RHEOLOGICAL PROPERTIES OF REDUCED-FAT GAZIANTEP CHEESE

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This thesis is dedicated to my mother.

ABSTRACT

RHEOLOGICAL PROPERTIES OF REDUCED-FAT GAZIANTEP CHEESE

KAHYAOĞLU, Talip M.Sc. in Food Engineering Supervisor: Assist. Prof. Dr. Sevim KAYA January 2002, 76 pages

The effects of fat reduction, heat treatments and storage temperatures (13 and 25°C) on the physical and chemical properties of Gaziantep cheese were investigated. Three different heat treatment temperatures (75, 85 and 95°C) were applied to three different fat (50.4, 33.4, 13.5%) containing cheese samples. With and without heat treatment, samples were analyzed with respect to viscoelastic parameters, color, meltability and texture. Decreasing fat content of the cheeses increased the hardness and elasticity. Heat treatments also imparted elasticity to the cheese. It was found that melting temperature of the cheese inversely related with fat content and temperature of the heat treatment. The gel strength constants of Gaziantep cheese were found in the range of 0.18-0.22. Differences in physical properties of the cheese in which fat content was reduced by 30 % with full-fat Gaziantep cheese were in an acceptable level. Heat treatment can be used for improving physical properties of fat-reduced Gaziantep cheese. Whiteness (L-value) significantly changed by the effect of fat reduction and heat treatment. It was observed that increase in storage temperature decreased whiteness and increased hardness of the samples.

Key Words: Gaziantep cheese, reduced-fat cheese, cheese rheology, viscoelastic properties, creep test, meltability, color.

ÖZET

AZ YAĞLI GAZİANTEP PEYNİRİNİN REOLOJİK ÖZELLİKLERİ

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Yağ miktarının azaltılması, ısıl işlemler ve saklama sıcaklığının (13 ve 25°C) Gaziantep peynirinin fiziksel ve kimyasal özelliklerine olan etkisi incelendi. Üç farklı ısıl işlem sıcaklıkları (75, 85 ve 95°C) üç farklı yağ (%50,4, %33,4, %13,5) içeren peynir örneklerine uygulandı. İsıl işlem uygulanan ve uygulanmayan örneklerin, viskoelastik parametre, renk, erime ve dokusal özellikleri açısından analiz edildi. Peynirlerin yağ miktarının azaltılması peynirin sertliğini ve elastikiyet özelliğini arttırdı. İsıl işlemde peynirlere elastikiyet kazandırdı. Peynirin erime sıcaklığının yağ miktarı ve ısıl işlemin sıcaklığı ile ters orantılı olduğu bulundu. Gaziantep peynirlerinin jel mukavemet sabitleri 0,18-0,22 arasında bulunmuştur. Yağ miktarı %30 oranında düşürülmüş Gaziantep peynirleri ile tam yağlı Gaziantep peynirlerinin fiziksel özellikleri arasındaki fark kabul edilebilir seviyededir. İsıl işlem yağ miktarı azaltılmış Gaziantep peynirlerinin fiziksel özelliklerini geliştirmek amacıyla kullanılabilir. Beyazlık (L-değeri) yağ miktarıyla ve ısıl işlemle önemli oranda değişmiştir. Saklama sıcaklığının artması beyazlığı azaltıp ve sertliği arttırdığı gözlendi.

Anahtar kelimeler: Gaziantep peyniri, az yağlı peynir, peynir reolojisi, viskolastik parametreler, sünme testi, eriyebilirlik, renk.

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LIST OF ABBREVIATIONS

 ω : Frequency

 σ : Shear stress

γ : Shear strain

η : Viscosity

75D : Heat treated at 75°C

Heat treated at 85°C

95D : Heat treated at 95°C

ANOVA : Analysis of Variance

AOAC : Association of Official Analytical Chemists

d : Day

db : Dry base

FDM : Fat in Dry Matter

FFC : Full Fat Cheese

FFM : Full Fat Milk

G : Modulus of Elasticity

G': Storage modulus

G": Loss modulus

h : Height

 h_0 : Initial height

HT : Heat treatment

Hz : Hertz

J : Compliance

L : Length

LFC : Low Fat Cheese

LFM : Low Fat Milk

LSD : Least Significant Difference

MNFS : Moisture in Non Fat Substance

NHT : Non Heat Treated

Pa : Pascal

RFC : Reduced Fat Cheese

RFM : Reduced Fat Milk

s : Second

SNF : Solid Non Fat

t : Time

 $tan \delta$: Tan delta

TPA : Texture Profile Analysis

CHAPTER I

INTRODUCTION

1.1 General Objective

The reduction of fat intake in diet is effective on decreasing the risk of the coronary heart disease and related health problems. Thus the requirement of fat reduction in people's diet is important now than ever since most people suffer from cardiovascular disease [1]. The reduction of fat intake has been recommended by many organizations such as US Department of Agriculture (USDA 1995) [2]. For that reason the food industry has responded by developing reduced-fat version of traditionally high-fat foods, to provide healthier diet to the consumers, and to achieve this, there is a considerable interest in the food industry in the improvement of reduced-fat foods [3].

Mainly dairy products are interested for fat reduction progresses. Especially in cheese industry there are great attempts for making high quality reduced-fat cheese since cheese is naturally high-fat food and its consumption through the world is high. Thus, many of reduced-fat cheeses have been produced by cheese manufacturer [4].

Cheese is increasingly used also as an ingredient in prepared food to add texture, flavor and color. Therefore, for a particular application, chemical and physical properties of cheese should be proper. Unfortunately, reduction of fat causes some negative effects on the flavor, body and texture of cheese. As a result of this, many commercial reduced-fat cheeses exhibit poor flavor and texture. Mainly reduction of the fat content of cheese also alters the parameters defining texture and rheology [5-7].

The rheological characterization of cheese is important as a means of determining body and texture for quality and identity as well as a means of studying its structure as a function of composition, processing techniques, and storage conditions. Since rheology deals with the deformation and flow of matter, it is an

essential measurement for the characterization of physical properties of the cheese under various conditions. Like most solid foods, cheese is viscoelastic in nature meaning that it exhibits both solid (elastic) and fluid (viscous) behavior. The viscoelastic properties of cheese can be as important as flavor, and determine the consumer acceptability [8].

Cheese consists of a dispersed phase of fat globules embedded in a continue matrix of protein phase. These structural components are responsible for the viscoelasticity of cheese. So reduction of fat content in cheeses results unacceptable physical properties [5]. To determine the unacceptability, evaluation of rheological methods has assumed even greater importance [9]. Due to these reasons many researches have been done to characterize the rheological behaviors of Cheddar and Mozzarella cheeses, which are the most popular cheeses at USA and Europe [10-12]. Besides of these, there were so many reports giving the rheological properties of some local cheeses under different conditions [13-16]. For example, Antaniou et al. [13] classify the French cheeses according to their textural properties and Bertolo et al. [14] investigated the textural properties of Gouda cheese.

Unfortunately there are few studies on the rheological properties of Turkish cheeses. The most of the existing studies were empirical and mainly hardness was measured by penetrometers in those studies [17,18]. The characterizations of rheological properties of Turkish cheeses are necessary for standardization and improving products quality.

Gaziantep cheese is unripened, semi-hard cheese, which is produced traditionally in the southeast part of Turkey [19]. For consumer demand textural properties of Gaziantep cheese are important. Dipping process (defined as heat treatment throughout the study), putting fresh cheese into hot whey or water for a short time period, is generally applied to Gaziantep cheese. This process imparts elasticity to cheese and also it has pasteurization effect [20]. For that reason the heat treatment temperature is important for making proper Gaziantep cheese. Its whole effects on the rheological properties of Gaziantep cheese should be investigated.

At the light of given facts, in this study, following objectives were targeted:

- 1. To evaluate the some physical properties of Gaziantep cheese such as meltability.
- 2. To observe the effect of fat reduction on the chemical and physical properties of Gaziantep cheese.

- 3. To determine the effects of heat treatment temperature, storage temperature and storage time on the rheological behaviors of Gaziantep cheese.
- 4. To investigate whether it is possible to define certain relationship between measured mechanical parameters and chemical attributes of cheeses.

1.2 Cheese Age

The following definition is given by The Food and Agricultural Organization 'Cheese is the fresh or maturated product obtained by the drainage (of liquid) after coagulation of milk, cream, skimmed or partly skimmed milk, butter milk or a combination of them'. Cheese is among nature's most important contributions to civilization and it has been a popular food for centuries. The literature concerning cheese reveals almost 2000 names applied to cheese, and periodically more names appear as new varieties are made [21]. Cheese represents perhaps one of the oldest means of food preservation and is made wherever animals are milked, whether the animal is cow, buffalo, reindeer goat, sheep, horse, camel, ass, yak or llama. Cheese is highly nutritious because it contains almost all of protein, usually most of the fat, essential vitamins and minerals and other nutrients of milk in a concentrated form.

As early as 9000 B.C. people in the regions that is known as Turkey, Iran and Iraq consumed milk from sheep, goat or camel. The fresh milk would spoil quickly after collecting so it was either consumed fresh or allowed to sour naturally for longer storage means as fermented products such as yogurt, cheese, and butter. One ancient legend tells the story of an Arabic merchant who put his supply of milk in a pouch made from a sheep's stomach as he set out on a trip across the dessert. The rennet in the lining of the pouch, combined with the heat of the sun, caused the milk to separate into curds and whey. The legend says that he satisfied his thirst with the whey and his hunger with the flavorful curd. Today, cheese is the most popular dairy product and the great portion of the total milk produced is used in cheese manufacturing [22]. The cow later became the major source of milk for other regions. Europe, the United States and the Oceanic countries have developed into the main producers of cheese from cow's milk. Milk from almost any mammal can be fermented into cheese, but these can differ greatly in taste, texture, appearance and cost. There is a cheese for every taste-preference and a taste-preference for every cheese.

Today the cheese term includes a very heterogeneous group of products that differ in composition, conditions used in manufacturing and storage, sensory characteristics and physical attributes. In spite of this variety, scientists have discovered a substantial number of commonalities between cheese types, which have greatly assisted our understanding of certain basic properties that can be controlled [23].

1.3 Classification of Cheese

Several different formal schemes are used to classify cheeses. Cheeses may be grouped according to unique manufacturing or processing procedures, consistency or rheology (softness or hardness), country of origin, general appearance (size, shape, color), source of milk, and chemical analysis.

Cheeses may be categorized according to manufacturing procedures such as the method by which the curd is formed (by acid and/or coagulating enzyme) or the ripening agent (bacteria, mold, yeast, unripened) as shown in Table 1. [24]. However, only a few cheeses (blue, Camembert, brick, Swiss) are characterized by distinctive ripening agents. Cheeses may also be classified according to rheology, or softness and hardness. However, there are no objective measurements of the softness or hardness of cheese. Some cheeses such as brick, classified as semi-soft, may actually be harder than rindless Swiss or washed-curd Cheddar, which are described as hard cheeses. Although formal cheese classifications provide useful information, universal standards of classification are needed.

According to their fat content the same varieties of cheese may be classified as reduced-fat, low-fat and non-fat. In this classification, maximum fat contents are determined by either a percentage reduction from the reference food (reduced-fat, light) or a maximum amount of fat per serving (low-fat, non-fat) [25]. For reduced-fat cheese fat content must be reduced by 25 % from reference cheese. Low-fat cheese contains 3 grams or less of fat per reference amount (28 g) or for low-fat cheese fat content should be reduced by 50% or more. Non-fat cheese contains 0.5 g or less of fat per 50 grams or 1 % fat on a wet basis. These standards may change due to the type of cheese and local legal standards.

Table 1. Classification of Cheeses by Manufacturing Process [24]

| Distinctive Process | Characteristics | Example Cheeses |
|---|---|--|
| Curd particles matted together | Close texture, firm body | Cheddar |
| Curd particles kept separate | Slightly open texture | Colby, Monterey, Jack |
| Bacteria-ripened throughout interior | Gas holes or eyes with eye formation throughout cheese | Swiss (large eyes), Edam or Gouda (small eyes) |
| Prolonged curing period | Granular texture; brittle body | Parmesan, Romano |
| Pasta filata | Plastic curd; stringy texture | Mozzarella |
| Mold-ripened throughout interior | Visible veins of mold (blue- green or white); piquant, spicy flavor | Blue, Gorgonzola, Roquefort |
| Surface-ripened mainly by bacteria and yeasts | Surface growth; soft, smooth waxy body; mild to robust flavor | Brick, Limburger |
| Surface-ripened mainly by mold interior | Edible crust; soft, creamy pungent flavor | Brie, Camembert |
| Curd coagulated mainly by acid | Delicate soft curd Neufchatel | Cottage, Cream |

1.4 Manufacture of Cheese

Basically cheese is a milk concentrate, the basic solids of which consist mainly of protein, actually casein, and fat. Cheese is a less perishable foodstuff than milk. Raw milk or pasteurized milk, skim milk, recombined or reconstituted milk; skim milk or ultrafiltered milk is used for manufacturing of most kinds of cheese. It is a continuous para-casein matrix with entrapped moisture and fat. In contrast to most dairy products, cheese represent a dynamic biological system: throughout the manufacturing and ripening process, a series of counter and /or successive biochemical events occur, that, if balanced, yield a product of desirable flavor, odor

and texture. No two cheeses are ever identical, even batches of the same variety. The basic steps for cheese manufacturing are shown in Figure 1.

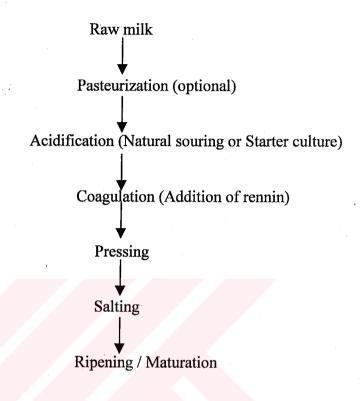


Figure 1. The basic stages of cheesemaking

The vast variety of cheeses can be divided into manufacturing and ripening phases. Basic manufacturing steps are: acidification of milk; coagulation of milk; removal of whey (cutting, cooking, stirring, pressing, salting); shaping (molding, pressing); and salting. Cheesemaking is basically a dehydration process whereby the fat and casein are concentrated six to twelve times, depending on the cheese variety. The amount of water retained in the product is regulated by the extent and combination of five steps (manufacturing steps) plus milk composition. The moisture content, the salt content and the micro flora regulate the biochemical changes during the subsequent ripening period. These factors, in turn, lead to the flavor, odor and texture of the finished product [26].

Pasteurization is a heat treatment of milk to destroy pathogenic bacteria that cause human disease. Pasteurization does not sterilize the milk. Some pathogenic bacteria can survive pasteurization. Many nonpathogenic bacteria and bacterial

spores will remain in the milk. Milk left standing will eventually sour because of these bacteria. Acidification, the first step and most basic operation, is defined as the progressive development of acidity throughout the manufacturing stage, and, for some varieties, in the early stages of ripening as well. This operation also known as ripening of milk is usually brought about by use of a starter [27].

Acid production is the key to the production of good quality cheese. It affects coagulant activity, curd strength, syneresis, pH and growth of non-starter microorganisms. Coagulant activity and the amount of coagulant retained in the curd affect the rate of proteolysis during ripening. The curd strength affects the yield: if the curd too fragile it shatters, leading to significant losses of fat protein in the whey. Syneresis controls the moisture content that regulates the bacterial growth and enzyme activity, which, in turn, influence the rate and pattern of ripening. The pH affects the rate of solubilization of the colloidal calcium phosphate, which affects casein susceptibility to proteolysis and influence the rheological properties of the cheese. If it is too acid, the cheese is crumbly; if too basic, the cheese is pasty and sticky. Finally, the growth of many non-starter microorganisms, especially food poisoning and gas producing ones, is controlled by the lactic-starter organisms; as a result, properly made cheese is inherently a very safe product.

