

**UNIVERSITY OF GAZİANTEP
GRADUATE SCHOOL OF
NATURAL & APPLIED SCIENCES**

**PROPERTIES AND APPLICATION OF WHEY
PROTEIN ISOLATE BASED FILMS CONTAINING
CLOVE AND APRICOT ESSENTIAL OILS**

**M. Sc. THESIS
IN
FOOD ENGINEERING**

**BY
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**Properties and Application of Whey Protein Isolate Based
Films Containing Clove and Apricot Essential Oils**

**M. Sc. Thesis
in
Food Engineering
University of Gaziantep**

**Supervisor
Prof. Dr. Sevim KAYA**

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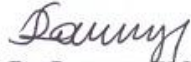
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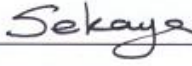
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ABSTRACT

PROPERTIES AND APPLICATION OF WHEY PROTEIN ISOLATE BASED FILMS CONTAINING CLOVE AND APRICOT ESSENTIAL OILS

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M. Sc. in Food Eng.

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The targets of the study were to examine the effect of clove and apricot essential oils on the mechanical, antimicrobial and barrier properties of whey protein (WPI) based edible films and to investigate the applicability of the films to a food system. Clove and apricot essential oils were added to WPI solutions were coded as C-WPI and A-WPI, respectively. Kaşar cheese were selected as the food system. Cheese sample were sliced and dipped into WPI, C-WPI, A-WPI solutions and uncoated samples were prepared as control, then they were stored at 4°C for 28 day. Texture profile and microbiological analysis of the samples were evaluated to determine changes in structural properties of the cheese and antimicrobial properties of the films during storage.

As it was aimed, both essential oils were bring in films antimicrobial property, further clove had more pronounced effect than apricot. It was determined that addition of essential oils into edible films decreased tensile strength and elastic modulus but increased percent elongation at break. But more pronounced effect of addition of the oils was observed for elastic modulus values than tensile strength. The elastic modulus value of C-WPI decreased to one-fifth of the WPI value. According to the results of texture profile analysis and rheological analysis essential oils incorporated edible films did not show a distinct differences with respect to WPI based film coated and uncoated cheese samples.

Key Words: WPI, active films, essential oils, mechanical properties, texture profile analysis.

ÖZET

KARANFİL VE KAYISI ESANSİYEL YAĞLARI İÇEREN PEYNİR ALTI SUYU PROTEİN BAZLI YENİLEBİLİR FİMLERİN ÖZELLİKLERİ VE UYGULANMASI

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Bu çalışmanın amacı, esansiyel yağların yenilebilir filmlerin mekanik, antimikrobiyal ve bariyer özelliklerine etkisi ve filmlerin gıda üzerine uygulanabilirliğini incelemektir. Karanfil ve kayısı yağları peynir altı suyu protein (WPI) çözeltisine eklenmiş ve bunlar sırasıyla C-WPI ve A-WPI olarak kodlandırılmıştır. Gıda örneği olarak da kaşar peyniri seçilmiştir. Peynirler dilimlenmiş; WPI, C-WPI ve A-WPI çözeltilerine batırılarak kaplanmış örnekler hazırlanmıştır. Ayrıca kaplanmamış peynirler kontrol numunesi olarak kullanılmıştır. Bu numuneler 28 gün boyunca 4°C de depolanmıştır. Depolama süresince peynirin yapısal değişikliğini ve filmlerin mikrobiyal özellikleri tekstür profili analizi ve mikrobiyolojik analiz ile takip edilmiştir.

Amaçlandığı gibi esansiyel yağların filmlere antimikrobiyal özellik kazandırdığı ayrıca karanfil yağının kayısı yağından daha etkili olduğu doğrulanmıştır. Esansiyel yağların eklenmesi gerilme direnci ve modülüsü azaltmış fakat maksimum uzamayı arttırmıştır. Esansiyel yağların eklenmesinin modülüs üzerine olan etkisinin gerilme direnci üzerine olan etkisinden daha belirgin olduğu gözlenmiştir. Karanfil yağı içeren filmlerin modülüs değeri sade filmlerin değerinin beşte biri kadar azalmıştır. Tekstür analizi ve reolojik test sonuçlarına göre esansiyel yağ eklenmiş filmler ile kaplı paynirlerin, kaplanmamış ve sade film kaplanmış peynirlere göre bir fark göstermediği gözlemlenmiştir.

Anahtar Kelimeler: WPI, aktif film, esansiyel yağ, mekanik özellikler, tekstür analizi.

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NOMENCLATURE

EAB	Elongation at Break
EM	Elastic Modülüs
F_0	Initial Force at The Beginning of The Relaxation Test
$F(t)$	Decaying force,
FTIR	Fourier Transform Infrared Spectroscopy
Gly	Glycerol
k_1	Maxwell Constant
k_2	Maxwell Constant
PCA	Plate Count Agar
PC	Plate Count
RH	Relative Humidity
TGA	Thermogravimetric Analysis
TPA	Texture Profile Analysis
TS	Tensile Strength
WPC	Whey Protein Concentration
WPI	Whey Protein Isolate
HPMC	Hydroxypropyl Methylcellulose
BW	Beeswax
CarW	Carnauba Wax

CHAPTER I

INTRODUCTION

1. General Objective

Microbial contamination reduces the shelf-life of foods and increases the risk of foodborne illness. Traditional methods of preserving foods from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging, and adding antimicrobial agents or salts. Incorporation of bactericidal agents or growth inhibitors into food formulations or onto food surface by spraying, dipping, or coating without matrix may result in partial inactivation of the active substances and in rapid diffusion within the bulk of food respectively. The use of packaging film based on antimicrobial polymer could prove more efficient, by maintaining high concentrations on food surface with a low migration of active substance. Moreover, the development of new biodegradable or edible packaging material films has recently been undertaken for environmental aspects. The different properties and uses of spices were known even to the ancient people in prehistoric times. In the last century, several reports have appeared in the literature on the different uses of spices, such as in the flavoring of foods, bactericidal, bacteriostatic, fungistatic, antihypertensive, diuretic and antioxidative activity and other functions. Some spices have got antimicrobial effects and are being used extensively in some society.

Active packaging of food is a concept in which the packaging material changes the condition of the packed food by prolonging the shelf life and/or enhancing the quality of the packed food (Ahvenainen, 2003). Active packaging is one of the innovative food packaging concepts that have been introduced as a response to the continuous changes in current consumer demands and market trends (Suppakul, 2003). It has been defined as ‘a type of packaging that changes the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food’ (Debevere, 1999). In general, active food

packaging can provide several functions that do not exist in conventional packaging systems (Labuza, 1996). The active functions may include scavenging of oxygen, moisture or ethylene, emission of ethanol and flavors, and antimicrobial activity (Floros et al., 1997).

