			
60	8			47,1050			
45	8				47,8513		
15	8					48,2625	
0	8						48,9300
Sig.		1,000	,050	,286	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,078.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.3 Tests of between-subjects effects

Dependent Variable: Protein

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	656,416[a]	31	21,175	99,489	,000
Intercept	37191,123	1	37191,123	174742,085	,000
Temperature	28,136	3	9,379	44,066	,000
Time Day	540,753	7	77,250	362,960	,000
Temperature * Time Day	87,527	21	4,168	19,583	,000
Error	6,811	32	,213		
Total	37854,349	64			
Corrected Total	663,227	63			

a. R Squared = ,990 (Adjusted R Squared = ,980)

Table B.3.1 Protein

Duncan[a,b]

Temperature	N	Subset 1	Subset 2	Subset 3
15,00	16	23,2800		
5,00	16		23,6481	
25,00	16			24,6050
37,00	16			24,8919
Sig.		1,000	1,000	,088

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,213.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.3.2 Protein

Duncan[a,b]

		Subset					
Time Day	N	1	2	3	4	5	6
0	8	19,3650					
15	8	19,6025					
45	8		23,5137				
30	8			24,7075			
75	8				25,2800		
105	8					26,2338	
60	8					26,5250	
90	8						27,6225
Sig.		,311	1,000	1,000	1,000	,216	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,213.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.4 Tests of between-subjects effects

Dependent Variable: Water Soluble Nitrogen

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	17,777[a]	31	,573	102,548	,000
Intercept	71,550	1	71,550	12794,716	,000
Temperature	6,534	3	2,178	389,466	,000
Time Day	8,572	7	1,225	218,968	,000
Temperature * Time Day	2,672	21	,127	22,753	,000
Error	,179	32	,006		
Total	89,507	64			
Corrected Total	17,956	63			

a. R Squared = ,990 (Adjusted R Squared = ,980)

Table B.4.1 Water soluble nitrogen

Duncan[a,b]

		Subset			
Temperature	N	1	2	3	4
15,00	16	,7525			
5,00	16		,8487		
25,00	16			1,0494	
37,00	16				1,5788
Sig.		1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,006.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.4.2 Water soluble nitrogen

Duncan[a,b]

		Subset						
Time Day	N	1	2	3	4	5	6	7
0	8	,2400						
15	8		,7800					
30	8			,9537				
45	8				1,1725			
60	8					1,2650		
75	8					1,3025	1,3025	
90	8						1,3550	1,3550
105	8							1,3900
Sig.		1,000	1,000	1,000	1,000	,323	,170	,356

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,006.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.5 Tests of between-subjects effects

Dependent Variable: Trichloroacetic Acid Soluble Nitrogen (TCA-SN)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6,473[a]	31	,209	88,022	,000
Intercept	11,858	1	11,858	4998,500	,000
Temperature	3,935	3	1,312	552,951	,000
Time Day	1,368	7	,195	82,363	,000
Temperature * Time Day	1,170	21	,056	23,489	,000
Error	,076	32	,002		
Total	18,407	64			
Corrected Total	6,549	63			

a. R Squared = ,988 (Adjusted R Squared = ,977)

Table B.5.1 Trichloroacetic acid soluble nitrogen (TCA-SN)

Duncan[a,b]

Temperature	N	Subset 1	Subset 2	Subset 3
5,00	16	,1993		
15,00	16	,2325		
25,00	16		,4700	
37,00	16			,8200
Sig.		,062	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,002.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.5.2 Trichloroacetic acid soluble nitrogen (TCA-SN)

Duncan[a,b]

		Subset			
Time Day	N	1	2	3	4
15	8	,2110			
0	8	,2500			
30	8		,3775		
60	8		,4025		
45	8		,4138		
90	8			,5588	
75	8			,5850	
105	8				,6450
Sig.		,119	,169	,289	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,002.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.6 Tests of between-subjects effects

Dependent Variable: TBARS (2-Thiobarbituric Acid Reactive Substances)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	,002[a]	31	6,605E-5	16,038	,000
Intercept	,002	1	,002	531,731	,000
Temperature	1,016E-5	3	3,386E-6	,822	,491
Time Day	,002	7	,000	66,311	,000
Temperature * Time Day	,000	21	5,987E-6	1,454	,166
Error	,000	32	4,118E-6		
Total	,004	64			
Corrected Total	,002	63			

a. R Squared = ,940 (Adjusted R Squared = ,881)

Table B.6.1 TBARS (2-Thiobarbituric acid reactive substances)

Duncan[a,b]

		Subset
Temperature	N	1
5,00	16	,0052
15,00	16	,0059
25,00	16	,0059
37,00	16	,0063
Sig.		,170

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4,12E-006.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.6.2 TBARS (2-Thiobarbituric acid reactive substances)

Duncan[a,b]

		Subset			
Time Day	N	1	2	3	4
75	8	,0016			
105	8	,0018			
90	8	,0021			
15	8	,0031	,0031		
60	8		,0043		
30	8		,0043		
45	8			,0119	
0	8				,0177
Sig.		,191	,250	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4,12E-006.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.7 Tests of between-subjects effects