Milk coagulation is the main process in cheesemaking: the milk forms a gel that entraps the fat [28]. Coagulation may be induced (1) by limited proteolysis by selected aspartate proteinases, usually chymosin (rennet), (2) acidification to isoelectric point of casein of approximately pH 4.6, (3) or acidification to about 5.2 with heating, (4) by addition of salts.

Rennet is a proteolytic enzyme and its role is to destabilize casein micelles and make them to coagulate. It is a traditional preparation made from the lining of the fourth stomach from very young, milk-fed calves. Rennin is the name given to the enzyme fraction of rennet. Rennin is a group of acid proteases. The most important enzyme in rennet is chymosin [29]. Similar enzymes, found in plants, microorganisms, and digestive tract tissues of other animals including chickens are also used for cheese production.

The properties of rennet curd are quite different from those of acid precipitated curds in that they have better syneresis properties that make it possible to produce low moisture curd without hardening [27]. Rennet coagulation occurs in two phases: an enzymatic phase where the Phe105-Met106 bond of κ -casein is hydrolyzed to

release the hydrophilic glycomacropeptide from the C-terminal end this occurs at 0-10°C, although it is usually higher in traditional cheesemaking.

For second phase (clotting) the temperature must be greater than 18°C. It is temperature dependent and does not take place until heat is available and even then only in the presence of available calcium ions [26]. The total effect of the primary and the secondary phases is the conversion of one of the milk protein fractions, casein, from a colloidal suspension to a fibrous network.

To remove whey of the cheese and for making compact curd cheese is pressed. The rate of pressing and pressure applied are adapted to each particular type of cheese. Pressing is also important for providing the true texture to the cheese for that reason that should be gradual at first, because initial high pressure compresses the surface layer and can lock moisture into pockets in the body of cheese.

Salt has its greatest role in ripening where it controls water activity, microbial growth and activity, enzyme activity and physical changes in the cheese proteins that influence texture and solubility through possible changes in protein conformation. There are four ways of applying salt to the cheese; salting in whey, salting in curd, rind salting, salting in brine.

At the end of the series processes, cheese is ripened and goes through a whole series of processes of a microbial, biochemical and physical nature. These changes affect lactose, protein and fat contents of the final product [30]. During this time, bacteria continue to grow in the cheese and change its chemical composition. Bacteria use the curd as food and release waste products, resulting in flavor and textural changes in the cheese. The biochemical changes in ripening are regulated by the moisture content, salt concentration, and the species of microbes present. These changes include glycolysis, lypolysis and proteolysis. Proteolysis is the most important event in ripening [31]. The type of bacteria active at this stage in the cheesemaking process and the length of time at that the cheese aged, determine the type and quality of cheese being made.

1.5 Chemical and Physical Properties of Cheese

The basic properties of cheese may be divided into two groups: chemical and physical properties [32]. They can help us to classify cheeses and many schemes have been proposed for classifying purpose to make them as standard as possible for international trade purposes. These properties determine the quality of cheese and

they should be under control during manufacturing. For controlling, we need fully understanding of their effects and reasons.

1.5.1 Physical Properties of Cheese

Physical properties of cheese include all properties that help to describe both the macroscopic and microscopic nature of cheese. Macroscopic properties encompass textural attributes such as hardness, viscosity, meltability, springiness, adhesiveness and cohesiveness [33,34]. Measurement of these properties includes some difficulties due to the complexity and non-homogeneity of food products [35]. Two main methods have been used to evaluate the physical properties of cheese. These are empirical methods and dynamic rheological methods [36].

1.5.1.1 Basic Concepts of Cheese Rheology

Rheology is the science of deformation and flow of matter [36,37]. It investigates the consequent behavior of sample when a force applied on it. So all materials have rheological properties and many areas are related with rheological data such as geology, bioengineering, chemical engineering, and tribology (study of lubrication, friction and wear) as well as food science and technology.

In food industry the rheological data are used in many areas [36]:

- Evaluation of food texture by correlation sensory data;
- Shelf life testing;
- Process engineering calculations;
- Determining ingredient functionality in product development;
- Intermediate or final product quality control;

Before discussing the rheological behavior of cheese it is necessary to define a number of basic concepts. A force may be applied to a sample tangentially to one surface as Figure 2a. or normally as in Figure 2b. In either case the force divided by the area over which it is applied is known as the stress and is usually denoted by the Greek letter σ .

Stress, defined as a force per unit area and usually expressed in Pascal (N/m²). If the sample deforms as a result of the tangential stress the deformation is known as the shear strain, or more simply as shear and denoted by γ .

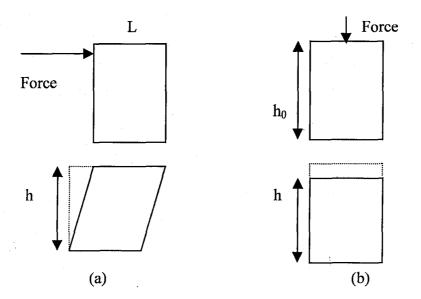


Figure.2. Effect of stress on a sample (a) in shear and (b) in compression.

There are several equations defining shear. These are;

| Shear strain | $\gamma = dL/h$ | Eq. (1) | |
|-----------------------|-----------------------|---------|---------------|
| In compression strain | $\gamma = dh/h_0$ | Eq. (2) | Cauchy strain |
| | $\gamma = \ln(h/h_0)$ | Eq. (3) | Hencky strain |

A modulus defined as the ratio of stress to strain while compliance is defined as the ratio of strain to stress. Rigidity (elasticity) is the distinctive characteristic of solids.

The modulus of elasticity as it is called is defined as the stress divided by strain and this is given the symbol G.

$$G = \sigma/\gamma$$
 Eq. (4)

A solid to which this equation applies for any value of stress and strain is known as an ideal or Hookean solid.

If the sample material that is being subject to the stress is a fluid it will not support any permanent stress and the strain will change progressively as long as the stress is applied. For a Newtonian fluid it will be the rate of change of the strain with which we are concerned. In this case it is customary to use the converse of fluidity for physical property. This is known as viscosity and is defined as stress divided by the rate of change of shear:

$$\eta = \sigma/(d\gamma/dt)$$
 Eq. (5)

Use of viscosity rather than fluidity ensures that both elasticity and viscosity vary in the same sense i.e. both increase as the resistance to deformation increases. A fluid for which the rate of strain always strictly proportional to the applied stress is known as a Newtonian fluid [38].

Some materials exhibit both elasticity and viscosity simultaneously. These are known as viscoelastic materials. Materials respond to an applied displacement or force by exhibiting either elastic or viscous behavior, or a combination of these, called viscoelastic behavior. Most polymers are viscoelastic, their mechanical properties showing a marked time- and temperature- dependence [39]. Cheese is also viscoelastic in nature; it exhibits both elastic (Hookean solid) and viscous (Newtonian fluid) behavior [40,41]. Unsteady state shear measurements provide a dynamic means of evaluating viscoelasticity. The two major categories of unsteady shear testing are transient and oscillatory.

Transient tests are used to evaluate the phenomena of creep and stress relaxation and start-up flow. These tests involve small strains and can be conducted with commercially available or easily constructed instruments. Creep test is the one way of measuring the viscoelasticity behavior of materials. In creep test, an instantaneous stress is applied to the sample and change in strain (called the creep) is observed over time. When the stress is released, some recovery may be observed as the material attempts a return to original shape. Creep experiments can also be conducted in uniaxial tension or compression type.

The idealized creep and recovery curves are illustrated in Figure 3. Subjected to a constant stress, strain in an ideal elastic material would be constant due to the lack of flow, and material would return to the original shape upon removal of stress. An ideal viscous material would show steady flow, producing linear response to stress with the inability to recover any of imposed deformation. Viscoelastic materials (e.g., cheese, bread dough) would exhibit a nonlinear response to strain and, due to their ability to recover some structure by storing energy, show a permanent deformation less than the total deformation applied to the sample. This strain recovery, creep recovery, is also called recoil [36].

Creep data may be described in terms of a creep compliance function:

$$J=f(t)=\gamma/\sigma_{constant}$$
 Eq. (6)

Compliance curves generated at different stress levels overlap when data are collected in the range of viscoelatic behavior. With a perfectly elastic solid J=1/G, the reciprocal of the shear modulus; however, different time patterns in experimental testing mean that J(t)=1/G(t). Equation (6) is presented in terms of shear deformation.

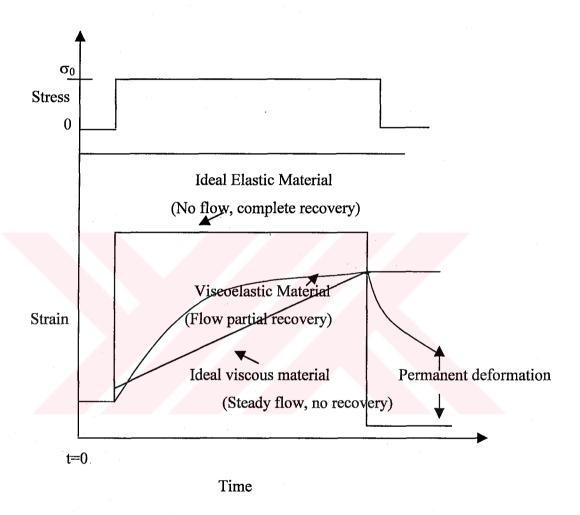


Figure 3. Creep and recovery curves.

In oscillatory instruments, samples are subjected to harmonically varying stress or strain. This testing procedure is the most common dynamic method for studying the viscoelastic behavior of food. Results are very sensitive to chemical composition and physical structure so they are useful in a variety of applications including gel strength evaluation, monitoring starch gelatinization, studying the glass transition phenomenon, observing protein coagulation or denaturation, evaluating curd formation in dairy products, cheese melting, texture development in bakery and meat products and shelf-life testing [42,43]. In oscillatory tests, materials are

subjected to deformation (in controlled rate instruments) or stress (in controlled stress instruments), which vary harmonically with time.

The frequency sweep is probably the most common mode of oscillatory testing because it shows how the viscous and elastic behavior of the material changes with the rate of application of strain and stress. In this test the frequency is increased while the amplitude of the input signal (stress or strain) is held constant. Frequency sweeps are very useful in comparing, sometimes called "finger printing" different food products or in comparing the effects of various ingredients and processing treatments on viscoelasticity.

1.5.1.2 Dynamic Rheological Methods

There is increasing need to expand fundamental understanding of material behavior. There is no doubt that empirical tests on food materials are important. They would not be employed in practice unless the results correlated in some useful fashion with end-use properties processing characteristics or serve as an index of product quality or in process control [36].

Nevertheless, empirical methods suffer from severe limitations: they are usually specific for given product or process or for a narrow range of properties; they lack predictive capability; results from one arbitrary test method can not be compared to those from another arbitrary test method. Such a work had been conducted by Park et al. [44]. They compared the four procedures for cheese meltability and reported that their results were not correlated. For that reasons fundamental rheological test have been adapted to the food systems that their results can be analyzed in a systematic fashion and predictions and correlations become feasible [42].

Fundamental rheological concepts have been used recently to determine these problems, to characterize the cheese textural properties and to evaluate some physical properties of cheese. The measurement of the rheological properties of cheese is performed for two reasons: 1) as a quality control method for cheesemakers, 2) as a technique for scientists to study cheese structure. The rheological properties of cheese can be as important as flavor, and are a large part of the total score awarded by the cheese grader [45]. Consequently, an objective instrumental method of determining rheological properties of cheese would be valuable. This also is true for scientific studies of cheese. Research into the origins of

cheese texture is an important part of dairy science, and investigations into cheese rheology have been conducted for over half a century.

Creep and Recovery, Strain or Stress sweep, and Frequency sweep tests have been used as a dynamic testing of cheese for last ten years. They offer very rapid results with minimal chemical and physical changes. But for cheese it has several inherent limitations since cheese is heterogeneous and non-isotropic. Therefore it is difficult to obtain a representative sample suitable for the small samples required for typical dynamic tests. Viscoelastic properties, G', G", and tanô have been reported for a variety of cheeses at various temperatures. The G' (storage modulus) is a measure of energy stored and subsequently released: G" (loss modulus) of the energy dissipitated per cycle of deformation and tanô of the dynamic character of the protein-protein in a gel network [46]. The material functions that are used for defining viscoelasticity and their equations are given in Table 2.

Dynamic rheology is a fundamental method that has been used for several applications in cheese research [47]. Therefore there have been many research publications on dynamic mechanical properties of cheese despite the fact that determining their rheological properties are complicated by structural nonhomogenity.

Tunick et al. [48] used viscoelastic properties as a means to distinguish the textural differences between Cheddar and Cheshire cheeses. They studied the strain sweep behavior of those cheeses with age. They observed the decreases with age in viscosity, elasticity, and body strength with Cheshire but for Cheddar cheese body breakdown was not seen under same condition. So they suggested this method could be used in identification of the two cheeses to prevent mislabeling. Hsieh et al.[49] reported the effect of some proteins on the viscoelastic properties of Mozzarella cheese. They concluded that proteins altered the viscoelastic properties of Mozzarella cheese.

Tunick et al. [50] compared the rheological properties of low-fat and full-fat Mozzarella cheese by using textural profile analysis, small amplitude dynamic oscillatory shear measurements, and meltability measurements. They reported that hardness of cheese increased with decreasing fat content and during storage the hardness decreased due to the proteolysis. But they found similar springiness values for low- and full-fat cheese and they reported that the loss tangent G"/G', increased with MNFS (moisture in non fat solid).

Table 2. Material functions used to describe viscoelastic behavior.

| Material Functions | Equations |
|---|---|
| Shear storage modulus | $G' = (\sigma_0/\gamma_0)\cos(\delta)$ |
| Shear loss modulus | $G'' = (\sigma_0/\gamma_0)\sin(\delta)$ |
| Complex modulus | $G^* = (\sigma_0/\gamma_0) = (G^{2} + G^{2})^{1/2}$ |
| Complex viscosity | $\eta^* = G^*/\varpi = (\eta^{2} + \eta^{2})^{1/2}$ |
| Dynamic viscosity | $\eta' = G''/\varpi$ |
| Out of phase component of the complex viscosity | η " = G'/ ϖ |
| Complex compliance | $J^* = 1/G^*$ |
| Storage compliance | $J' = G' / (G')^2 + (G'')^2$ |
| Loss compliance | $J'' = G''/(G')^2 + (G'')^2$ |
| Tan delta (phase angle) | $Tan(\delta) = G''/G'$ |

Ma et al. [51] measured the viscoelastic properties of reduced-fat and full-fat cheese. They concluded that the full-fat cheese exhibited a greater magnitude of elastic modulus and loss modulus than reduced-fat cheeses. Also they reported that the creep test differentiated viscoelastic differences of cheeses due to the reduction of fat content. Rheological results indicated significant protein matrix between reduced-fat and full-fat cheese. On the other hand Fife et al. [52] reported that increase in the fat and moisture content of Mozzarella cheeses are accompanied by a decrease in the modulus of elasticity (an indication of rigidity).