Anti-microbial packaging is a rapidly emerging technology. The need to package foods in a versatile manner for transportation and storage, along with the increasing consumer demand for fresh, convenient, and safe food products presages a bright future for anti-microbial packaging (Floros et al., 1997). However, more information is required on the rheological and microbiological effects of these systems on the packaged food especially on the issues of nutritional quality and human safety (Floros et al., 1997). So far, research on anti-microbial packaging has focused primarily on the development of various methods and model systems, whereas little attention has been paid to its preservation efficacy in actual foods (Han, 2000). Research is essential to identify the types of food that can benefit most from Anti-microbial packaging materials. It is likely that future research into a combination of naturally-derived anti-microbial agents, biopreservatives and biodegradable packaging materials will highlight a range of the merits of anti-microbial packaging in terms of food safety, shelf-life and environmental friendliness (Nicholson 1998; Rodrigues and Han 2000; Coma et al., 2001). The reported effectiveness of natural plant extracts suggests that further research is needed in order to evaluate their antimicrobial activity and potential side effects unpackaged foods. An additional challenge is in the area of odor/flavor transfer by natural plant extracts to packaged food products. Thus, research is needed to determine whether natural plant extracts could act as both an antimicrobial agent and as an odor/flavor enhancer. Moreover, in order to secure safe food, anti-microbial endments to regulations might require toxicological and other testing of compounds prior to their approval for use (Vermeiren et al., 2002).

Since microbial contamination of these foods occurs primarily at the surface, due to post-processing handling, attempts have been made to improve safety and to delay spoilage by use of antibacterial sprays or dips. However, direct surface application of antibacterial substances onto foods have limited benefits because the active substances are neutralized on contact or diffuse rapidly from the surface into the food mass.

Therefore, the use of packaging films containing antimicrobial agents could be more efficient, by slow migration of the agents from the packaging material to the surface of the product, thus helping maintain high concentrations where they are needed. If an antimicrobial compound can be released from the package during an extended period, the activity can also be extended into the transport and storage phase of food distribution. Antimicrobial substances incorporated into packaging materials can control microbial contamination by reducing the growth rate and maximum growth population and/or extending the lag-phase of the target microorganism or by inactivating microorganisms by contact.

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 (Kaya and Kahyaoglu, 2003).

Fresh kaşar cheese was selected as a food system to determine the effectiveness of active edible films usage. In the literature there are some related studies (Seydim, 2006, Carmen et al., 2010); however in this study the main objective was to analyze the changes of rheological properties of cheese samples coated with WPI-edible films. Kaşar cheese is a semi-hard Turkish traditional cheese which is one of the cheeses consumed most in Turkey. According to Turkish Standards, this cheese is classified as ‘fresh kaşar cheese’ and ‘old or matured kashar cheese’ in terms of ripening.

In present study clove and apricot essential oils were added into whey protein based edible films to determine the antimicrobial activity of films. Results indicates that presence of essential oils prevent the microbial growth. This means antimicrobial agents in clove and apricot essential oils are active on microorganisms.

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

1. To form active WPI based edible films containing essential oils of clove and apricot.
2. To evaluate the rheological, mechanical and physical properties of active-WPI based edible films.
3. To observe the applicability of active film on the coating of kaşar cheese by detecting antimicrobial effects of essential oils incorporated into WPI edible coating solutions and then measuring changes in rheological properties of uncoated and coated kaşar cheese samples with each of active-WPI films during storage.

CHAPTER II

LITERATURE SURVEY

2. Edible Films and Coatings

Packaging has a significant role in the food chain and it is integral part both of the food process and the whole food supply chain. Food packaging has to perform several tasks as well as fulfilling many demands and requirements. Traditionally, a food package makes distribution easier. It has protected food from environmental conditions, such as light, oxygen, moisture, microbes, mechanical stresses and dust. Other basic tasks have been to ensure adequate labeling for providing information e.g., to the customer, and a proper easy convenience to the customer, e.g., easy opening, reclosable lids and a suitable dosing mechanism. Basic requirements are good marketing properties, reasonable price, technical feasibility (e.g., suitability for automatic packaging machines, sealability), suitability for food contact, low environmental stress and suitability for recycle or refilling. A package has to satisfy all these various requirements effectively and economically. Some requirements and demands are contradictory to each other, at least to some extent. For these reasons, a modern food package should be optimized and integrated effectively with the food supply chain (Ahvenainen, 2003).

Edible films help meet the many challenges involved with the marketing of foods that are nutritious, safe, of high quality, stable and economical. Edible films can be used as barriers to gases and water vapor. For this purpose they can be positioned either on the surface of the food, e.g., as a coating on fruits, or within the food, where they separate components of markedly different water activity.

In the first instance, the function is to restrict loss of moisture from the fruit to the environment or to lessen absorption of oxygen by the fruit and thereby slow respiration. In the latter instance, the film may serve to stabilize water activity

gradients and preserve different textural properties possessed by different food components (Kester and Fennema, 1986).

Edible films and coating can also improve the mechanical handling properties, or structural integrity of a food product. Coatings can also provide some physical protection for food products which are susceptible to injury during transport, such as fresh fruits and vegetables (Krochta et al., 1994).

Edible films can be looked upon as an active packaging technology, and many of the antimicrobials or antioxidants mentioned above incorporated in the packaging material could as well be incorporated in an edible film meant to be eaten together with the product (Ahvenainen, 2003). Food additives, such as flavors, antimicrobial agents, antioxidants, and colors can be incorporated into edible films and used to control location or rate of release of these additives in a food (Krochta et al., 1994). Edible antimicrobial films and coatings have shown to be an efficient alternative in controlling food contamination. The growth of both deteriorating and pathogenic microorganisms can be prevented by incorporating antimicrobial agents into edible films or coatings (Baysal and Bilek, 2009).

There is a trend to select the antimicrobials from natural sources and to use generally recognized as safe (GRAS) compounds so as to satisfy consumer demands for healthy foods, free of chemical additives (Carmen et al., 2010). Several compounds have been proposed for antimicrobial activity in food packaging; more commonly antimicrobials used are organic acids, the polysaccharide chitosan, some polypeptides as nisin, the lactoperoxidase system, and some plant extracts and its essential oils among others (Seydim, 2006, Carmen et al., 2010).

Since ancient times, spices and herbs have been added to foods as seasoning additives due to their aromatic properties. In the development of antimicrobial edible films and coatings, essential oils from herbs and spices have been extensively used (Carmen et al., 2010). Essential oils extracted from spices and herbs are generally recognized as containing the active antimicrobial compounds. Eugenol, carvacrol, and thymol are phenol compounds, are found in cinnamon, cloves, sage, and oregano (Snyder, 1997). Eugenol (4-allyl-2-methoxyphenol), is a naturally occurring phenol

essential oil extracted from cloves, is known to be an antioxidant, a monoamine oxidase (MAO) inhibitor and known to have neuroprotective effects (Devi et al., 2010). The antimicrobial activity of plant extracts used as flavouring agents in foods is due to their essential oil fraction (Kotzekidou and Giannakidis, 2007). The essential oil fraction is particularly high in cloves, and eugenol comprises 95% of the fraction (Snyder, 1997). Moreover, the hydrophobic nature of eugenol enables it to penetrate the lipopolysaccharide of the Gram-negative bacterial cell membrane and alters the cell structure, which subsequently results in the leakage of intracellular constituents (Devi et al., 2010).