Dependent Variable: Color L* Values

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	262,513[a]	31	8,468	75,341	,000
Intercept	415101,551	1	415101,551	3693125,632	,000
Temperature	108,619	3	36,206	322,125	,000
Time Day	43,341	7	6,192	55,085	,000
Temperature * Time Day	110,553	21	5,264	46,837	,000
Error	3,597	32	,112		
Total	415367,660	64			
Corrected Total	266,110	63			

a. R Squared = ,986 (Adjusted R Squared = ,973)

Table B.7.1 Color L* values

Duncan[a,b]

Temperature	N	Subset 1	Subset 2	Subset 3
37,00	16	78,6869		
5,00	16		80,4306	
15,00	16		80,6600	
25,00	16			82,3644
Sig.		1,000	,062	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,112.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.7.2 Color L* values

Duncan[a,b]

		Subset			
Time Day	N	1	2	3	4
0	8	78,7000			
45	8		80,2338		
105	8		80,2488		
15	8		80,5650	80,5650	
60	8			80,7963	
30	8			80,8488	
90	8				81,2850
75	8				81,6063
Sig.		1,000	,070	,119	,064

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,112.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.8 Tests of between-subjects effects

Dependent Variable: Color a* Values

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	145,793[a]	31	4,703	174,478	,000
Intercept	43,412	1	43,412	1610,541	,000
Temperature	104,752	3	34,917	1295,408	,000
Time Day	16,783	7	2,398	88,946	,000
Temperature * Time Day	24,258	21	1,155	42,855	,000
Error	,863	32	,027		
Total	190,067	64			
Corrected Total	146,655	63			

a. R Squared = ,994 (Adjusted R Squared = ,988)

Table B.8.1 Color a* values

Duncan[a,b]

		Subset		
Temperature	N	1	2	3
15,00	16	-1,7869		
5,00	16	-1,7319		
25,00	16		-1,1212	
37,00	16			1,3456
Sig.		,350	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,027.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.8.2 Color a* values

Duncan[a,b]

		Subset				
Time Day	N	1	2	3	4	5
0	8	-1,6300				
15	8		-1,2600			
30	8		-1,2075			
45	8			-,9125		
60	8			-,7887		
75	8				-,5750	
90	8					-,1887
105	8					-,0263
Sig.		1,000	,527	,141	1,000	,056

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,027.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.9 Tests of between-subjects effects

Dependent Variable: Color b* Values

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	193,134[a]	31	6,230	44,624	,000
Intercept	22504,125	1	22504,125	161188,098	,000
Temperature	92,127	3	30,709	219,957	,000
Time Day	3,320	7	,474	3,397	,008
Temperature * Time Day	97,687	21	4,652	33,319	,000
Error	4,468	32	,140		
Total	22701,727	64			
Corrected Total	197,602	63			

a. R Squared = ,977 (Adjusted R Squared = ,955)

Table B.9.1 Color b* values

Duncan[a,b]

Temperature	N	Subset 1	Subset 2	Subset 3
25,00	16	17,8062		
15,00	16	17,8519		
5,00	16		18,5900	
37,00	16			20,7588
Sig.		,732	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,140.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.9.2 Color b* values

Duncan[a,b]

		Subset		
Time Day	N	1	2	3
75	8	18,3963		
15	8	18,5238	18,5238	
30	8	18,5563	18,5563	
0	8	18,7100	18,7100	18,7100
90	8		18,8488	18,8488
45	8		18,9188	18,9188
105	8			18,9825
60	8			19,0775
Sig.		,134	,066	,087

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,140.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

Table B.10 Tests of between-subjects effects

Dependent Variable: Yellowness Index (YI)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	1352,984[a]	31	43,645	78,410	,000
Intercept	80729,147	1	80729,147	145034,496	,000
Temperature	793,266	3	264,422	475,049	,000
Time Day	35,299	7	5,043	9,059	,000
Temperature * Time Day	524,419	21	24,972	44,864	,000
Error	17,812	32	,557		
Total	82099,943	64			
Corrected Total	1370,796	63			

a. R Squared = ,987 (Adjusted R Squared = ,974)

Table B.10.1 Yellowness index (YI)

Duncan[a,b]

		Subset		
Temperature	N	1	2	3
15,00	16	33,0600		
25,00	16	33,0838		
5,00	16		34,3769	
37,00	16			41,5437
Sig.		,929	1,000	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,557.

a. Uses Harmonic Mean Sample Size = 16,000.

b. Alpha = ,05.

Table B.10.2 Yellowness index (YI)

Duncan[a,b]

		Subset		
Time Day	N	1	2	3
15	8	34,6388		
30	8	34,7263		
75	8	34,7650		
0	8	35,2750	35,2750	
45	8		35,8363	
60	8		35,9775	
90	8		36,0563	
105	8			36,8538
Sig.		,128	,063	1,000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = ,557.

a. Uses Harmonic Mean Sample Size = 8,000.

b. Alpha = ,05.

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