Ustunol et al. [53] used the dynamic complex modulus of Cheddar cheese with varying fat content as an index of meltability and they reported a good correlation with meltability obtained from Arnott's test. Guinee et al. [54] concluded that the micro structural changes with milk homogenization resulted in marked reduction in flowability, strectchability and fluidity of the melted cheese. They obtained these results by measuring the G' and G" of cheeses.

Drake et al. [55] tried to correlate the sensory texture analysis using loss and storage moduli and proposed that sensory attributes of processed cheese can be described by fundamental rheological tests. Dynamic measurements have also been used to examine the effect of storage conditions [56] and cooking temperatures [57] on the rheology of Mozzarella cheese. Yun et al. [57] reported that the average

values for either storage or loss modulus from the strain sweep of Mozzarella cheeses, were not affected by different cooking temperatures.

1.5.1.3 Empirical Methods

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Empirical methods are a valuable and well-established part of food industry. Since they do not measure fundamental properties, they may appropriately be called indexers [36]. The food industry uses many empirical instruments to measure the physical behavior of food products such as Penetrometers, Consistometers, and Texture Analyzers. Also for assessing meltability, some empirical methods have been used. These include the Schreiber, Olson and Price and Arnott method [58]. These methods and devices are not used to determine fundamental properties, but results may find diverse applications: quality control, correlation to sensory data, or even serve as official standards of identity.

The first rheological measurements of cheese were empirical; the cheese grader would press the surface with thumb to judge firmness and elasticity. The readiness of Cheddar curd for cutting was determined by the cheesemaker using a dairy thermometer to cut through the set curd. Progressively, the development of simple instruments followed. These instruments had in common five components: (1) a means of mechanically deforming cheese, (2) a means of recording the force, (3) a means of recording the deformation, (4) a means of measuring time during deformation, and (5) a test cell to hold the sample [34]. A ball compressor replaced the grader's thumb: its hemisphere was pressed into the surface of a cheese sample and the depth of penetration and recovery time recorded. A penetrometer was developed that provided force measurements as a needle was pushed into the cheese; results were similar to those obtained by the ball compressor.

The first instrument to examine texture by imitation of the chewing of food, the Volodkevich bite tendometer, was developed in the 1930s. The first attempt to imitate mastication by instrumental means was the MIT denture tendometer in which a set of dentures was motorized and a force-time curve was obtained during the simulated chewing action by means of strain gauges mounted in the articular. The difficulty with this instrument was that little information could be obtained.

Texture refers to human sensation of food derived from its rheological behavior during mastication and swallowing. Obtaining a quantitative description of texture using instrumental data is very complicated because no instruments duplicate human capabilities. There are two methods to evaluate food texture: sensory and instrumental. A double compression test, the most recognized instrumental means of characterizing the texture of solid and semi solid foods. Among these many empirical and imitative tests the most popular one is TPA [59-62]. Generating and interpreting texture profile information, with instrumental or sensory means, is called TPA [36].

In TPA, analyses of the force-time curve led to the extraction of seven textural parameters—five measured and two calculated from the measured parameters. These seven parameters were named as follows: Fracturability: (also called brittleness), hardness, cohesiveness, adhesiveness, springiness, gumminess, chewiness.

Numerous studies have focused on analyzing the influences of compositional and manufacturing parameters on textural attributes and for correlating sensory texture attributes of cheese with instrumental texture profile analysis. Several attempts have been made to correlate objective measurements with sensory textural attributes for a wide variety of cheese. Most of the studies employed the measurement of mechanical parameters derived from the development of the General Foods TPA. Some of these studies reported that high or satisfactory correlations were achieved for a number of mechanical parameters and sensory attributes [63]. Other studies reported, however, that very poor correlations were obtained for any mechanical and sensory parameter examined using the TPA technique for the types of cheese they examined [64].

Drake et al. [46] reported that among TPA parameters, hardness, springiness and gumminess were highly correlated with sensory firmness. Rudan et al. [2] concluded that as the fat content of the cheese decreased, the TPA hardness, cohesiveness, and springiness increased. However, Bryant et al. [47] concluded that for cheddar cheese, as fat in cheese decreased, hardness and springiness increased and adhesiveness and cohesiveness decreased. Also Rudan et al. [65] reported that homogenization of milk or cream did not affect the TPA parameters, meltability, and apparent viscosity.

Tunick et al. [56] studied the effects of cooking temperatures, moisture level and storage time on the textural properties of reduced-fat Mozzarella cheese. They reported that proteolysis during refrigerated storage causes partial breakdown of the casein network, and so hardness of the samples decreases with storage time. Raphaelides et al. [64] evaluated the textural properties of ultrafiltered Teleme

cheese by TPA tried to find a correlation between sensory and instrumental results but very poor correlations were obtained for any mechanical and sensory parameters examined using TPA technique.

1.5.1.4 Meltability

Meltability is one of the properties of cheese at elevated temperatures. The physical properties of melted cheese are highly complex and give rise to at least five different functional attributes, namely meltability, stretchability, elasticity, free oil formation and browning/blistering. Stretchability is the ability of the melted cheese to form fibrous strands that elongate without breaking under tension (sometimes called stringiness). Elasticity refers to the resistance to elongation of the fibrous strands as they are stretched. Free oil formation is the tendency of free oil to separate from the melted cheese body and form oil pockets, particularly at the cheese surface.

Meltability may be defined as the ease with cheese flows or spreads upon heating [66]. For process cheese, cheese analogs and various natural cheeses meltability is one of the most important physical properties and in dairy industry there are many empirical methods to determine it. The most popular methods for measuring meltability are the Schreiber and Arnott test [67,68]. Basically all these methods based on the measurements of the change in diameter or height of the cheese sample after exposing the definite time and temperature in an oven.

Measuring meltability using one of the empirical methods was not so successful yet, since it was suggested that there was not any correlation between the results of empirical test [44]. This was not totally unexpected since flow of cheese, under such circumstances, can hardly be characterized by single an empirical parameter. Although this, the simplicity and speed of such empirical methods attractive, especially for quality assurance in an industrial environment. However, for research purposes their lack of objectivity is a distinct disadvantage. This problem may come from the complexity of melting phenomena, it related both heat transfer, thermal phase change characteristics of cheese, composition and microstructure so they are highly interdependent and transient properties.

To solve these problems scientists have tried to correlate and find the relationship between meltability and rheological properties of cheeses. Tunick et al. [69] proposed the use of differential scanning calorimetry help to assess cheese meltability. Kuo et al. [70] have been proposed that the creep test, the viscoelasticity

index, can be used to distinguish the meltability of cheddar cheese of different ages and fat levels. They used transient tests for estimating softening point of cheese. Mounsey et al. [58] used the maximum tan δ as a meltability index and good correlation between maximum tan δ and empirical test.

1.5.1.5 Color

Appearance is an important factor in the consumer acceptance and directly related to product quality. The appearance or color is influenced by how it reflects, absorbs, or transmits light, which in turn is related to physical and chemical nature of the food [72]. The color is specific for each variety of the cheese, and the whiteness is a characteristic of Gaziantep cheese [73]. Fat imparts to the cheese whiteness by scattering light so fat reduction causes deficiency in cheese color [74]. For proper production this problem should be eliminated.

1.5.2 Chemical Properties of Cheese

Recent developments in cheese marketing have resulted in a demand for cheese of greater uniformity of composition than in the past. Such uniformity best achieved by a grading system based on the compositional analysis since the quality of cheeses directly related with chemical properties of cheese [75,76]. Mainly pH, moisture-protein ratio, fat in dry matter (FDM) and maturation are the chemical properties of cheeses and they affect and determine the rheological behaviors of cheeses.

1.5.2.1 pH

Every cheese variety has a characteristic pH range. With in this range the quality of the cheese is dependent upon both its composition and the production methods. The pH value is important in that it provides an indication of the extent of acid production throughout the cheese making process. The effects of pH on cheese manufacturing and maturation are well recognized. The most significant effect of pH is imparting brittleness when pH value less than 5.0 in hard cheeses. Other properties such as softness in semi-soft or soft cheeses and meltability of all cheese types are affected also by pH. Dramatic changes in the properties of cheeses occur as the pH is reduced from 5.4 to 4.9 which result from several factors including solubilization of most of the colloidal calcium phosphate, alteration in cheese microstructure with a

reduction in protein aggregate size and alterations in bonding between and within the cheese protein network. As the pH decreases towards that of the isoelectric point of casein the protein assume an increasingly more compact conformation and the cheese becomes shorter in texture and fractures and small deformations [77].

1.5.2.2 Moisture-Protein ratio

There is a general agreement on the qualitative effects of water and protein on the textural properties of cheese with the casein matrix imparting rigidity and water taking the rigidity of cheese. Basically, cheeses consist of an aggregation of water. fat and protein (mainly casein). Since protein is considerable denser than either fat or water, it occupies one-sixth of the total volume. Nevertheless, it is largely the protein matrix give rise to the rigid form of the cheese. Any modification of the nature or the amount of protein present in cheese will modify its texture. The protein content of the cheese mainly affects the hardness of cheeses; Rodriguez et al. [78] reported that as the protein content of the gels increased, the protein matrix became denser. This could account for the increased hardness of cheeses with higher total protein [79,80].

1.5.2.3 Fat in Dry Matter

Fat in cheese exists as physically distinct globules, dispersed in the aqueous protein matrix. In general, increasing the fat content results slightly softer cheese, as does an increase in moisture content, since the protein framework is weakened as the volume fraction of protein molecules decreases. Relatively large variations in the fat content, however, necessary before the texture of cheese significantly affected. Commercial cheese with high FDM usually has a high MNFS and this causes a decrease in firmness.

The filled composite gel method has been used for understanding the relation between fat and moisture in cheese [2]. According to filled gel composite method cheese can be thought of as a composite material made up of fat and whey surrounded and supported by a three-dimensional protein network. Although both fat particles and whey (mostly water) represent the filler within casein matrix, the distribution of two is not the same. As determined by electron microscopy, the water appears to be distributed in columns between protein fibers; emulsified fat droplets were found within these columns. Furthermore, some of the water is believed to be bound to, thus interact with, the casein matrix. Both the properties of the filler and the properties of the prope the protein network, as well as any interaction between the two, should determine the physical properties of the composite.

The general trends for changes in cheese composition and structure and predicted changes in cheese rheological properties, based on the filled gel composite model, are outlined and summarized in Figure 4.

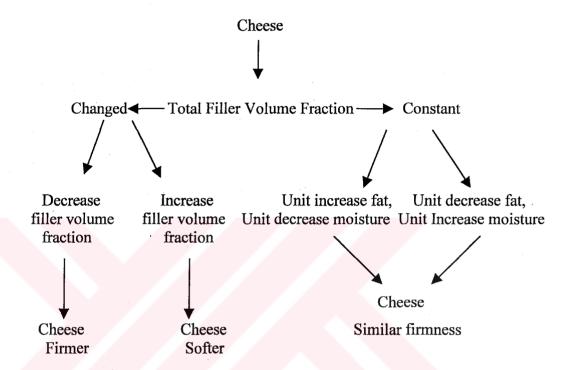


Figure 4. Influence of total filler volume fraction and the expected effect on the rheological properties of cheese based on the filled gel composite model.

1.5.2.3 Maturation

The first nomadic manufacturer of cheese undoubtedly noted changes in the physical properties of cheese during storage or maturation [23]. As several processes occur simultaneously ripening is a complex of multiple biochemical degradation and synthesis reactions [81]. Considerable changes in texture occur during maturation as a consequence of proteolysis. A major event during initial stages of maturation that affects the physical properties of cheese is the cleavage of the Phe23-Phe24 bond of α -casein by milk clotting enzymes [23]. The rubbery texture of green cheese changes relatively rapidly as the framework of α_{s1} -casein molecules is cleaved by the residual coagulant. A group of Cheddar cheeses examined over a period of nearly a year increased in hardness and decreased in elasticity with the age of the cheese. In part,

this is caused by the loss of structural elements but another feature of proteolysis is probably important [82].

Clearly, the change in texture during ripening depends upon the extent of proteolysis which, for any individual cheese, is determined by the duration and temperature of maturation. The main factor that influences the rate of proteolysis appears to be salt in moisture (S/M). A direct relationship between S/M and residual protein was established whereas the correlation between moisture and residual protein was relatively weak. A cheese with a low S/M value has a higher rate of proteolysis and is correspondingly softer in texture than with a high S/M value [83]. Meltability is also affected from maturation. During maturation the meltability of Cheddar increase due to proteolysis [84].

1.6 Reduced- and Low-Fat Cheese Making Technology

The popularity of cheese is, at an all-time high. Today's consumers are using more cheese than ever cook, enhance and snack, with cheese usage divided equally among home, food service and prepared foods. The vast majority of cheese is not eaten by itself, but as part of another food. This greatly influences the types and amounts of cheese the food industry requires. Because cheese is an integral part of food products, it becoming increasingly for cheese manufacturers to produce their cheese according to the functionalities required for the end use. Usage is highly depends on cheese functionality requirements, for example, flavor, texture, melt, browning and shredding ability [85].

Cheese is primarily used for its organoleptic contributions to a food; it also provides functionality and nutrition to finished food. With consumers having general understanding about the health implications associated with high fat diets, more cheesemakers are becoming involved in the manufacture of reduced-fat cheese. For many cheesemakers, making cheese better means making consistent, highly functional, lower-fat cheese.

Fat performs many important functions within the food [86]. For cheese, fat contributes to taste, texture, appearance and functionality. And also mainly affect the nutritional value. Fats are responsible body, texture, aroma and flavor of cheese. The reduction of fat in cheese cause many deficiencies therefore much research is focused on the optimization of the sensory and functional qualities of reduced-fat

food [5,25,55]. For optimization qualities of reduced- and low-fat cheese three strategies have emerged:

- 1. Modifications of cheese making procedure: This is simplest and most economic method of improving the flavor and texture of reduced fat cheeses. Critical parameters considered in make-procedure modifications are moisture and acid concentrations. Increasing the moisture content can provide some lubricity or creamy mouth feels and texture provided by fat. Retention of moisture in cheese curd is a key requirement toward offsetting the cost and textural problems with higher solid content in low-fat cheese.
- 2. Use of adjunct culture: Adjunct culture is a culture used in addition to standard lactic acid culture normally used to make a particular type of cheese. Adjunct cultures are traditionally used to improve or accelerate flavor development in full-fat cheeses. They can improve the flavor of reduced and low-fat cheeses through increased proteolysis, specifically amino peptidase activity, which reduces the bitterness and increases the concentrations of desirable flavor peptides and precursors flavor volatiles [86]. The selection of adjunct cultures to enhance the development of cheese flavor is on the basis of enzymatic activity or their capacity to produce particular flavor compounds
- 3. Use of fat replacers: Fat replacers can be divided into two groups: fat mimetics and fat substitutes. Fat mimetics are polar water-soluble compounds used to partially replace the sensory and functional characteristics of fat [55,87-89]. And they are carbohydrate- or protein-based materials that mimic the properties of natural fats, which may improve the characteristic of low-fat cheeses by binding water and improving texture and yield. Fat substitutes are fat-soluble and have the similar physical and functional properties of fats but which reduce the calorie content of the food.

None of these fat-reduction strategies can fully replace all the properties and functions of fat however; research advances within three areas continue to improve the flavor and texture of reduced- and low-fat cheeses.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials

Cow's milk was obtained from a local farm in Gaziantep at spring season, and analyzed for initial chemical parameters. All data given were the mean of at least two trials. Hexane, potassium sulfate (crystal extra pure), boric acid (crystal extra pure), sodium hydroxide and sulphuric acid (95-98% extra pure) were purchased from Merck Chemical Co. (Darmstadt, Germany) and deionised water was used for all experiments.