Cloves (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) are the aromatic dried flower buds of a tree in the family Myrtaceae. Cloves are used as a spice in cuisines all over the world. Clove oil, an herbal extract, contains eugenol which when tested on various agar media has antimicrobial properties and has been shown to inhibit *L. monocytogenes*, *Campylobacter jejuni*, *Salmonella Enteritidis*, *Escherichia coli* and *Staphylococcus aureus* (Mytle, 2004). The chemical analysis of cloves resulted in the identification of 36 components, with a high concentration of eugenol (88.58%), eugenyl acetate (5.62%), β -caryophyllene (1.39%), 2-heptanone (0.93%), ethyl hexanoate (0.66%), humulenol (0.27%), α -humulene (0.19%), calacorene (0.11%) and calamenen (0.10%).

Fruits from the family *Rosaceae* (the two cherry cultivars, sour cherry, apricot and hawthorn) had also a strong inhibitory effect on *C. jejuni* but, except for sour cherry and apricot, had a weak or no effect on *S. ser. Typhimurium*. Other authors also found good inhibition results with cherry (Lee et al. 2003), and apricot (Rashid et al. 2007). *Prunus armeniaca* L. (*Rosaceae*) is an important medicinal edible plant species commonly known as ‘apricot’. The plant is rich in mono- and polysaccharides, polyphenols, fatty acids and sterol derivatives, carotenoids, cyanogenic glucosides, and volatile components due to its appealing smell. *P. armeniaca* has been also investigated for various biological activities such as antimicrobial, antimutagenic, inhibitory activity against several enzymes, cardioprotective, anti-inflammatory and antinociceptive as well as antioxidant activity. Among these activities, antioxidant activity of apricot has been studied

extensively and the plant displayed a high antioxidant effect in both *in vitro* and *in vivo* test systems (Erdoğan and Kartal, 2010).

2.1 Components of Edible Films

Components of edible films and coatings can be divided into three categories: hydrocolloids, lipids and composites. Hydrocolloids include polysaccharides such as proteins, cellulose derivatives, alginates, pectins, starches, and others. Lipids include waxes, acylglycerols, and fatty acids. Composites contain both lipid and hydrocolloid components (Krochta et al., 1994).

2.1.1 Lipids

Lipid materials, due to their hydrophobic character, provide better moisture barriers than polysaccharides and proteins, but they offer little resistance to gas transfer and have poor mechanical strength (Martinez et al., 2010). Lipid films are often used as a barrier to water vapor, or as coating agents for adding gloss to confectionery products (Krochta, et al., 1994). Their use in a pure form as free-standing film is limited, because most lack sufficient structural integrity and durability (Giancone, 2006). Waxes are commonly used for coating fruits and vegetables to retard respiration and lessen moisture loss. Formulations for wax coatings vary greatly and the compositions are often proprietary (Krochta et al., 1994). Acetylated mono glycerides are frequently added to wax formulations to add pliability to the coating (Giancone, 2006).

2.1.2 Hydrocolloids

Hydrocolloids are hydrophilic polymers, of vegetable, animal, microbial or synthetic origin, that generally contain many hydroxyl groups and may be polyelectrolytes (for example alginate, carrageenan, carboxymethylcellulose, gum arabic, pectin and xanthan gum) (Skurtys et al., 2010). Hydrocolloid films have good barrier properties to oxygen, carbon dioxide, and lipids. These films can be used in application where control of water vapor migration is not the objective (Krochta et al., 1994).

Hydrocolloids used for films and coatings can be classified according to their composition, molecular charge, and water solubility. In terms of composition, hydrocolloids can be either carbohydrates or proteins. Film forming carbohydrates include starches, plant gums (for example, alginates, pectin, and gum arabic), and chemically modified starches. Film forming proteins include gelatin, casein, soy protein, whey protein, wheat gluten, and zein (Krochta et al., 1994).

2.1.2.1 Polysaccharide-based edible films

Polysaccharide-based coatings are colorless, have an oily-free appearance and a low caloric content and can be used to increase the shelf life of fruits, vegetables, shell fish or meat products avoiding or significantly reducing dehydration, oxidative rancidity and darkening of the surface (Carneiro-da-Cunha, 2009). Polysaccharide-based coatings are expected to be a good oxygen barrier due to their tightly packed, ordered hydrogenbonded network structure although they do not behave well as moisture barriers because of their hydrophilic nature (Oms-Oliu, 2008).

2.1.2.2 Protein- based edible films

The ability of different proteins to form films and coatings is highly dependent on their molecular characteristics: molecular weight, conformations, electrical properties (charge vs pH), flexibilities, and thermal stabilities (Carmen et al., 2010).

Animal (milk and whey proteins) and plant (such as soy and zein proteins) proteins (Skurtys et al., 2010) and/or polysaccharide-based films have good mechanical behaviour, but their barrier properties against water vapor (WV) is poor (Talens et al., 2010) due to their hydrophilic character (Stading et al., 2001).

Soy protein

Gel forming property is one of the most important functional properties of soy proteins. Gelation is also related to the swelling ability, water holding capacity, and viscosity of re-hydrated soy protein isolates. Considerable researches have been concentrated on the using soy protein based edible films (Cui et al., 2004). Soy protein films have poor moisture barrier properties due to their hydrophilic properties

and the substantial amounts of hydrophilic plasticizer used in film preparation (Gennadios et al., 1994). In spite of this, there is considerable interest in edible and/ordegradable soy films due to their environmentally friendly nature and to their potential in the novel food packaging industry (Kim et al., 2002). Soy protein isolate is abundant, inexpensive, biodegradable, and nutritional raw material (Cao et al., 2007).

Zein

Zein (corn protein) belongs to the characteristic class of proteins known as prolamines, which occur specifically in cereals (the equivalent of hordein in barley and gliadin in wheat) (Cheryan and Shukla, 2000). Zein forms films with high tensile strength and low water vapor permeabilities compared to other protein-based films. It also has a desirable heat seal property (Ghanbarzadeh and Oromiehi, 2008).

The properties of protein-based films can be improved by laminating additional film layers with desired film properties such as corn zein films (Ghanbarzadeh, Oromiehi, 2009; Pol, Dawson, Acton, Ogale, 2002). Corn zein produces a film with high tensile strength and low water vapor permeabilities when compared to other protein-based films, and the resulting films have desirable heat seal characteristics (Cho 2002; Park & Weller, 1996). It is also expected that the lamination of corn zein layers onto soy protein isolate films will produce oxygen barrier bilayer films that will enable pouches to be readily formed by heat sealing of the corn zein layer (Cho, Lee and Rhee, 2010).