2.2 Cheese Manufacture and Experimental Design

Gaziantep cheeses used for this study were manufactured in a local plant. Milk samples with varied amount of fat were standardized using the route represented in Figure 5.

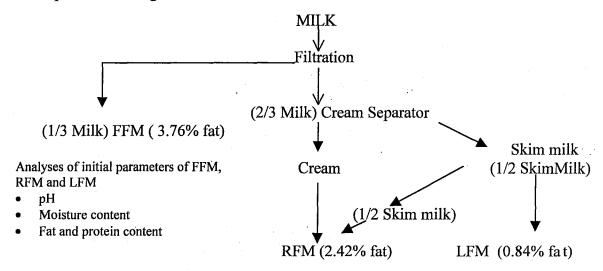


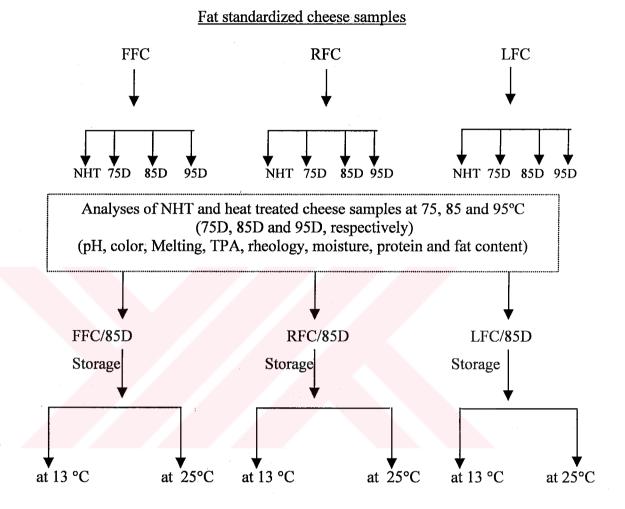
Figure 5. Schematic presentations of full-, reduced-, and low-fat milk preparation and applied analysis.

Raw milk (100 L) was filtered via cheese cloth. One-third of the milk defined as full fat milk (FFM) was used to prepare full-fat cheese (FFC). The rest of the milk passed through cream separator to lower the fat content of milk below 1%. After cream separation, half of the skim milk (LFM) was used to prepare low-fat cheese (LFC). For reduced fat cheese some of obtained cream was added to rest of the skim milk so RFM milk was obtained. All milk samples were then heated up to 72°C and pasteurized at that temperature for 15 sec. After pasteurization, they were cooled to 37°C and at that temperature 20 ml of commercial rennin (Mayasan A.Ş.) was added into each batch (20mL/30L). They were incubated at 30°C for one-hour. Then, curd was cut, mixed and poured into specially prepared cloth bags and left to remove its whey. After whey separation, they were pressed for 3 hours using 40 kg weights per 1 m².

Experimental design and applied analyses for cheese samples are displayed schematically in Figure 6. Each cheese lots were divided equally into four parts by knife. For three parts heat treatments were applied at three different temperatures (75°C, 85°C, 95°C), last part remained unheated. In heat treatments, first whey was heated up to 75°C at that temperature cheese blocks were dipped into whey and waited for 100 seconds. Then same procedures were applied for 85 and 95°C respectively. Prepared cheese blocks were packaged with plastic films to prevent water loss and immediately brought to laboratory for analyses. In laboratory, cheese samples were left at room conditions for nearly one-hour to come equilibrium with room temperature (20±2°C). The physical (color and melting), chemical (pH, moisture content), textural (TPA) and rheological analyses of cheese blocks were carried out in the same day. Some samples were stored in refrigerator and next day analyzed for fat and protein contents. The cheese making was replicated on two different days.

Some of the heat-treated cheese blocks at 85°C (FFC/85D, RFC/85D, LFC/85D samples) were stored in jars, filled with 20% brine, at 13 and 25°C. This brine concentration was selected since it was advised for Gaziantep cheese storage [19]. The three cheese blocks LFC/85D, RFC/85D, FFC/85D divided into 12 parts and 36 cheese blocks were obtained. These cheese blocks were cut into equal dimensions (5x5x5 cm) to eliminate variation in salt diffusion rates that might affect the textural properties. These cheese blocks were stored at 13°C and 25°C in the cold

room and temperature-controlled oven (T12-Heraues Instruments, Germany) respectively. The used temperatures were selected as taking into account traditional storage conditions.



Analyses of FFC, RFC and LFC samples heat treated at 85°C (85D), after 2, 4, 6, 14 and 28 days storage (Melting, moisture content, TPA, color)

Figure 6. Schematic presentations of experimental design and applied analysis.

Hardness, color, meltability and moisture contents of cheese samples were measured in duplicate after 2, 4, 6, 14, 28 d storage at 13 and 25°C.

2.3 Chemical Analyses

Moisture and fat content of milk were determined using Lactan 1.4 Milk Analyzer (Uysallar Inc. Co). Moisture content of cheese was determined using oven method (16 h at 105°C) [90]. Protein contents of cheese and milk samples were determined by Kjeldahl method [91]. Extraction method was used to determine the fat content of cheese, as defined in AOCS Official method [92].

The pH of milk and cheese was measured using Nel model pH-meter, cheese was dispersed in distilled water (5mL water/ 5gr cheese) [19].

2.4 Dynamic Mechanical Analysis

Gaziantep cheese samples were cut into disk-shaped slices by using special mould (3.0 \pm 0.3 mm thick and 35 \pm 1 mm diameter) and covered with stretch film to prevent dehydration. Samples were equilibrated to room temperature for at least one hour prior to testing. Viscoelastic measurements were performed at 20 \pm 1°C with a rheometer HAAKE RheoStress RS coupled with a Peltier/Plate TCP/P temperature control unit (HAAKE GmbH, Karlshure) using a cone and plate system (d:35 mm, α : 2°). Circulator DC10 was used to control temperature within range 10-70°C. Samples were loaded between plates with a gap of 2.5 mm and sat undisturbed for 5 min to allow sample relaxation.

For dynamic oscillatory tests, the storage modulus (G') and loss modulus (G") were measured during a frequency sweep varying from 0.01 to 15 Hz at a constant stress of 200 Pa, which was within the linear viscoelastic region of the cheeses. These moduli represent the amount of energy elastically stored and recovered per cycle (elastic component, G') and the amount of energy lost per cycle by viscous dissipation (G").

Creep and recovery tests were run at the stress value 200 Pa and creep time was 120 sec at 1 Hz. For temperature frequency test a range between 10-70°C was selected.

Data reported are averages of two measurements of two replicates and obtained values were analyzed using a RheoWin Data Manager (RheWin Pro V.2.64).

2.5 Meltability

Meltability of cheeses was determined using the Arnott test [44]. Cheese samples were cut into cylinders (25±1 mm in diameter and 10±0.5 mm in height) with a knife and specially designed mould tool. Each specimen was placed in the center of a glass dish. The dishes were heated in an oven at 100°C for 15 min. After cooling to room temperature for 30 min, the height and diameter of the melted cheese samples were measured. The meltability of the cheese was calculated by averaging the height of the melted cylinders and reported in cm.

2.6 Color Analysis

The color measurements were done using HunterLab ColorFlex (A60-1010-615 Model Colorimeter, Hunter Lab, Reston, VA). The instrument was standardized each time with a white and black ceramic plate. The color values were expressed as L (whiteness or darkness), a (redness/ greenness), b (blueness) and YI (yellowness index).

2.7 Texture Profile Analysis

For texture profile analysis (TPA), cheeses were cut into cylinders with height 10±0.5 mm and 25±1 mm diameter, wrapped with plastic, and waited for equilibration to room temperature (~20°C). TPA tests were performed using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). Test conditions were: P/25 aluminum cylinder probe (25 mm diameter); test speed 1 mm/s; pretest speed 5 mm/s, post test speed 1 mm/s; compression (strain) 25%; time pause, 5 s. Data collection and calculation were done using the Texture Expert Exceed Version 2V3 (Stable Micro Systems, 1998).

2.8 Statistical Analysis

The design of the experiment was a 3 X 4 factorial in a complete randomized design with two replications of cheese making. The three levels of fat content (FFC, RFC, LFC) and four different type heat treatment (NHT, 75D, 85D, 95D) were the main effects. LSD test was used for multiple comparisons at 5% significance level. SPSS for Windows software was used to analyze the data. All statistical results were tabulated and given in the Appendix for each test separately.

CHAPTER III

RESULTS AND DISCUSSION

The present study was initiated to study some physical and chemical properties of Gaziantep cheese and relation of them. The results of experimental studies and the treatment of the resultant data are given in graphical and tabular forms and discussed separately.

3.1 Composition of Milk

Chemical composition of the milks used for cheese manufacture is given in Table 3. The fat content of the standardized milks ranged from 3.76 to 0.84 % to achieve desired reductions in fat content of the cheeses. The protein contents of the milks gradually decreased as the fat content of milk increased. As expected, the moisture contents of milks increased with decreasing fat content. These changes were found to be significant statistically (P<0.05). However, the variation in SNF content and pH values of milk samples was not significant (P>0.05) (Table A1, A2).

Table 3. Chemical compositions of FFM, RFM and LFM samples used for the cheese production.

| Component | FFM | RFM | LFM |
|---------------------------|--------------------|--------------------|-------------------|
| Water content (%) | 87.12 ^a | 88.47 ^b | 89.98° |
| Fat content (%) | 3.76 ^a | 2.42 ^b | 0.84° |
| Protein content (%) | 3.12 ^a | 3.13 ^a | 3.18 ^c |
| Solid non-fat content (%) | 9.12 ^a | 9.11 ^a | 9.08 ^a |
| pH | 6.54 ^a | 6.55 ^a | 6.52 ^b |

a,b,c Means within same row with no common superscript differ (P<0.05).

3.2 Composition of Cheese

Cheese production was performed in a dairy plant in order to provide standard conditions and minimize personal and methodical error during production.

The chemical composition of cheese samples produced from the standardized milks with varied fat contents is shown in Table 4. Reduction in fat increased the moisture and protein contents relatively. As the fat content was reduced, MNFS of cheese samples were decreased significantly (P<0.05) (TableA3, A4); this could be explained as the moisture did not replace the fat on an equal basis.

The filled gel composite model had been used by Rudan et al. [2] to understand the changes in the composition of cheese as a result of fat reduction. The reduction of MNFS is consistent with the filled gel composite model. Similar results were found in literature [33,72]. Water in cheese is found as either free or bound to the protein since fat, the other major component is hydrophobic [35]. The protein contents of fat-reduced cheeses were higher than that of FFC (Figure 7) and probably due to this, more water remains in fat-reduced cheeses.

Table 4. Compositional analyses of Gaziantep cheese samples with varied fat level and heat treatments at different temperatures.

| Type | HT (°C) | pН | Protein (%, db) | Moisture (%) | Fat (%, db) | MNFS (%) |
|------------|---------|------|--------------------|--------------------|-------------|--------------------|
| | NHT | 6.41 | 25.40 ^a | 51.24 ^a | 50.45 | 67.95 ^a |
| FFC | 75D | 6.41 | 26.01 ^b | 47.93 ^b | 46.57 | 63.27 ^b |
| | 85D | 6.44 | 26.54° | 47.95 ^b | 46.23 | 63.14 ^c |
| | 95D | 6.45 | 27.02 ^d | 46.78° | 45.13 | 61.57 ^d |
| | NHT | 6.41 | 29.04 ^e | 53.17 ^d | 33.42 | 63.03 ^e |
| RFC | 75D | 6.43 | 29.47 ^f | 50.50 ^e | 31.55 | 59.85 ^f |
| | 85D | 6.45 | 29.58 ^g | 50.52 ^e | 30.96 | 59.82 ^g |
| | 95D | 6.48 | 30.23 ^h | 49.48 ^f | 31.42 | 58.76 ^h |
| | NHT | 6.42 | 32.90 ^k | 57.42 ^g | 13.57 | 60.94 ^k |
| LFC | 75D | 6.43 | 32.97 ¹ | 53.83 ^h | 12.5 | 57.13 ¹ |
| | 85D | 6.47 | 33.20 ^m | 53.55 ^k | 12.25 | 56.79 ^m |
| Table Toll | 95D | 6.47 | 33.41 ⁿ | 49.63 ⁿ | 10.14 | 52.53 ⁿ |

a,b,c,d... Means within same column with no common superscript differ.

The pH values of all samples were in the range of 6.41-6.47, these values were higher than those presented in literature (approximately 5-5.5), but similar results were reported by Kaya [19]. These higher pH values could be due to absence of fermentation process in fresh cheese.

Application of heat treatment decreased the moisture content of the samples (Figure 8). This may be due that water could not be entrapped and bind by protein matrix since curds were freshly formed and whey separation easily occurred.

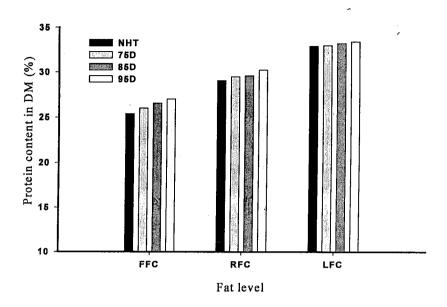


Figure 7. Effects of fat content and heat treatments on the protein contents of the Gaziantep cheese samples.

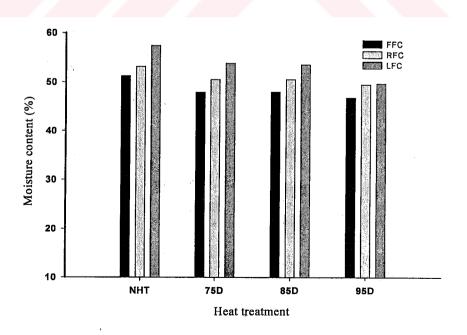


Figure 8. Effects of fat content and heat treatments on moisture content of Gaziantep cheese.

The results (Table 4) also showed that increasing the heat treatment temperature significantly increased the water loss from the cheese samples (P<0.05) (Table A5), as known that heating (scalding process) increased the whey separation [93].

Some of the stored FFC/85D, RFC/85D, LFC/85D samples in 20 % brine at 13°C and 25°C were analyzed with respect to chemical composition. The changes in moisture content of cheese as a function of storage time for both temperatures studied were shown in Figures 9-10. Storage time and temperature had significant (P<0.05) (Table A6) effects on moisture content. Moisture contents of samples decreased suddenly during first five day of storage then decreasing continued gradually. This may be due to high salt intake at the beginning of storage. Moisture content values of cheeses stored at 25°C were lower than in cheeses stored at 13°C owing to temperature dependence of the water diffusion coefficient [14]. These results are in agreements with literature [19]. The effect of storage temperature on moisture contents of FFC is higher than RFC and LFC (Figures 11-13). It is interesting to indicate that when fat contents of cheese were reduced, effect of storage temperature on the moisture content of the cheese was reduced.

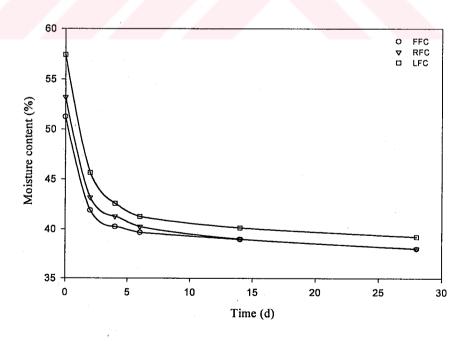


Figure 9. Effects of storage time on the moisture content of brined FFC, RFC and LFC samples stored at 13°C.

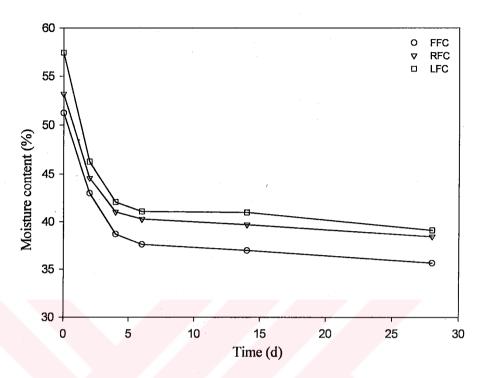


Figure 10. Effect of storage time on the moisture content of brined FFC, RFC and LFC samples stored at 25°C.

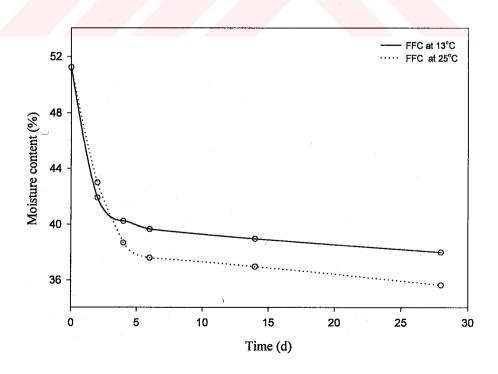
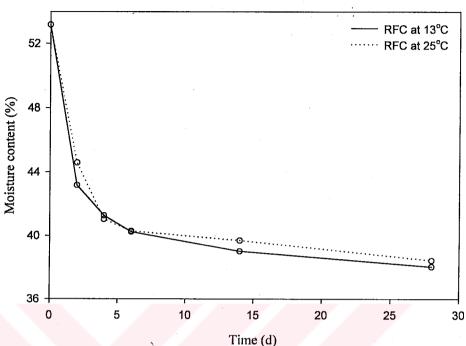


Figure 11. The effects of storage temperature on the moisture content of brined FFC.



Time (d)
Figure 12. The effects of storage temperature on the moisture content of brined RFC.

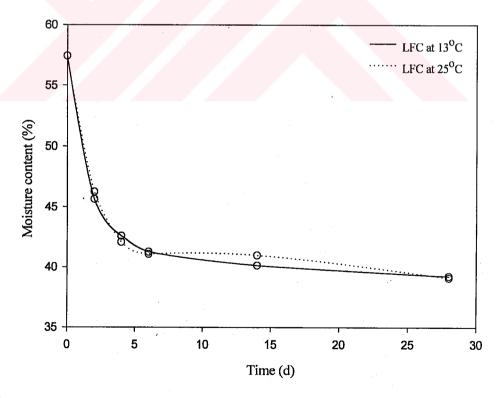


Figure 13. The effects of storage temperature on the moisture content of brined LFC.

3.3 Dynamic Rheological Measurements

3.3.1 Frequency Sweep

Frequency sweeps tests were used to distinguish whether the fat reduction and temperatures of heat treatment applied were effective on cheese characteristics or not. The dynamic viscoelastic properties of Gaziantep cheeses were measured within the linear range (shear stress = 200 Pa at 20°C) [46].

The results of frequency sweep tests (ω = 0.02-15 Hz) on the NHT of FFC, RFC and LFC samples are presented in Figure 14. For all NHT samples, G' was greater than G" at any given point which indicates a dominant contribution of the elastic component to the viscoelasticity.

Both G' and G" of the NHT cheeses were dependent on frequency and demonstrated similar trends. However the magnitude of elastic and loss modulus of LFC were greater than that of elastic and loss moduli of RFC and FFC. This could be explained as a reduction in fat resulted in an increase in the protein content; this increased the elastic (or solid-like) character of cheese [59]. Thus, the LFC could be considered to retain more of its solid like viscoelastic structure than the FFC. This kind of response has also been reported by other researchers [54,55,56]. But our results were not similar to those obtained by Ma et al. [51], who reported that the full-fat Cheddar cheeses produced stronger structure than the fat-reduced cheese at sample temperature of 20°C.

The NHT samples, shown in Figure 14, were solid-like gels with rheological spectra resembling that of weak gel. Typical characteristics of a weak gel were observed: G' was greater than G" throughout the frequency range, moduli showed a slight dependence on ω with increasing ω . Such behavior has been reported in biopolymer gels such as 2% agar gel, 1.4% xanthan solution and 15% bovine serum albumin gels [94].

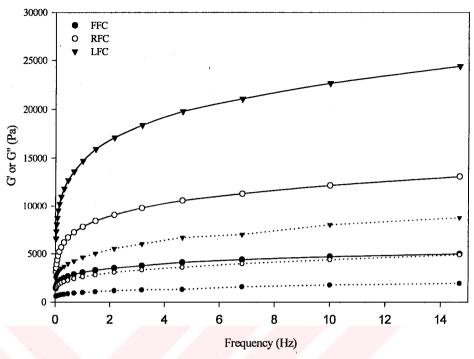


Figure 14. Mechanical spectra for NHT samples of FFC, RFC and LFC.

G' (solid line) and G"(dotted line).

The observed changes in rheological properties with the application of heat treatments were shown in Figure 15-17. For all samples G' is higher than G" in whole frequency range. The similar trends with NHT samples were observed. When frequency was increased elastic and loss modulus increased sharply at lower frequencies (f<1.5 Hz), but slightly increased at higher frequency (f>1.5 Hz). It was found that the elastic and loss modulus of cheese samples increased when heat treatments were applied. The solid-like characters of heat-treated samples are greater than non-heat-treated samples. Furthermore, increasing dipping temperatures increased the G' and G" values. Reports [95,96] indicated that the less moisture in cheese matrix led to less hydration of protein, less freedom of movement for protein molecules, larger amounts of intact casein and a firmer casein matrix. At the light of this fact, it was understood that the application of heat treatment resulted decreasing moisture contents of cheeses (Table 4). This may be a reason for increasing in cheese elasticity.

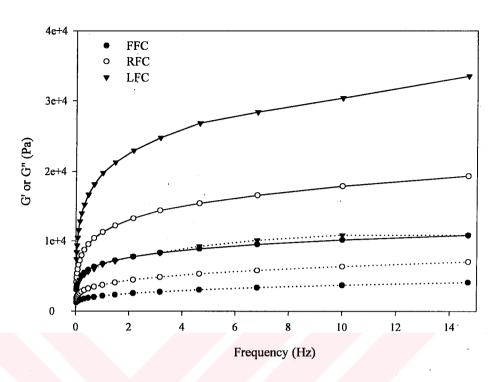


Figure 15. Mechanical spectra for FFC, RFC and LFC heat-treated at 75°C.

G' (solid line) and G"(dotted line).

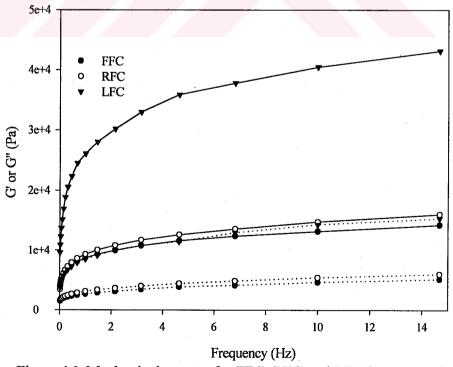


Figure 16. Mechanical spectra for FFC, RFC and LFC heat-treated at 85°C.

G' (solid line) and G" (dotted line).

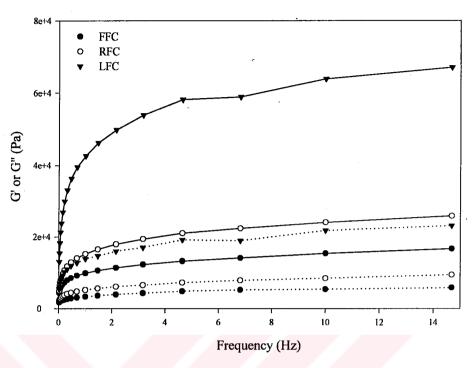


Figure 17. Mechanical spectra for FFC, RFC and LFC heat-treated at 95°C.

G'(solid line) and G"(dotted line).

The effect of dipping temperatures and fat amount on the elastic modulus of all samples were represented in Figure 18. When temperatures of heat treatment increased the magnitudes of G' and G" increased also. The heat treatment increases the moisture loss of cheese and water acts as a lubricant or a plasticizer between different proteins. Therefore, lower moisture increases hardness. The combined effects of fat and moisture on the textural characteristics of cheese are very significant [2]. With respect to the filled gel composite method, the fat and whey (mostly moisture) represent the filler within casein network. They are both responsible for the viscous properties of cheese. If the fat has no interaction (molecular bonding, colloidal forces, or friction) with the matrix, then, as its volume fraction is decreased, there is a more matrix to deform per unit volume, and consequently, the composite should get harder or more elastic [2].

It was suggested that the fat content is more effective on the rheological properties of cheese than moisture content [35]. From the results of this study, it was found that moisture content was more effective than fat content in LFC samples than RFC and FFC samples (Figure 18). When fat content decreased the effect of

moisture content increased on the solid character of the cheese. Also this effect might be due to changes in the structure of the proteins or probably proteins contribute more to cheese elasticity in cheese with lower fat than FFC [97]. When fat content decreased, the protein becomes compact and so heating may easily affect the protein structure [57]. As seen in Figure 18, for the case of NHT samples of RFC was less elastic than heat-treated FFC at 85 and 95°C. And also NHT-LFC was less elastic than heat-treated RFC at 95°C. This shows that by adjusting heat treatment temperatures; the deficiencies in physical properties of cheese due to effect of fat reduction may be eliminated during cheese production.

The magnitudes of G', G", and of all cheeses from our frequency sweep experiment were similar to the values for Cheddar obtained by Drake et al. [46] and lower than for Mozzarella cheese obtained by Yun et al [57]. In the experiments G' was higher than G" in the frequency sweep (Fig.7-14). This indicates that Gaziantep cheese samples from both experiments were in plateu region of viscoelastic spectrum [53]. This plateu region separates the short time response (i.e. transition region) where the chain architecture has little effect, from the long time response (i.e. terminal region).

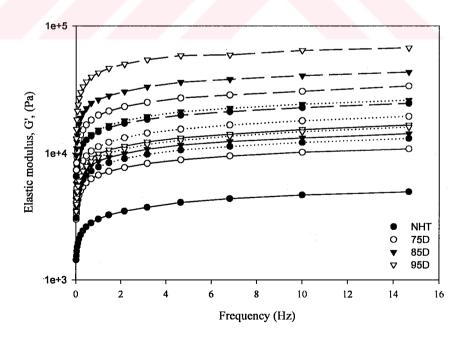


Figure 18. Effects of fat, and heat treatment temperatures on the elasticity of Gaziantep cheeses. FFC (solid line), RFC (dotted line), LFC (long dash line).

The values for tan δ of all Gaziantep cheeses (at frequencies ranging from 0.01 to 15 Hz) were between 0.3 and 0.4. This range is the same with the values for Mozzarella cheeses obtained by Yun et al [57]. When comparing the values of tan δ with the results of Yun et al. [57], Gaziantep cheese samples were similar to those of amorphous polymers of high molecular weight below its glass transitions temperature (polymethyl methacrylate) or very lightly cross linked amorphous polymer (styrene-butadiene random copolymer).

From the frequency sweep data, G' and G'' were transformed to interpret the data by the power-law model, i.e., $P = a\omega^b$, where can be G' or G'', while a and b are constants. The values of the constants a and b were shown in Table 5. The mean values of the constant b for G' and G'' of FFC in our experiment ranged from 0.18-0.20, similar to the values of Yun et al. [57], which ranged from 0.16 to 0.18. For LFC and RFC, the values of constant b are 0.20-0.22, slightly higher than those values. The a values of Gaziantep cheeses are similar to the gel's a values but b values are similar to concentrated solution's value.

Table 5. Power law model of G' and G" of FFC, RFC and LFC samples with or without heat treatments (tested at 200 Pa and 20° C, r^2 = 0.97-0.99).

| Cheese | | | | Heat treatment | |
|--------|-------|--------------------------------------|---|---|---|
| type | (Pa)* | NHT | 75D | 85D | 95D |
| FFC | G' | $3.02 \text{X} 10^3 \text{w}^{0.18}$ | $6.66 \times 10^3 \varpi^{0.18}$ | $8.61 \times 10^3 \text{m}^{0.18}$ | $9.76 \times 10^3 \text{ m}^{0.19}$ |
| | G" | $10X10^3 \varpi^{0.2}$ | $2.22X10^3 \varpi^{0.2}$ | $2.83 \text{X} 10^3 \text{w}^{0.2}$ | $3.39 \mathrm{X} 10^{3} \mathrm{\varpi}^{0.19}$ |
| | G' | $7.6 \text{X} 10^3 \text{w}^{0.2}$ | $1.1 \text{X} 10^4 \text{w}^{0.21}$ | $1.46 \text{X} 10^4 \text{w}^{0.22}$ | $9.66 \times 10^3 \varpi^{0.21}$ |
| RFC | G" | $2.68 \text{X} 10^3 \text{w}^{0.2}$ | $3.78 \times 10^3 \varpi^{0.21}$ | $5.13X10^3 \varpi^{0.22}$ | $3.22 \times 10^3 \varpi^{0.22}$ |
| | G' | $1.46 \text{X} 10^4 \text{w}^{0.19}$ | $1.91 \times 10^4 \varpi^{0.21}$ | $2.53 \text{X} 10^4 \text{m}^{0.21}$ | $4.03 \times 10^4 \varpi^{0.21}$ |
| LFC | G" | $4.81 \text{X} 10^3 \text{w}^{0.21}$ | $6.55 \mathrm{X} 10^3 \mathrm{\varpi}^{0.20}$ | $8.51 \mathrm{X} 10^3 \mathrm{\varpi}^{0.21}$ | $1.35 \text{X} 10^3 \text{w}^{0.20}$ |

Viscoelastic Property

3.3.2 Creep and Recovery Test

P.C. YÜKSEKÖĞRETIM KURULU DOKÜMANTASYON MERKEZİ

Creep is defined as 'the slow deformation of a material, usually measured under a constant stress'. In a creep test selected shear stress is 'instantaneously' applied to a sample and the resultant strain monitored as a function of time. After some predetermined time the stress is removed and the strain is again monitored. Since the actual change of strain will be dependent upon the applied stress, it is usual to talk about the compliance rather than the strain. The compliance is defined simply

as the ratio of the strain to the applied stress and is denoted by the letter J (J = strain/stress). By using this notation, creep curves may be directly compared even if they were not measured under the same applied stress.

For creep and recovery tests, the constant stress (200 Pa) and 120-second creep time were selected. The response curves for FFC, RFC, and LFC samples of non heat treated were shown in Figure 19. It was observed that the tested cheeses responded differently in creep and recovery test. The NHT-LFC had a lower compliance than FFC and RFC. The smaller compliance of the LFC indicated that it was more rigid than the others. These results were in agreement with rigidity determined from the oscillation experiment.

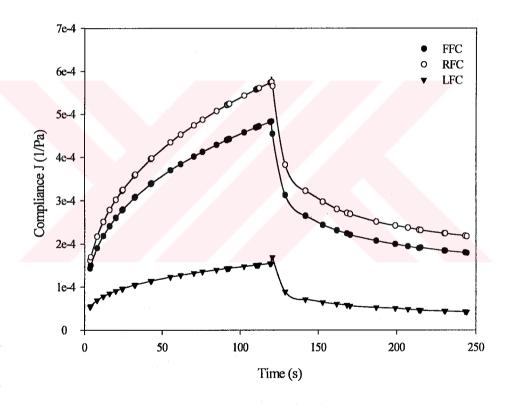


Figure 19. Creep and recovery curves of NHT samples of FFC, RFC and LFC at constant stress (200 Pa) and 120-second creep time.