Gelatin

Gelatin is a protein derived from collagen, the major constituent of animal connective tissue (Gilsenan and Murphy, 2000). The physical properties of gelatins are related not only to the molecular weight distribution but also to the amino acid composition (Skurtys et al., 2010). The amino acid content and sequence varies from one source to another but always consists of large amounts of proline, hydroxyproline and glycine (Gilsenan and Murphy, 2000). The use of gelatin as an edible film or coating was studied considerably until the sixties, which resulted in many patents, mainly in the pharmaceutical area (Park, Whiteside and Cho, 2007). The properties and film forming ability of gelatins are directly related to the

molecular weight, i.e. the higher the average molecular weight, the better the quality of the film (Skurtys et al., 2010). There has been an increased interest these last years on the knowledge focused on fish gelatin (Gomez-Guillén and Montero, 2001; Gudmundsson & Hafsteinsson, 1997; Haug 2004; Muyonga 2004). However, there is not enough information available about the application on films (Jongjareonrak, Benjakul 2006). Othersides, the main problem of marine gelatins are their inferior rheological properties, which may limit their application field (Norland, 1990, Leuenberger, 1991;).

Casein

The caseins are a family of phosphoproteins synthesised in the mammary gland in response to lactogenic hormones and other stimuli and secreted as large colloidal aggregates termed micelles, which are responsible for many of the unique physical properties of milk (Ginger and Grigor, 1999). Casein content of bovine milk represents approximately 80% of milk proteins. The principal casein fractions are α_{s1} -, α_{s2} -, β - and K-casein and their relative proportion is estimated to 45.0%, 12.0%, 35.0% and 8.0%, respectively.

Casein, a little organized protein, produced smooth structured films, whereas the gelatin films presented a fibrous structure (characteristic of collagen fibrils), which appears to be responsible for the greater tensile strength, greater elasticity and lower water vapor permeability of this film (Chambi and Grossso, 2005). Little work has centered on individual casein fractions due to high manufacturing costs; however, Ward (1998) developed a cost-elective method for isolating β -casein. The β -casein is the most hydrophobic protein in milk, and edible β -casein films are expected to have lower water vapor permeability than other milk protein films (McHugh and Krochta, 1994). Chemical, structural, and geometric properties, They observed that b-casein has the potential to produce desirable films for food and packaging use due to low WVP and high tensile strength and elongation values however, due to temperature sensitive solubility of β -casein, commercial scale production of β -casein films could be limited.

Whey protein

Milk proteins casein and whey proteins, are some of the most common source of proteins used to obtain films and coatings.

Whey is the yellow-green liquid that separates from the curd during manufacture of cheese and casein. Whey protein has been used in confectionery, bakery and ice cream products, infant formula, health foods, and sports bars. Recent investigations aimed to find new uses of whey protein, have made use of the ability of processed whey protein (80 to 90% by weight) to form films and coatings on the surface of food products (Perez et al., 2006).

Whey protein concentrates (WPC) and whey protein isolates (WPI) are finding more use as food ingredients in varies applications due to their nutritional quality and functional properties, such as water binding, viscosity, gelation, etc. (Gunaskaran and Ak, 2000).

Whey protein isolate produces totally water-soluble coatings but heat denatured solutions of whey protein isolate produce coatings in which the protein is insoluble. Industrially produced whey protein concentrate (WPC) have a protein content between 25 and 80% whereas the whey protein isolate (WPI) have protein content above 90% (Skurtys et al., 2010). The WPI is generally richer in β -Lactoglobulin (β -LG) and α -Lactalbumin (α -LA). α -LA is a 123 amino acid globular protein which represents about 20% of total whey protein, and has four intrachain disulfide bridges which confer hydrophobicity and foaming properties to the molecule (Perez et al., 2006).

Whey protein is a byproduct of cheese making. It can be used for production of biodegradable films. Whey protein films have low tensile strength and high water vapor permeability due to the high proportion of hydrophilic amino acid in their structures. Mechanical and barrier properties of the whey protein films might be improved by laminating a good structured whey protein film with films that having high tensile strength and hydrophobic properties (Ghanbarzadeh and Oromiehi, 2008).

Whey protein isolate produces transparent, flexible, edible films with excellent oxygen, and aroma barrier properties at relatively low humidity. These films are water insoluble, however, due their hydrophilic nature they are less effective moisture barriers (Hui et al., 2007). Whey protein films produced without addition of any plasticizers are very brittle therefore the addition of plasticizers provides flexibility to the films but also increases their water vapour permeability (Ozdemir and Floros, 2007). The making of protein-based films generally needs the incorporation of a minimal content of plasticizer to reduce its brittleness. Film plasticizers function by weakening intermolecular forces between adjacent polymer chains. This results in an increased film extensibility and flexibility with decreased elasticity, mechanical resistance, and barrier property of the films. (Gounga, 2007) The most commonly used plasticizers are: polyols (propylene glycol, glycerol, sorbitol, polyethylene glycol), oligosaccharides (sucrose) and water (Skurtys et al., 2010).

The plasticizing effect of glycerol (Gly) can be attributed to its occurrence in between polymer molecules hence decreasing the protein–protein interactions and increasing the mobility of polypeptide chains allowing the films to be less resistant and more elastic (Gounga, 2008). McHugh and Krochta (1994) studied the behavior of WPI films made of high Gly content and found significantly low Tensile strength (TS) and high elongation at break (EAB).

McHugh et al., (1994) developed films from commercial whey protein isolate by heat denaturing the whey protein at temperatures between 75 and 100°C. Heating of WPI is believed to induce disruption of the native conformation of globular whey proteins, resulting in the exposure to the solvent of free sulphhydryl groups (SH) (Pereira et al., 2010), normally occluded within β -lactoglobulin (β -LG) and bovine serum albumin (BSA), and hydrophobic amino acid side chains. SH may rapidly interchange with existing disulfide bonds to generate new inter- and intramolecular disulfide bonds. During heating, the role of sulphhydryl/disulfide interchange may promote intermolecular disulfide bond formation, which together with intermolecular protein-protein interactions (such as entropic forces, dipolar, and electrostatic interactions) between unfolded ‘reactive’ proteins determines protein aggregation. For the production of edible protein films, the formation of aggregates in the film-

forming solutions (before drying) is required to form stiff and stretchable films (Pereira et al., 2010).

2.1.3 Composites

Composite films can be formulated to combine the advantages of the lipid and hydrocolloid components and lessen the disadvantages of each (Krochta et al., 1994). When a barrier to water vapor is desired, the lipid component can serve his function while hydrocolloid component provides the necessary durability (Giancone, 2006). These films can be used as coatings for processed fruits and vegetables. (Krochta et al., 1994)

2.2 Film Applications

2.2.1 Dipping

This method lends itself to food products that require several applications of coating materials or require a uniform coating on irregular surface. After dipping, excess coating material is allowed to drain from product and it is then dried or allowed to solidify (Giancino, 2006). This method has been used to apply films of acetylated monoglycerides to meats, fish and poultry and apply coating of wax to fruits and vegetables (Krochta, 2002).

2.2.2 Spraying

Films applied by spraying can be formed in a thinner, more uniform manner than those applied by dipping. Spraying, unlike dipping, is more suitable for applying a film to only one side of a food to be covered. This is desirable when protection is needed on only one surface, e.g., when a pizza crust is exposed to a meat sauce (Krochta, 2002). Spraying can also be used to apply a thin second coating, such as the cation solution needed to cross-link alginate or pectin coatings (Giancino, 2006). In the food industry, coating by spraying is the conventional method generally used when the coating-forming solution is not very viscous. Indeed, highly viscous

solution cannot be or very uneasily sprayed, and thus only dipping techniques can be apply, giving high thickness to the coating (Skurtys et. al., 2010).

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2.2.3 Casting

This technique, useful for forming free-standing films, is borrowed from methods developed for not edible films. For formation of a film the film-forming biopolymers are first dissolved in the solvent. If heating or pH adjustment enhances film formation and/or properties, this is done next. If a composite film or coating based on emulsion desired, a lipid material, and possibly a surfactant, is added. Next, the mixture is heated to above the lipid melting point and then homogenized. Degassing is an important step to eliminate bubble formation in the final film or coating. Finally, the edible film or coating is formed by applying the prepared formulation to the desired casting or product surface and allowing the solvent to evaporate. Providing heated air at low humidity and high velocity increases drying rates (Giancino, 2006).

Coating is simple and allows film thickness to be controlled accurately on smooth, flat surfaces. Casting can be accomplished by controlled- thickness spreading or by pouring. Controlled- thickness spreading requires a spreader with a product reservoir and an adjustable gate, the height of which can be set accurately and with good reproducibility. The spreader simply drawn over the receiving surface, depositing a layer of the film-forming solution of the desired thickness, which is subsequently dried. Alternatively, the film-forming solution can be poured into a confined area of a level receiving surface and subsequently dried (Krochta, 2002; Giancino, 2006).

2.3 Properties of Edible Films

2.3.1 Mechanical Properties

Films based on biopolymer complexes are viscoelastic materials that possess characteristics of both solids and liquids. Interactions between the biopolymers, the solvent, plasticizers, lipids, and other additives dispersed in the space of the matrix, also contribute to the mechanical properties of the film (Martinez et al., 2010).

Edible films can control moisture, gases, and lipid migration and can be supporters of additives and nutrients. For their formulation, there can be used polysaccharides, proteins, and lipids and they must result neutral with respect to color and flavor. An important component is the plasticizer which enhances flexibility and extensibility (Carmen et al., 2010).

Mechanical properties are important to edible films, because adequate mechanical strength ensures the integrity of the film and its freedom from minor defects such as pinholes. Quantitative information of mechanical parameters of edible films is essential for their adequate design. Tensile strength (or more accurately, ultimate tensile strength) is the ultimate tensile strength that the film can sustain. Elongation is usually taken at the point of break and is expressed as the percentage of change of the original gauge length of the specimen (Martinez et al., 2010).

Tensile strength (TS) expresses the maximum stress developed in a film during a tensile test. TS offers a measure of integrity as well as heavy duty use potential for films and the percentage elongation at break (EAB) is a quantitative representation of a film's ability to stretch. On increasing protein concentration an increase in TS and EAB was observed. On the other hand, increasing glycerol led to a decrease in TS and elastic modulus while EAB increased. An elastic modulus (EM), or modulus of elasticity, is the mathematical description of an object or substance's tendency to be deformed elastically when a force is applied to it (Gounga et al., 2008).

2.3.2 Textural Properties of Edible Films

Texture of foods is a highly subjective human experience with foods during their consumption. It is, in essence, the 'eating quality' of foods encompassing many properties of foods that excite our senses of sight, touch and sound (Gunaskaran and Ak, 2000). Since texture consist of a number of different physical sensations, it is preferable to talk about 'textural properties', which infers a group of related properties, rather than 'texture', which infers a single parameter (Bourne, 2002).

The principle of the TPA tests is illustrated in Figure 2.1: A 'bite size 'sample of food of standard size and shape is placed on the baseplate and compressed and

decompressed two times by a platen attached to the drive system. To imitate the chewing action of the teeth there should be a high compression (Bourne, 2002).

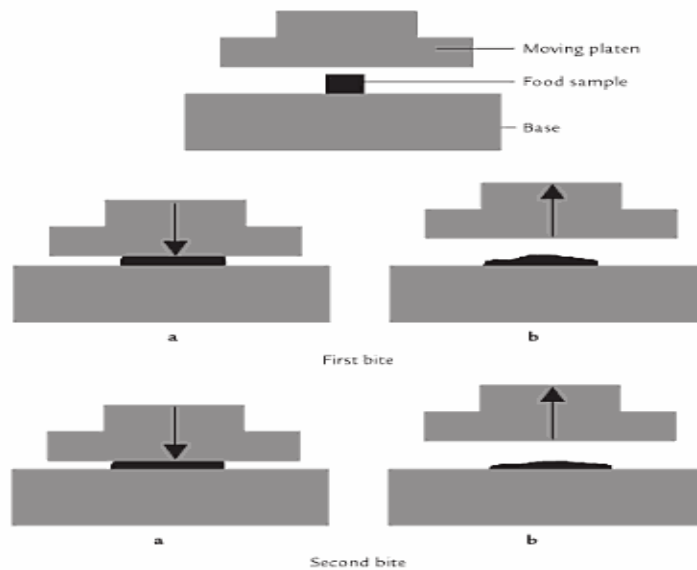


Figure 2.1 Schematic diagram of the two compressions required for the texture profile analysis test. (a) Downstroke actions during the first and second bites; (b) upstroke actions during the first and second bites.

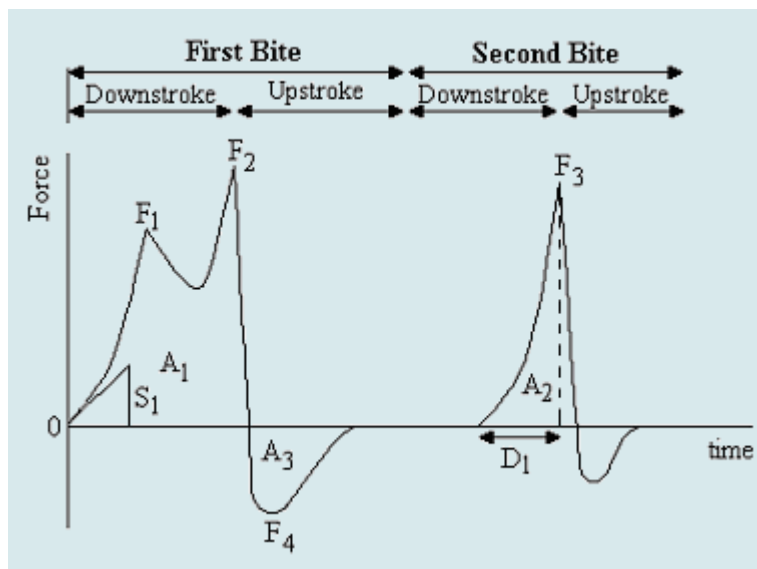


Figure 2.2 A generalized texture profile analysis curve.