It was determined that there was not any proportional relationship between RFC and FFC samples. As shown in Figure 19 for non heat-treated samples the compliance values of RFC were higher than FFC. The RFC and FFC samples dipped at 75°C showed nearly same response to the applied stress (Figure 20) but again LFC had smaller compliance than others. All LFC samples showed smaller compliance

than RFC and FFC samples at any conditions (Figure 21,22). These results showed that the differences in viscoelastic behavior between RFC and FFC could not be detected by creep and recovery test. Drake et al. [46] observed no differences in creep and residual strain among the FCC and RFC containing lecithin. But interestingly they concluded that creep strain of RFC samples were higher than those of FFC. However in this study it is found that creep strain of LFC was lower than FFC and RFC. Kuo et al. [70] also found similar results with our study. These confusing results may be due to effect of protein rather than fat. Since protein structures can easily changes for varied cheeses under different conditions. To determine the effect of fat content on the creep behavior of cheese exactly more controlled studies are needed. The structural relation between fat and protein should be determined for understanding their effect.

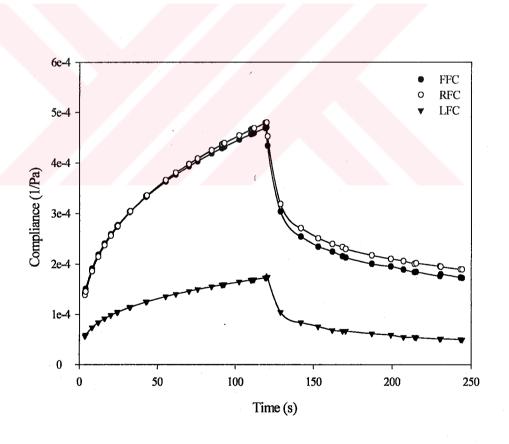


Figure 20. Creep and recovery curves of FFC, RFC and LFC heat-treated at 75°C at constant stress (200 Pa) and 120-second creep time.

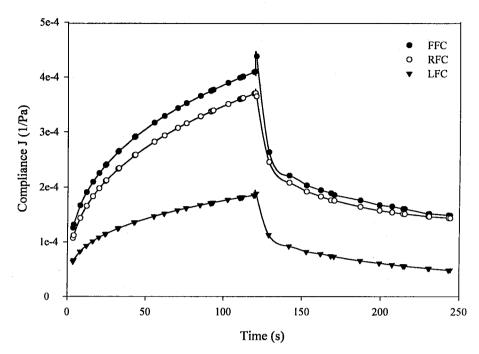


Figure 21. Creep and recovery curves of FFC, RFC and LFC heat-treated at 85°C, at constant stress (200 Pa) and 120-second creep time.

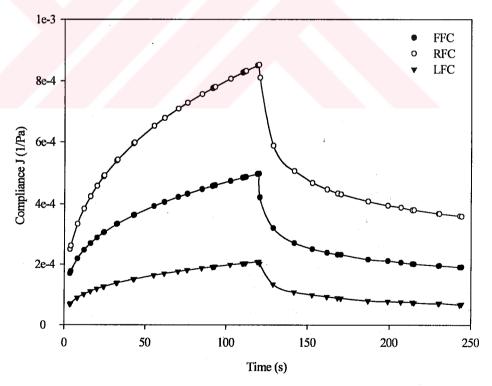


Figure 22. Creep and recovery curves of FFC, RFC and LFC heat-treated at 95°C, at constant stress (200 Pa) and 120-second creep time.

The effect of heat treatments on the creep behavior of LFC was shown in Figure 23. It was interestingly found that when heat treatment temperatures increased the compliance of the samples increased also. This suggests that elasticity of the heat-treated samples decreased when the temperatures were increased. This result was not confirmed the frequency sweep results. In frequency sweep test, it was found that heat treatment increased the elasticity of the samples (Figure 18). This may be due to the application of stress during the test. In frequency sweep tests the stress applied continuously with increasing the rate of application and deformation was measured as a function of frequency. On the other hand in creep test constant stress applied to the samples instantaneously and deformation was observed. So when cheeses responded the applied stress undistributed protein network structure may be dominant [72]. During application of heat treatments the protein structure may be weakened so less elastic behavior for heat-treated cheeses were observed with time.

The results of the creep test were shown for RFC and FFC in Figure 24 and 25, respectively.

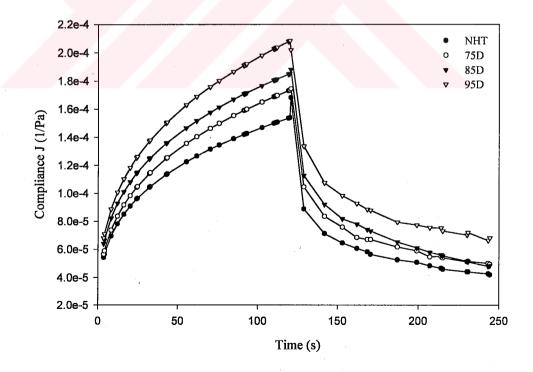


Figure 23. Creep and recovery curves of LFC with different heat treatment temperatures, at constant stress (200 Pa) and 120-second creep time.

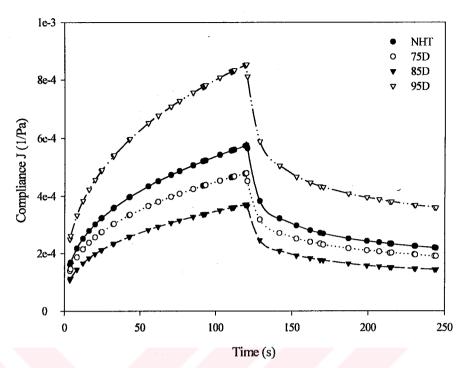


Figure 24. Creep and recovery curves of RFC with different heat treatment temperatures, at constant stress (200 Pa) and 120-second creep time.

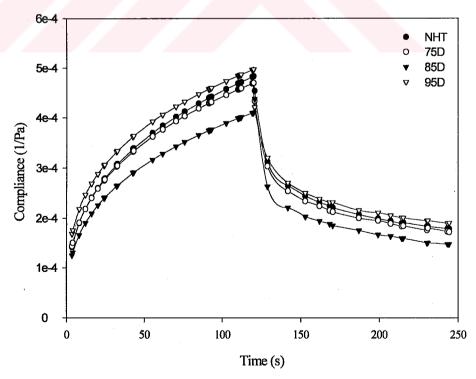


Figure 25. Creep and recovery curves of FFC with different heat treatment temperatures at constant stress (200 Pa) and 120-second creep time.

It was not observed any proper effect of heat treatments on the elasticity of RFC and FFC. All FFC samples showed nearly same behavior in creep test. But RFC samples did not response uniformly.

3.3.3 Temperature sweep test

Rheological scanning during the thermal processing is significant for monitoring the physical properties changes in the cheese that relate to molecular changes [36]. Temperature sweep tests were applied to all Gaziantep cheese samples between 10-70°C to determine any physical changes i.e. melting of fat, during heating. For discussing the test results, elastic modulus (G') and phase angle (δ) parameters were selected. The value of δ shows viscous character of the materials.

The changes in elastic modulus during temperature sweep test for non heat-treated Gaziantep cheeses are shown in Figure 26. For all cheeses, increasing the temperature resulted in a decrease in G' and increase in the phase angle (phase lag) δ (Figure 27-29). The high value of δ shows that material has more viscous character. The changes in G' and δ indicate a phase transition from an unheated cheese, largely elastic in rheological response (δ = 15-18° at 10°C), to a melted cheese, which is more viscous in character (δ = 35-80° above 60°C). G' decreased rapidly as the temperature was raised from 10 to 35-50°C. The decrease in G' indicated softening of the cheese. This may be due to the liquefaction of the fat phase, which is fully liquid at 35-40°C [54].

To observe the rheological attributes of cheese samples at high temperatures, log G' versus T graphs of FFC samples (with and without heat treatments) were plotted in Figure 30. The heat-treated FFC samples showed sharp deviation from G' values at high temperatures but NHT-FFC sample showed uniform decreasing. This deviation could be explained with complete melting (or changing the structure) of the samples. Except the 95D-RFC the RFC samples showed uniform decreasing (Figure 31). But all low-fat samples represented uniform and nearly the same response to the test (Figure 32). These results may comment, as the heat treatments were mainly effective for fatty parts.

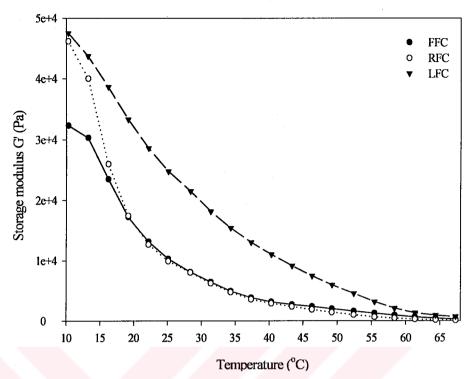


Figure 26. Storage modulus as a function of temperature for NHT of FFC, RFC and LFC.

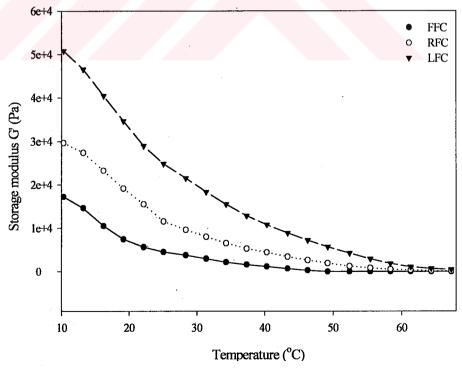


Figure 27. Storage modulus as a function of temperature for heat-treated FFC, RFC and LFC at 75°C.

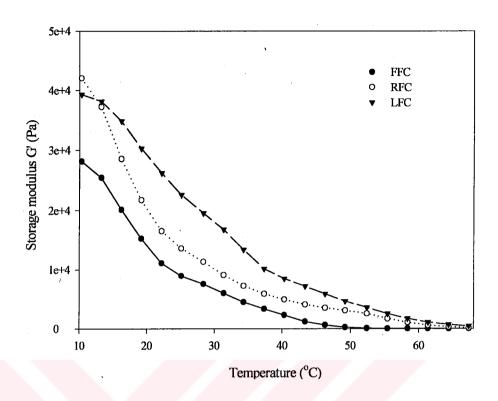


Figure 28. Storage modulus as a function of temperature for heat-treated FFC, RFC and LFC at 85°C.

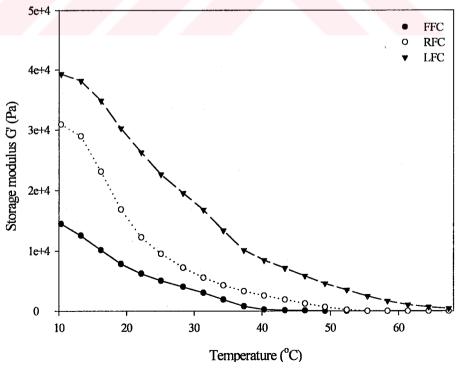


Figure 29. Storage modulus as a function of temperature of FFC, RFC and LFC heat-treated at 95°C.

The phase angle δ increased slowly from 16-18° to 20-25° when temperatures raised from 10°C to 40°C and thereafter more quickly reaching maximum values (Figure 33), the magnitude of which were affected by fat content and heat treatment for FFC, and to a lesser extend by heat treatment for RFC and LFC cheese.

Since $\tan \delta = G''/G'$ when the G'=G'', $\tan \delta$ equals 1.0. This means that solid and liquid characters are the same extent at this point that is called as crossover temperature [57]. This temperature might be accepted as the beginning of the melting. In Table 7, melting temperatures of all cheese samples were listed. Increasing the fat content decreased the melting temperatures. The marked increase in δ occurred at that temperatures especially in the FFC, indicating a rapid increase in fluidity of the melting sample, which may result from the coalescence of nonglobular fat that is fully liquid at 40°C. For 95D-FFC this temperature was below 40°C. The heat treatments accelerate the coalescence of nonglobular fat particles, this may be due to that during the dipping process in hot whey some fat globules may liquefy and entrapped by casein matrix and when again heating is applied the liquefying of fats become easy. But to clear identification, further study is needed and the microstructure of the cheese should be investigated by scanning electron microscopy.

Table 7. The melting temperatures (when $\tan \delta = 1.0$) of FFC, RFC and LFC samples without and with heat treatments.

| Heat treatment | Cheese type | | | |
|----------------|-------------|------|------|--|
| (°C) | FFC | RFC | LFC | |
| NHT | 56.1 | 59.2 | 67.0 | |
| 75D | 46.3 | 55.3 | 67.0 | |
| 85D | 44.5 | 55.2 | 67.5 | |
| 95D | 36.8 | 49.2 | 64.3 | |

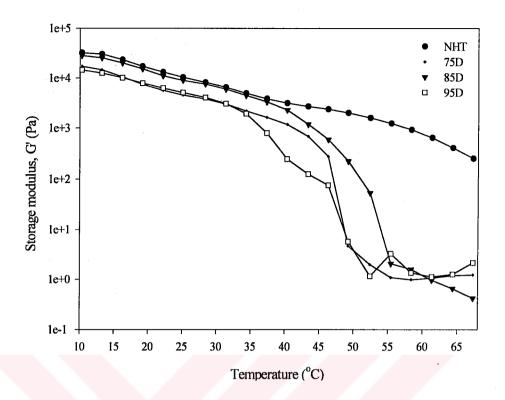


Figure 30. Storage modulus as a function of temperature for FFC samples that exposed to different heat treatments.

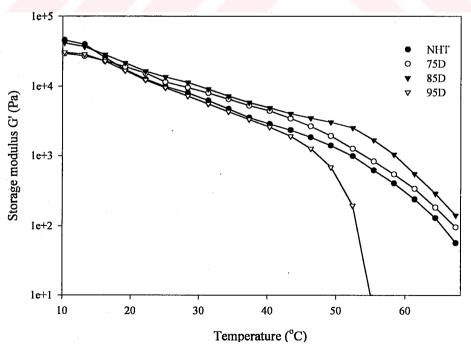


Figure 31. Storage modulus as a function of temperature for RFC samples that exposed to different heat treatments.

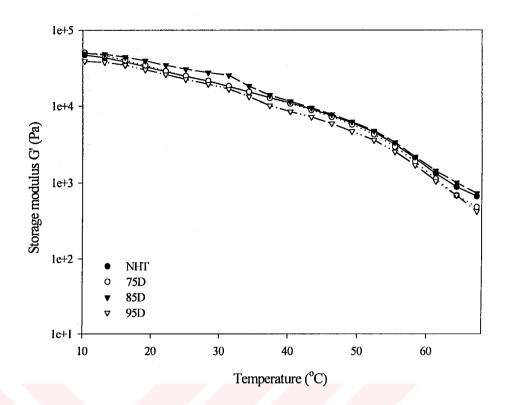


Figure 32. Storage modulus as a function of temperature for LFC samples that exposed to different heat treatments.

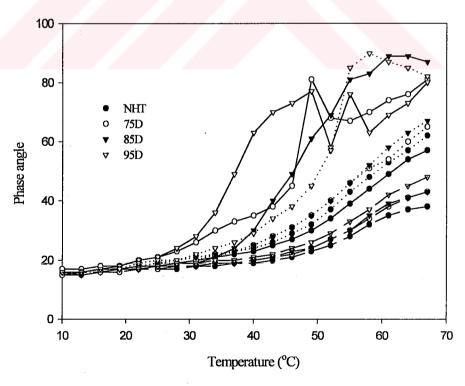


Figure 33. Phase angles of the Gaziantep cheese samples treated with different heat treatments (FFC-solid line, RFC-dotted line, LFC-dashed line).

3.4 Meltability

The meltability of the Gaziantep cheese containing varied fat amounts with and without heat treatments at different temperatures were represented in Figure 34. As the fat content of the cheese decreased, the meltability of the cheese decreased. Decreasing fat content result in a lower MNFS, causing the protein matrix to become firmer and more likely to support its own weight when heated [12]. Tunick et al. [12] and Rudan et al. [2] also found that Mozzarella cheese meltability decreased as the fat content decreased. But Fife et al. [52] found that fat content had no influence on the meltability of Mozzarella cheese.

There was a significant difference (P<0.05) (Table A7) between the meltability values obtained from heat-treated and non-heat treated Gaziantep cheese samples (Table 8). It was found that effect of heat treatment temperatures (in the studied range) had no proper effect on the melting characteristics of Gaziantep cheese. For FFC and RFC heat treatment caused decreasing meltability but for LFC the dipping at 95°C resulted increasing the meltability of cheese. It was observed that the melting properties of Gaziantep cheese were not affected with age (data are not shown). This may be evidence of the lack of proteolysis of Gaziantep cheese during the storage since for some cheeses meltability increases during storage time because of the proteolysis, breakdown of α_{s1} -casein, solubilization of the resulting fragments, and release of the fat [50,84]

There are some conflicting reports where most of the reports suggested that fat amount is one of the important criteria effecting melting and some scientists found that melting are not related with fat content of the cheese. Since the meltability is related to heat transfer and thermal phase change characteristics of the solid cheese and rheological or flow properties of the melt. These characteristics are highly interdependent and transient properties [7,3]. Some scientists reported that estimating of the melting characteristics of cheeses is difficult due to not only the compositional differences properties (the ratio of the major components, fat, water, protein) but also the different structural properties [44]. Further study should be done for complete understanding of melting phenomena or needed to develop new concept to define melting properties.

Table 8. Meltability of NHT and heat-treated FFC, RFC and LFC samples.

| Cheese type – | Heat treatment | | | |
|---------------|-------------------|-------------------|-------------------|-------------------|
| | NHT | 75D | 85D | 95D |
| FFC | 68.5ª | 58.0 ^b | 59.0 ^b | 54.5 ^b |
| RFC | 39.5° | 46.0 ^d | 44.5 ^d | 40.0 ^e |
| LFC | 18.5 ^f | 14.0 ^g | 14.0 ^g | 26.5 ^h |

a,b,c Means within table with no common superscript differ (P<0.05).

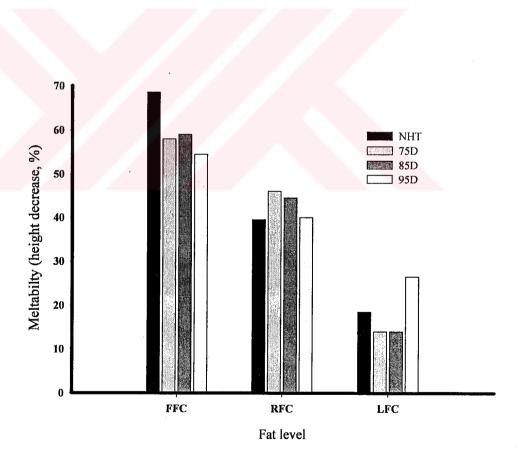


Figure 34. Effect of fat content and the heat treatments on meltability of FFC, RFC and LFC.

3.5 Color Analysis

Color change, with respect to whiteness (L-value), of Gaziantep cheeses were shown in Figure 35. The L-value corresponds to whiteness, and higher L-values indicate whiter products. When fat content of cheese was increased the L-values also increased (P<0.05) (Table A10) since fat can contribute to the L-value of dairy products by scattering light [98]. This result was expected and agreed with the literature reports [74].

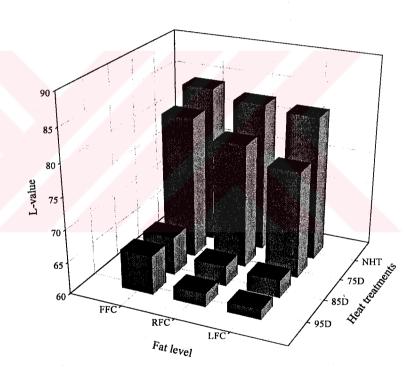


Figure 35. Changes in whiteness (L-value) of NHT or heat-treated FFC, RFC and LFC.

Rather than fat reduction, effects of heat treatment on the whiteness of Gaziantep cheese were interestingly high. When heat treatment temperature was increased the L-value of the cheese decreased (P<0.05) (Table A10-12). This result was unexpected since the differences in fat contents of the heat –treated and non heat-treated samples for each type of Gaziantep cheese samples studied were less

than those of fat reduced and fatty cheese. The changes in L-values with heat treatment were probably related with changes in protein structure during heating. When cheese is heated, some of the protein in the serum phase interacts to form gels particles, which scatter light, and cause the cheese become whiter [98]. After heat treatment color analyses were done when samples come to equilibrium with the room temperature (20°C).

It was observed that during cooling whiteness of the samples especially lowfat samples decreased continuously. This may be due to that when the cheese cools, the proteins in the cheese serum dissociate, no longer scatter light and the cheese is less white. In addition, there may also be reversible interactions between the proteins in the cheese whey and casein matrix during heating and cooling. Although high differences between fat contents of the FFC/95D, RFC/95D and LFC/95D, the Lvalues of the samples were 66, 62 and 61.5 respectively. These results suggest the contribution of the whey to L-value may be greater than after heating than before heating. This means that before heat treatments the effect of fat content on the Lvalues is high and mainly determines the whiteness of the cheeses. But after heating, proteins in the cheese serum may influence the whiteness of cheese. The higher moisture content of the heat-treated LFC supports this idea. Metzger et al. [98] studied to observe whiteness change during heating and cooling of Mozzarella cheese with controlling the proteins and they suggests the mentioned reasons for their results. And they concluded that protein interactions were responsible for changes. Fife et al. [52] reported that when LFC was cooled, slightly greenish tint was observed. This greenish tint associated with fewer light-scatterings centers (less fat globules) so whiteness of cheese decreased.

The changes of L-values of Gaziantep cheese in salt solution (20% salt) during 28-day storage at 13°C were shown in Figure 36. There was an interesting trend in color during the short time brining. First, L- value of the cheese samples decreased steeply but then increased due to possibly uneven salt distribution in the cheese structure [73]. After reaching the equilibrium of salt distribution, L-values of samples did not change. The same trend was observed for the cheese stored at 25°C (Figure 37). But L-values of the cheese stored at 25°C were slightly higher than the L-values of the samples stored at 13°C. This probably differences in the salt content

and moisture content of the samples, because salt diffusion and moisture loss increased with increasing temperature [83].

The a,b and YI values of samples are not shown. There were no statistically differences for these values among the samples. Only the slightly increases in b value was observed for the LFC samples. These means LFC samples were yellowier than others, which may be resulted in slightly more retention of lactose in cheese [98].

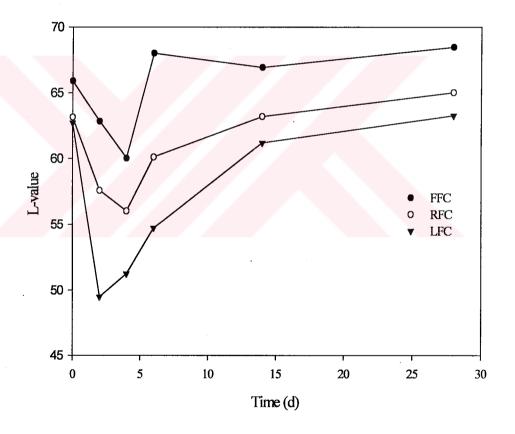


Figure 36. Changes in whiteness (L-value) of FFC, RFC and LFC stored at 13°C and in 20% salt content.

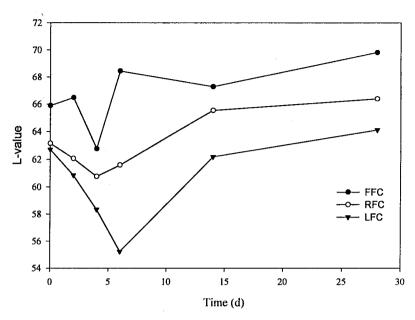


Figure 37. Changes in whiteness (L-value) of FFC, RFC and LFC stored at 25°C and in 20% salt content.

3.6 Texture Profile Analysis

Hardness (force necessary to attain a given deformation) is the most commonly evaluated for determination of cheese texture [95]. Textural hardness values of FFC, RFC and LFC without and with heat treatments are given in Figure 38. It was found that reducing the fat content significantly increased the hardness (P<0.05) (Table A13-15). Although LFC and RFC samples had higher moisture contents, their hardness also increased. This showed that fat imparts softness to the cheese higher than water. It was reported that diffusion coefficient of salt (NaCl) is directly related to the temperature and moisture content and inversely related to the fat content [7]. It was obvious that the denser protein matrix causes the increasing the hardness of fat- reduced cheeses. This effect was more pronounced at higher heat treatment temperatures. When heat treatment temperatures was increased the differences between the hardness of the cheese samples increased (Figure 38). This may be due to the heat treated samples containing less MNFS (Table 4) than their NHT counterparts, leading to less hydration of protein, less freedom of movement for the protein molecules, larger amount of intact caseins, and a firmer casein matrix [24]. These results are in agreement with literature [1,32].

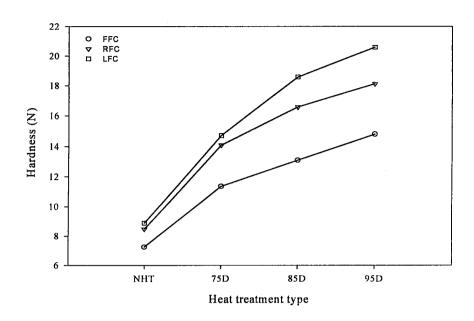


Figure 38. Effect of fat reduction and heat treatments on the textural hardness of Gaziantep cheese.

During 28-day storage the hardness values of the FFC/85D, RFC/85D and LFC/95D samples were measured. Changes in hardness during the storage at 13°C were shown in Figure 39. Within the first four days the hardness values of all samples increased sharply then continued increasing steadily. This may be due to the higher moisture loss and increasing the salt content at the beginning of the storage after that the rate of salt diffusion decreases and system may reach equilibrium so the rate of increasing the hardness decreased [97]. Softness in cheese samples was not observed during the storage, although for most kind of cheese decreasing in hardness was reported [32,35]. It was also reported that that decreasing in hardness due to breakdown of protein network during proteolysis [35]. The absence of softening during storage may be evident lack of fermentation process in Gaziantep cheese.

As expected, decreasing the fat content increased the hardness and this trend continued during the storage. The stored samples at 25°C (Figure 40) showed higher hardness than stored at 13°C. This may probably come from the differences between the moisture contents of samples. Samples stored at 25°C had lower moisture content than stored at 13°C (Figure 11).

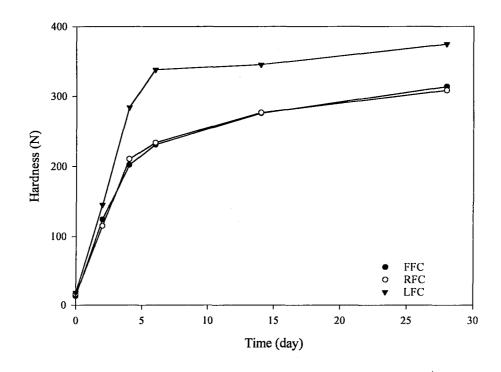


Figure 39. Changes in the hardness of FFC, RFC and LFC during storage at 13°C

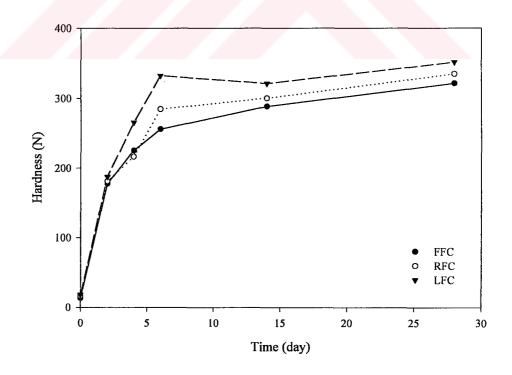


Figure 40. Changes in the hardness of FFC, RFC and LFC during storage at 25°C.

CHAPTER IV

CONCLUSIONS

Gaziantep cheese was studied to determine effect of fat content, heat treatments and storage conditions on its physical characteristics including rheological attributes, meltability, hardness and color. In the light of the results given in chapter III, this study revealed the following conclusions:

The reduction of fat contents caused increase in moisture and protein contents of the cheese. Heat treatments decreased the moisture contents of cheeses. Increasing the storage temperatures increased the moisture loss during brining.

The frequency sweep test was successful to determine the structural changes. The gel strength of the cheeses increased when fat content of cheese was decreased. Heat treatments also enhanced the structures of the cheeses. Power law model fitted all frequency sweep data with high correlation coefficients. The gel strength constants were between 0.18-0.22. The values for tan δ of all Gaziantep cheeses were between 0.3 and 0.4. This range is the same with the values for Mozzarella cheese. The structural changes in Gaziantep cheeses could not be detected using creep and recovery test.

Increasing fat content in the cheese and application of heat treatments decreased the melting temperature of Gaziantep cheese.

Whiteness (L-value) significantly changed by the effect of fat reduction and heat treatment.

Reducing fat content increased hardness of Gaziantep cheese. This effect was increased with the effect of heat treatment.

When the fat content of Gaziantep cheese is decreased to around 30%, the defects in its physical properties can be eliminated by modification of heat treatment.