The height of the force peak on the first compression cycle (first bite) was defined as hardness. Fracturability (originally called brittleness) was defined as the force of the significant break in the curve on the first bite (Bourne, 2002). The ratio of positive

force areas under the first and second compression (A_2/A_1) was defined as cohesiveness. The negative force area of first bite (A_3) represented the work necessary to pull the compressing plunger away from the sample and was defined as adhesiveness (Gunaskaran and Ak, 2000; Bourne, 2002). The distance that the food recovered its height during the time that elapsed between the end of the first bite and the start of second bite was defined as springiness (originally called elasticity) (Bourne, 2002). Two other parameters were derived by calculation from the measured parameters: gumminess was defined as the product of hardness x cohesiveness; chewiness was defined as the product of gumminess x springiness (Yaşar, 2006). In the original description of TPA chewiness was defined as the energy required masticating a solid food product and gumminess as the energy required to disintegrate a semisolid food to a state a state of readiness for swallowing (Bourne, 2002).

2.3.3 Rheological Properties of Edible Films

Food rheology is the study the deformation and flow of the raw materials, the intermediate products, and the final products of the food industry (Bourne, 2002). When subjected to external forces, solids (or truly elastic materials) will deform, whereas liquids (or truly viscous materials) will flow. However, contemporary rheology is more interested in the behavior of real materials with properties intermediate between those of ideal solids and ideal liquids (Gunaskaran and Ak, 2000).

Food rheology, the material science of foods, investigates the relationships among stress (s), strain (g) and time scale of foods to (1) understand the effects of processing on products, (2) probe the system structure, and (3) reveal critical aspects of food texture (Allen, 2003).

One of the fundamental tests to study viscoelastic response is stress relaxation. This test can be performed in (uniaxial) tension, compression, shear, bending, torsion, etc. (Gunaskaran and Ak, 2000). The relation between stress and strain is time-dependent, and can be expressed in terms of creep compliance (constant stress) or the stress relaxation function (constant strain) (Bhattacharya, 2005).

Uniaxial testing is the most popular configuration for evaluating mechanical and rheological properties of cheeses (Gunaskaran and Ak, 2000).

The stress-relaxation curves for gels have traditionally been described in terms of a discrete linear-Maxwell model (Mammarella, 2002).

The relaxation curve can be represented by

$$\frac{F_{(t)}}{F_{(0)}} = 1 - \frac{t}{k_1 + k_2 t}$$

Where F_0 is the initial force at the beginning of the relaxation test, $F_{(t)}$ is the decaying force, and k_1 and k_2 are the constants. The linear dependence of stress relaxation on strain is called as linear viscoelasticity (Mammarella, 2002).

2.3.4 Antimicrobial Activity of Edible Films

Numerous studies have been performed to establish the effectiveness of antimicrobials usage within films and coatings. The enumeration by plate count of microbial population, at selected times, from inoculated surface agar plates in contact with film disk containing the antimicrobial is a test useful to model wrapping of foods and obtained results may suggest what can happen when the film enters in contact with a contaminated surface (Coma et al. 2003; Min et al. 2005a, Min et al. 2005b, Min et al. 2005c and Kristo et al.2008).

The film surface inoculation test is another assay frequently performed and consists in the enumeration by plate count of the microbial population inoculated on the surface of a film disk in contact with a semisolid media such as agar that model a certain food product. This assay is useful to simulate surface contamination. Results obtained may suggest what happens when microbial contamination occurs on coatings or films in contact with food and gives an idea of the barrier ability of the film to prevent an external contamination (Sanjurjo et al. 2006; Flores et al. 2007b; Vásconez et al. 2009).

Fan and others determined total viable counts (TVC) in plate count agar by the spread plate method for chitosan coating fish samples (Fan et al., 2008).

Populations of *L. monocytogenes*, mesophilic aerobic bacteria, lactic acid bacteria, and yeasts and molds were determined by plating appropriate dilutions in duplicate on Modified Oxford Agar (Difco/Becton Dickinson), Plate Count Agar (Difco/Becton Dickinson), MRS Lactobacillus Agar (Difco/Becton Dickinson), and Rose Bengal Agar (Difco/Becton Dickinson), respectively for cheddar cheese by Limjaroen, Ryser, Lockhart and Harte (2005).

Rojas and others, 2008 prepared serial dilutions of Fuji apples and then pour plated onto plate count agar (PCA).

2.3.5 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique extensively applied to various substances including liquid, gas and solid matter to identify the unknown materials. This technique measures the absorption of various infrared light wavelengths by the material of interest. These infrared absorption bands identify specific molecular components and structures. (Song et al., 2007).

FTIR technique not only can be used to make qualitative analyses for the substance identification, but also can be used to obtain the content of composition in the sample. In particular, FTIR has nearly no requirement of sample size. (Song et al., 2007)

2.3.6 Thermal properties of Edible Films

Thermal degradation studies of polymers are necessary as many applications depend on their thermal stability. In order to accomplish this goal, many techniques have been used including pyrolysis mass spectrometry, thermal volatilization analysis, etc. (Cervantes, 2006). Thermogravimetric analysis (TGA) was shown to be a rapid means of quantifying vapor pressure of selected organic compounds, including triglycerides (Goodrum, Geller and Lee, 1997).

Thermogravimetric analysis uses heat to force reactions and physical changes in materials. TGA provides quantitative measurement of mass change in materials associated with transition and thermal degradation. TGA records change in mass from dehydration, decomposition, and oxidation of a sample with time and temperature. Characteristic thermogravimetric curves are given for specific materials and chemical compounds due to unique sequence from physicochemical reactions occurring over specific temperature ranges and heating rates. These unique characteristics are related to the molecular structure of the sample (Cervantes, 2006).

CHAPTER III

MATERIAL & METHOD

3.1 Materials

WPI (BiPro, 98.1% protein) used to make films was supplied by Davisco International (Le Seur, MN). All used chemicals were reagent grade and the water was doubly distilled. Essential oils of clove and apricot were obtained from a local market in Gaziantep. Fresh kaşar cheese samples were obtained from the same firm and have the same production date.

3.2 Experimental Studies

The study was performed with in two sections. In the first section, WPI based edible films with and without essential oils were prepared and then their some mechanical and physical properties were detected. In the second section the applicability of the WPI edible films on food systems was investigated. To provide full covering of cheese samples with the edible films, dipping techniques were performed using WPI edible coating solutions which has the same composition of the films.

3.2.1 Whey Protein Isolate-Based Film Preparation

Aqueous solution of 5% (w/w) WPI were prepared by stirring WPI powder in distilled water until the WPI was completely dissolved. Solutions were placed in a water bath at 90°C and kept there for 30 min (Ozdemir and Floros (2007)). Solutions were then cooled to room temperature, and 8% (v/v) amount of glycerol was added. Clove and apricot essential oils were added with 5% ratios as essential oil concentration per film solution (100 ml of solution contains 4gr WPI, 7,07 ml glycerol and 4 ml essential oil). It was mixed vigorously for 2 min using a mixer, solutions kept at room temperature for 1.5 hours. 15 ml of solutions were spread on 15 cm² glass plates and allowed to dry at room conditions (20±2°C and 40±5% RH)

overnight and dried films were peeled from casting surface. Then dried films were left at $23 \pm 2^\circ\text{C}$ and $45 \pm 5\%$ RH for conditioning for one day.

3.2.1.1 Mechanical Properties and Some Physical Properties

Instron Testing Machine Model 441 ASTM D882-91 ASTM (1993) was used to measure mechanical properties, elongation at break, elastic modulus, tensile strength of edible films. Brightness and opacity of films was measured with BYK gardner micro-gloss 45 and diffusion systems M57D, respectively.

Brightness and opacity of films were measured with respect to ASTM D1003-61 and ASTM D2457-70.

3.2.1.2 Fourier Transform Infrared (FTIR) Spectroscopy

Transmission infrared spectra of the films were recorded at room temperature using Perkin Elmer Spectrum 100, FT-IR Spectrometer was used for the FT-IR measurements to investigate the expected changes in the main characteristic groups of WPI films. Films were cutted about 1 cm^2 to prepare suitable samples for measurement. Film samples were heated under nitrogen, in a tubular oven connected to the FTIR equipment. FTIR analysis was performed with a resolution of $4/\text{cm}$ in the range of $4,000\text{--}650/\text{cm}$.

3.2.1.3 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis were made with Perkin Elmer Precisely, TGA 4000. Samples for TGA measurements were prepared from several pieces cut from the films to a mass of about 6 mg and placed in capsules of the analyzer. Data were taken at temperatures between 20°C and 800°C . The heating rate was set at $10^\circ\text{C}/\text{min}$.

3.2.2 Application of WPI edible coating solutions with or without clove and apricot essential oils

WPI and active WPI coating solutions containing apricot and clove essential oils were used to coat kaşar cheese samples. Fresh kaşar cheese samples were sliced with dimensions of 2.5 cm width, 2.5 cm length and 1.3 cm height and stuck with toothpicks and then coated using dipping methods (Figure 3.1). They are dipped into film solutions two times, each lasting 1 minute. Schematic presentation of the experimental study was shown in Figure 3.2. Ten set of samples were prepared (Figure 3.3), each set was formed with five uncoated, five WPI film coated, five A-WPI (WPI based film containing apricot essential oil) coated, five C-WPI (WPI based film containing clove essential oil) coated cheese samples were placed on each set. Then two sets were used for microbiological analysis for first day and last day as initial and final measurements. Weight loss of uncoated and coated samples was determined using one set of samples. Other seven sets used for texture and rheological analysis for 1st, 2nd, 3rd, 7th, 14th, 21st and 28th days. The samples stored at 4°C during experiment.

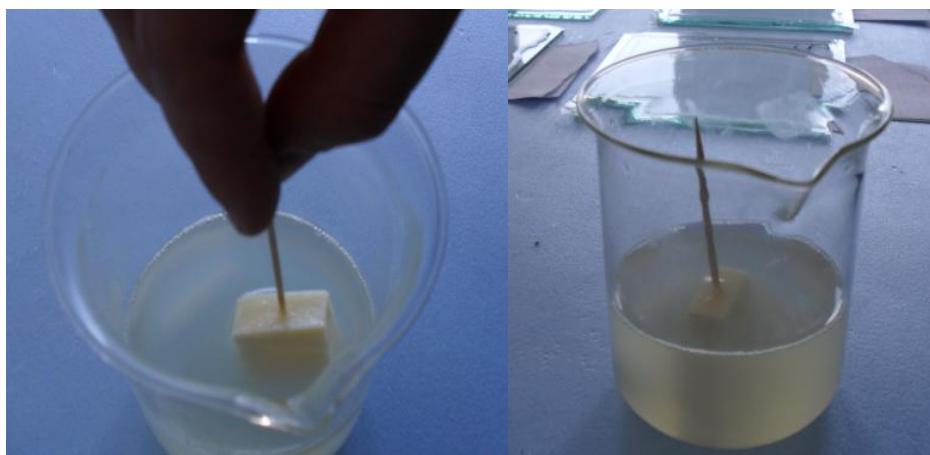


Figure 3.1 Application of dipping method for coating of cheese samples.

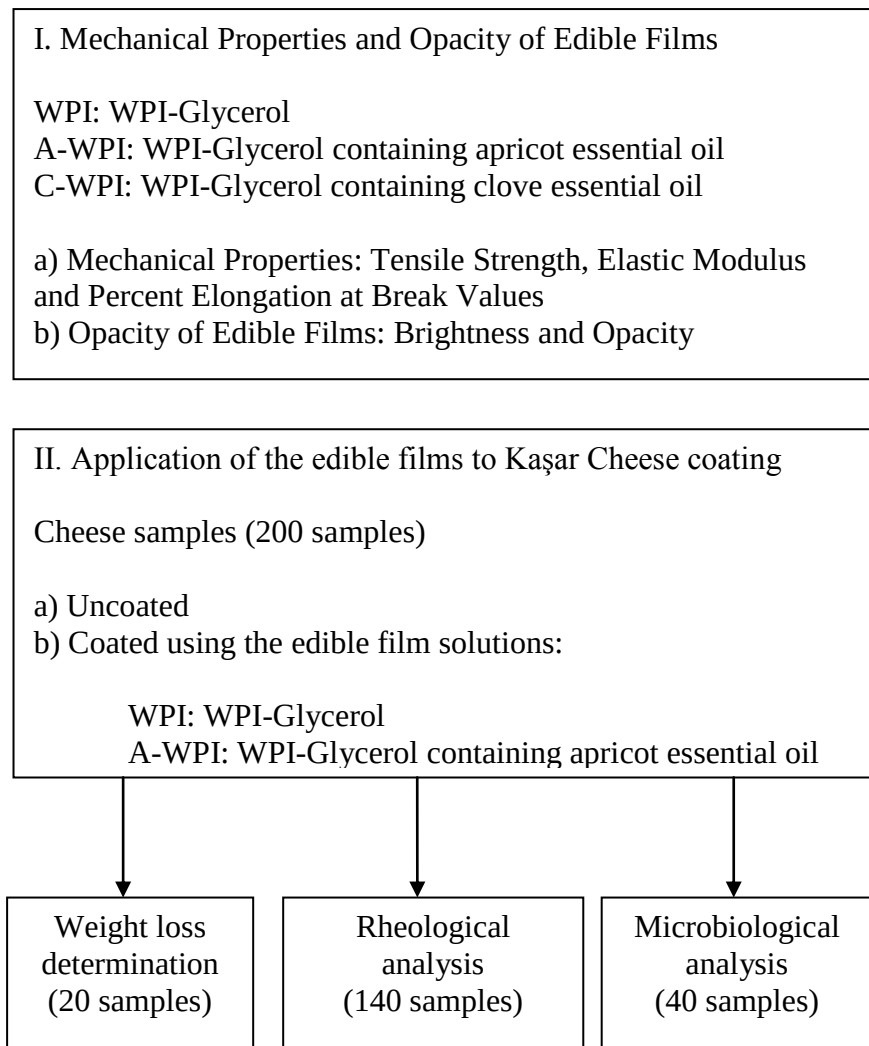


Figure 3.2 Schematic Representation of Experimental Design.



Figure 3.3 Appearance of a sample set containing five WPI, five C-WPI, five A-WPI and five uncoated cheese samples.