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APPENDICES

Table Al. ANOVA (one-way) for chemical composition of milk.

| | | Sum of Squares | df | Mean Square | F | P |
|------------------|----------------|----------------|----|-------------|--------|-------|
| Moisture content | Between Groups | 7.905 | 2 | 3.952 | 8177.2 | 0.000 |
| | Within Groups | 1.5E-03 | 3 | 4.8E-04 | | |
| | Total | 7.906 | 5 | | | |
| SNF | Between Groups | 1.2E-03 | 2 | 6.2E-04 | 3.7 | 0.155 |
| | Within Groups | 5.0E-04 | 3 | 1.7E-04 | | |
| | Total | 1.7E-03 | 5 | | | |
| Protein content | Between Groups | 3.4E-03 | 2 | 1.7E-03 | 103.0 | 0.002 |
| | Within Groups | 5.0E-05 | 3 | 1.7E-05 | | |
| | Total | 3.5E-03 | 5 | | | |
| pН | Between Groups | 1.2E-03 | 2 | 6.2E-04 | 3.7 | 0.155 |
| • | Within Groups | 5.0E-04 | 3 | 1.7E-04 | | |
| | Total | 1.7E-03 | 5 | | | |

Table A2. Multiple comparisons for components of milk.

| Dependent variable | | | MD | SE | P |
|--------------------|-----|-----|-----------|-------|-------|
| Moisture content | FFM | RFM | -1.325* | 0.022 | 0.000 |
| | | LFM | -2.810* | 0.022 | 0.000 |
| | RFM | FFM | 1.325* | 0.022 | 0.000 |
| | | LFM | -1.485* | 0.022 | 0.000 |
| | LFM | FFM | 2.810* | 0.022 | 0.000 |
| | | RFM | 1.485* | 0.022 | 0.000 |
| SNF | FFM | RFM | 1.5E-02 | 0.013 | 0.329 |
| | | LFM | 3.5E-02 | 0.013 | 0.073 |
| | RFM | FFM | -1.5E-02 | 0.013 | 0.329 |
| | | LFM | 2.0E-02 | 0.013 | 0.219 |
| | LFM | FFM | -3.5E-02 | 0.013 | 0.073 |
| | | RFM | -2.0E-02 | 0.013 | 0.219 |
| Protein content | FFM | RFM | -1.0E-02 | 0.004 | 0.092 |
| | | LFM | -5.5E-02* | 0.004 | 0.001 |
| | RFM | FFM | 1.0E-02 | 0.004 | 0.092 |
| | | LFM | -4.5E-02* | 0.004 | 0.002 |
| | LFM | FFM | 5.5E-02* | 0.004 | 0.001 |
| | | RFM | 4.5E-02* | 0.004 | 0.002 |
| PH | FFM | RFM | 1.5E-02 | 0.013 | 0.329 |
| | | LFM | 3.5E-02 | 0.013 | 0.073 |
| | RFM | FFM | -1.5E-02 | 0.013 | 0.329 |
| | | LFM | 2.0E-02 | 0.013 | 0.219 |
| | LFM | FFM | -3.5E-02 | 0.013 | 0.073 |
| | | RFM | -2.0E-02 | 0.013 | 0.219 |

^{*} The mean difference is significant at the 0.05 level.

Table A3. ANOVA (two-way) for moisture content, protein content and MNFS by heat treatments and fat level

| Source | Dependent | Sum of | df | Mean | F | P |
|----------------|------------------|-----------|-----|---------|-----------|-------|
| | Variable | Squares | | Square | | |
| Heat treatment | Moisture content | 83.659 | 3 | 27.886 | 2535.120 | 0.000 |
| 75D, 85D, 95D | Protein content | 3.914 | 3 | 1.305 | 724.802 | 0.000 |
| • | MNSF | 124.549 | . 3 | 41.516 | 9049.851 | 0.000 |
| Fat level | Moisture content | 104.663 | 2 | 52.332 | 4757.414 | 0.000 |
| FFC, RFC, LFC | Protein content | 189.394 | 2 | 94.697 | 52609.343 | 0.000 |
| | MNSF | 203.925 | 2 | 101.963 | 22226.203 | 0.000 |
| Interaction | Moisture content | 12.439 | 6 | 2.073 | 188.469 | 0.000 |
| | Protein content | 0.766 | 6 | 0.128 | 70.960 | 0.000 |
| | MNSF | 12.097 | 6 | 2.016 | 439.475 | 0.000 |
| Error | Moisture content | 0.132 | 12 | 1.1E-02 | | |
| | Protein content | 2.2E-02 | 12 | 1.8E-03 | | |
| | MNSF | 5.5E-02 | 12 | 4.6E-03 | | |
| Total | Moisture content | 62708.561 | 24 | | | |
| | Protein content | 21290.664 | 24 | | | |
| | MNSF | 87919.419 | 24 | | | |

Table A4. Multiple comparisons of moisture content, protein content and MNFS of cheese by fat level.

| Dependent variable | | | MD* | SE | P |
|--------------------|-----|-----|---------------------|-------|-------|
| Moisture content | FFC | LFC | -5.115 [*] | 0.052 | 0.000 |
| | | RFC | -2.514* | 0.052 | 0.000 |
| | LFC | FFC | 5.115* | 0.052 | 0.000 |
| | | RFC | 2.601* | 0.052 | 0.000 |
| | RFC | FFC | 2.514* | 0.052 | 0.000 |
| | | LFC | -2.601* | 0.052 | 0.000 |
| Protein content | FFC | LFC | -6.880* | 0.021 | 0.000 |
| | | RFC | -3.338* | 0.021 | 0.000 |
| | LFC | FFC | 6.880* | 0.021 | 0.000 |
| | | RFC | 3.543* | 0.021 | 0.000 |
| | RFC | FFC | 3.338* | 0.021 | 0.000 |
| | | LFC | -3.543* | 0.021 | 0.000 |
| MNSF | FFC | LFC | 7.140* | 0.034 | 0.000 |
| | | RFC | 3.606* | 0.034 | 0.000 |
| | LFC | FFC | -7.140* | 0.034 | 0.000 |
| | | RFC | -3.534* | 0.034 | 0.000 |
| | RFC | FFC | -3.606* | 0.034 | 0.000 |
| | | LFC | 3.534* | 0.034 | 0.000 |

^{*} The mean difference is significant at the 0.05 level.

Table A5. Multiple comparisons of moisture content, protein content and MNFS of cheese by heat treatments

| Dependent Variabl | е | | MD | SE | P |
|-------------------|-----|-----|----------|-------|-------|
| Moisture content | 75D | 85D | 8.5E-02 | 0.061 | 0.186 |
| Worsture content | | 95D | 2.033* | 0.061 | 0.000 |
| | | NHT | -3.175* | 0.061 | 0.000 |
| | 85D | 75D | -8.5E-02 | 0.061 | 0.186 |
| | | 95D | 1.948* | 0.061 | 0.000 |
| | | NHT | -3.260* | 0.061 | 0.000 |
| | 95D | 75D | -2.033* | 0.061 | 0.000 |
| | | 85D | -1.948* | 0.061 | 0.000 |
| | | NHT | -5.2083 | 0.061 | 0.000 |
| | NHT | 75D | 3.175* | 0.061 | 0.000 |
| | | 85D | 3.260* | 0.061 | 0.000 |
| | | 95D | 5.208* | 0.061 | 0.000 |
| Protein content | 75D | 85D | -0.290* | 0.024 | 0.000 |
| | | 95D | -0.737* | 0.024 | 0.000 |
| | | NHT | 0.367* | 0.024 | 0.000 |
| | 85D | 75D | 0.290* | 0.024 | 0.000 |
| | | 95D | -0.447* | 0.024 | 0.000 |
| | _ | NHT | 0.657* | 0.024 | 0.000 |
| | 95D | 75D | 0.737* | 0.024 | 0.000 |
| | | 85D | 0.447* | 0.024 | 0.000 |
| | | NHT | 1.103* | 0.024 | 0.000 |
| | NHT | 75D | -0.367* | 0.024 | 0.000 |
| | | 85D | -0.657* | 0.024 | 0.000 |
| | | 95D | -1.103* | 0.024 | 0.000 |
| MNSF | 75D | 85D | 0.165* | 0.039 | 0.001 |
| | | 95D | 2.448* | 0.039 | 0.000 |
| | | NHT | -3.892* | 0.039 | 0.000 |
| | 85D | 75D | -0.165* | 0.039 | 0.001 |
| | | 95D | 2.283* | 0.039 | 0.000 |
| | | NHT | -4.057* | 0.039 | 0.000 |
| | 95D | 75D | -2.448* | 0.039 | 0.000 |
| | | 85D | -2.283* | 0.039 | 0.000 |
| | | NHT | -6.340* | 0.039 | 0.000 |
| | NHT | 75D | 3.892* | 0.039 | 0.000 |
| | | 85D | 4.057* | 0.039 | 0.000 |
| | | 95D | 6.340* | 0.039 | 0.000 |

^{*} The mean difference is significant at the 0.05 level.

Table A6. ANOVA (three-way) for moisture content of cheese by fat content, storage time and storage temperature.

| Source | Sum of Squares | Df | Mean Square | F | Sig. |
|-------------------------|----------------|----|-------------|-----------|-------|
| Storage time | 2136.129 | 5 | 427.226 | 83678.620 | 0.000 |
| Storage temperature | 9.783 | 1 | 9.783 | 1916.136 | 0.000 |
| Fat content | 82.131 | 2 | 41.066 | 8043.337 | 0.000 |
| Time* Temperature | 7.992 | 5 | 1.598 | 313.077 | 0.000 |
| Time* Fat | 73.828 | 10 | 7.383 | 1446.027 | 0.000 |
| Temperature* Fat | 35.017 | 2 | 17.509 | 3429.324 | 0.000 |
| Time * Temperature *fat | 35.555 | 10 | 3.555 | 696.398 | 0.000 |
| Error | 0.184 | 36 | 5.1E-03 | | |
| Total | 135393.404 | 72 | | | |

Table A7. ANOVA (two-way) for meltability of cheese by fat content and heat treatment.

| Source | Sum of Squares | df | Mean Square | F | P |
|---------------------|----------------|----|-------------|---------|-------|
| Fat content | 7033.000 | 2 | 3516.500 | 715.220 | 0.000 |
| Heat treatment | 34.167 | 3 | 11.389 | 2.316 | 0.127 |
| Fat *Heat Treatment | 452.333 | 6 | 75.389 | 15.333 | 0.000 |
| Error | 59000.000 | 12 | 4.917 | | |
| Total | 46460.000 | 24 | | | |

Table A8. Multiple comparisons for meltability of cheese by fat content

| , | | MD | SE | P |
|-----|-----|---------|-------|-------|
| FFC | LFC | 41.75* | 1.109 | 0.000 |
| | RFC | 17.50* | 1.109 | 0.000 |
| LFC | FFC | -41.75* | 1.109 | 0.000 |
| | RFC | -24.25* | 1.109 | 0.000 |
| RFC | FFC | -17.50* | 1.109 | 0.000 |
| | LFC | 24.25* | 1.109 | 0.000 |

^{*} The mean difference is significant at the 0.05 level.

Table A9. Multiple comparisons for meltability by heat treatments

| | · · · · · · · · · · · · · · · · · · · | Mean Difference | SE | P |
|-----|---------------------------------------|-----------------|-------|-------|
| NHT | 75D | 2.833* | 1.280 | 0.047 |
| | 85D | 3.000* | 1.280 | 0.037 |
| | 95D | 1.833 | 1.280 | 0.178 |
| 75D | NHT | -2.833* | 1.280 | 0.047 |
| | 85D | .1667 | 1.280 | 0.899 |
| | 95D | -1.000 | 1.280 | 0.450 |
| 85D | NHT | -3.000* | 1.280 | 0.037 |
| | 75D | -0.167 | 1.280 | 0.899 |
| | 95D | -1.167 | 1.280 | 0.380 |
| 95D | NHT | -1.833 | 1.280 | 0.178 |
| | 75D | 1.000 | 1.280 | 0.450 |
| | 85D | 1.167 | 1.280 | 0.380 |

Table A10. ANOVA (two-way) for L-value of cheese by fat content and heat treatment.

| Source | Sum of Squares | df | Mean Square | F | Sig. |
|----------------|----------------|----|-------------|---------|-------|
| Fat | 65.8 | 2 | 32.924 | 25.977 | 0.000 |
| Heat treatment | 1852.8 | 3 | 617.607 | 487.296 | 0.000 |
| Fat* heat | 6.5 | 6 | 1.088 | 0.858 | 0.551 |
| Error | 15.2 | 12 | 1.267 | | |
| Total | 127126.5 | 24 | | | |

Table A11. Multiple comparisons for the L-value of the cheese by fat content.

| | | Mean Difference | Std. Error | P |
|-----|-----|-----------------|------------|-------|
| FFC | LFC | 3.871* | 0.563 | 0.000 |
| | RFC | 2.988* | 0.563 | 0.000 |
| LFC | FFC | -3.871* | 0.563 | 0.000 |
| | RFC | -0.884 | 0.563 | 0.142 |
| RFC | FFC | -2.986* | 0.563 | 0.000 |
| | LFC | 0.884 | 0.563 | 0.142 |

^{*} The mean difference is significant at the 0.05 level.

^{*} The mean difference is significant at the .05 level.

Table A12. Multiple comparisons for L-value of cheese by heat treatment.

| | | Mean Difference | SE | P |
|-----|-----|-----------------|-------|-------|
| NHT | 75D | 3.327* | 0.650 | 0.000 |
| | 85D | 18.740* | 0.650 | 0.000 |
| | 95D | 19.403* | 0.650 | 0.000 |
| 75D | NHT | -3.327* | 0.650 | 0.000 |
| | 85D | 15.413* | 0.650 | 0.000 |
| | 95D | 16.077* | 0.650 | 0.000 |
| 85D | NHT | -18.740* | 0.650 | 0.000 |
| | 75D | -15.413* | 0.650 | 0.000 |
| | 95D | 0.663 | 0.650 | 0.328 |
| 95D | NHT | -19.403* | 0.650 | 0.000 |
| | 75D | -16.077* | 0.650 | 0.000 |
| | 85D | -0.663 | 0.650 | 0.328 |

Table A13. ANOVA (two-way) for hardness of cheese by fat content and heat treatment.

| Sum of Squares* | df | Mean Square | F | Sig. |
|-----------------|--------------------------------------|---|---|--|
| 64.018 | 2 | 32.009 | 60.225 | 0.000 |
| 317.360 | 3 | 105.787 | 199.038 | 0.000 |
| 12.498 | 6 | 2.083 | 3.919 | 0.021 |
| 6.378 | 12 | 0.531 | | |
| 5052.318 | 24 | | | |
| | 64.018 317.360 12.498 6.378 | 64.018 2 317.360 3 12.498 6 6.378 12 | 64.018 2 32.009 317.360 3 105.787 12.498 6 2.083 6.378 12 0.531 | 64.018 2 32.009 60.225 317.360 3 105.787 199.038 12.498 6 2.083 3.919 6.378 12 0.531 |

^{*} Type III

Table A14. Multiple comparisons for hardness of cheese by fat content.

| | | Mean Difference | SE | P |
|-----|-----|-----------------|-------|-------|
| FFC | LFC | -3.9413* | 0.365 | 0.000 |
| | RFC | -2.5650* | 0.365 | 0.000 |
| LFC | FFC | 3.9413* | 0.365 | 0.000 |
| | RFC | 1.3763* | 0.365 | 0.003 |
| RFC | FFC | 2.5650* | 0.365 | 0.000 |
| | LFC | -1.3763* | 0.365 | 0.003 |

^{*} The mean difference is significant at the 0.05 level.

^{*} The mean difference is significant at the 0.05 level.

Table A15. Multiple comparisons for the hardness of cheese by heat treatment

| | | • | | |
|-----|-----|---------|-------|-------|
| | | MD | SE | P |
| 75D | 85D | -2.532* | 0.421 | 0.000 |
| | 95D | -4.285* | 0.421 | 0.000 |
| | NHT | 5.353* | 0.421 | 0.000 |
| 85D | 75D | 2.532* | 0.421 | 0.000 |
| | 95D | -1.753* | 0.421 | 0.001 |
| | NHT | 7.885* | 0.421 | 0.000 |
| 95D | 75D | 4.285* | 0.421 | 0.000 |
| | 85D | 1.753* | 0.421 | 0.001 |
| | NHT | 9.638* | 0.421 | 0.000 |
| NHT | 75D | -5.353* | 0.421 | 0.000 |
| | 85D | -7.885* | 0.421 | 0.000 |
| | 95D | -9.638* | 0.421 | 0.000 |



Based on observed means.

The mean difference is significant at the 0.05 level.