3.2.2.1 Microbiological Analysis of uncoated and coated kaşar cheese samples with and without essential oils

The total microbial count of cheeses were determined by standart plate count using spread method. PC agar was prepared using following procedure: 500 ml of distilled water was added on 8.75 gr of plate count agar powder and boiled until solution was become clear. This clear solution was sterilized at $120\pm 1^{\circ}\text{C}$ for 20 min. About 20 ml of sterilized solution was poured into petri dishes than they were allowed to solidify. PC agar was prepared for 20 petri dishes for four sample groups; uncoated cheeses, WPI coated cheeses, A-WPI and C-WPI coated cheeses. 25 g of each kind of cheese samples were mixed with 250 ml sterilized ($120\pm 1^{\circ}\text{C}$ for 20 min) 0.1% peptone water and homogenized for 20 minutes. 10^{-1} dilutions of samples were used during experiment. Petri dishes were incubated at 37°C during 48 hours and results were expressed as colony forming unit per gram of sample (cfu/g sample). This procedure was applied for initial and final measurements. Coated samples were waited one day to make them stable and initial measurement was made. Final measurement was made at 28th day.

3.2.2.2 Textural Analysis of uncoated and coated kaşar cheese samples with and without essential oils

3.2.2.2.1 Texture Profile Analysis (TPA)

TA.XT2; Texture Analyzer (Stable Microsystems, Godalming, UK) was used for measuring texture profiles of the cheese samples. Measurements were made at 1st, 2nd, 3rd, 7th, 14th, 21st and 28th days. Measurement conditions were adjusted as test speed 1 mm/s; post test speed 1 mm/s; 10% strain. Rectangular shaped probe was used with dimension 4x5cm. Temperature of texture analyzer was adjusted as 10°C . Temperature control was made by using Peltier apparatus XT/PP. This apparatus provide acceptable surface temperature to measure small and weak products like adhesive which sensitive to compression.

3.2.2.2.2 Stress Relaxation Test

Stress Relaxation curves were determined Texture Analyzer (TA.XT2, Stable Microsystems, Godalming, UK). Measurements were made at 1st, 2nd, 3rd, 7th, 14th, 21st and 28th days. All measurements were made with 10% strain. Pre-test speed, test speed and post test speed were adjusted as 5 mm/s. Hold time was fixed as 300 second. Rectangular shaped probe was used with dimension 4x5cm. Temperature of texture analyzer was adjusted as 10°C. Temperature control was made by using Peltier apparatus XT/PP. This apparatus provide acceptable surface temperature to measure small and weak products like adhesive which sensitive to compression.

3.2.2.3 Determination of Weight Loss of uncoated and coated kaşar cheese samples with and without essential oils

After film solution dried on cheese samples the initial weights were obtained. Five samples of each C-WPI, A-WPI, and WPI coated and uncoated cheese were numbered and placed on refrigerator (4°C). Cheese samples were weighted periodically with a sensitive scale (Precisa XB220A with 0,0001g sensitivity)

3.3 Statistical Analysis

Analysis of variance (ANOVA) was conducted to determine effect of essential oils on edible films and effect of these film types on cheese structure and microbial growth by using SPSS software (v.16.0.0). One way ANOVA was used to compare means of parameters and Duncan test was applied as post-Hoc test.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 General

The WPI based edible films containing essential oils was produced and their mechanical, thermal, microbial and structural properties were determined. All WPI based film samples were applied on a food samples to measure these properties. Mold growth on the surface is an important problem in order to eliminate microorganisms growth some kind of additives are widely used. Active packaging especially having antimicrobial properties are becoming an important packaging for such food product. For this reason kaşar cheese was selected. The results of experimental studies and the treatment of the resultant data given in graphical and tabular forms are discussed separately for each sample.

4.2 Mechanical Properties and Some Physical Properties

Tensile strength expresses the maximum stress was measured in a film during a tensile test. Tensile strength offers a measure of integrity as well as heavy duty use potential for films and the percentage elongation at break is a quantitative representation of a film's ability to stretch (Gouna et al., 2008). Results of mechanical properties of the studied films (WPI, C-WPI and A-WPI) after reaching equilibrium are shown in Table 4.1.

WPI film showed the highest tensile strength of 1.86 MPa and elastic modulus of 30.94 MPa. The TS values of WPI film were 1.87 MPa, which were similar to the reported value for WPI-Glycerol (1:1) films as 1.94 MPa (Kaya and Kaya, 2000), they found the modulus of elasticity of the film was 20.87%. However modulus of elasticity was found as higher than given value in literature.

Table 4.1. Mechanical properties of WPI-based edible films after equilibrium.

Film Type	Elongation at break (%) $\pm$ SE	Tensile Strength (MPa) $\pm$ SE	Elastic Modulus (MPa) $\pm$ SE
WPI	84.85 $\pm$ 7.21	1.87 $\pm$ 0.12	30.94 $\pm$ 2.20
C-WPI	164.34 $\pm$ 24.84	1.15 $\pm$ 0.07	7.17 $\pm$ 1.05
A-WPI	99.73 $\pm$ 13.43	1.01 $\pm$ 0.07	1.17 $\pm$ 0.39

WPI: whey protein isolate based edible films

C-WPI: clove essential oil incorporated whey protein isolate based edible films

A-WPI: apricot essential oil incorporated whey protein isolate based edible films

The TS values of A-WPI and C-WPI films were 1.01–1.87 MPa, which were lower than that of control film. Addition of essential oils to edible films effect the mechanical properties of the films (Table A3, Table A5). Addition of essential oils to edible films decreases tensile strength and elastic modulus but increase percent elongation at break. Since EM value of C-WPI and A-WPI films were nearly one fourth of WPI it can be said that the more pronounced effect of addition of the oils was observed for elastic modulus values than tensile strength. The decreasing of tensile strength can be attributed to the presence of essential oils between polymer molecules hence decreasing the protein–protein interactions and increasing the mobility of polypeptide chains allowing the films to be more resistant and less elastic. McHugh and Krochta (1994) studied the behavior of WPI films made of high glycerol content and found significantly low tensile strength and high elongation at break.

It was reported that the addition of lipid or essential oil to packaging films decreases tensile strength and elastic modulus significantly (Pérez-Gago and Krochta, 2000; Yang and Paulson, 2000; Rojas-Graü et al., 2006, 2007; Kyriaki G. et al., 2009).

Essential oil addition induces the development of a heterogeneous film structure, featuring discontinuities which may affect the stretching ability of the film based on the characteristics of the oils added. Since the essential oils used in the study are liquids at room temperature, it will be present in the film in the form of oil droplets that can easily be deformed, enhancing the film's extensibility (Fabra et al., 2008). This means that essential oils of clove and apricot were bound with whey protein

isolate and this provided increase in percent elongation of the films. Similar results have been reported on the plasticizing effect of soybean oil in whey protein films (Fang et al., 2002). It is also worth noting that the differences among the samples became more apparent in the case of percent elongation values, indicating the importance of essential oil characteristics, i.e. with respect to solubility in water. The immixability of the oils in the WPI solutions could be clarified with gloss (opacity) value (Table 4.2), the opacity value of the apricot was the highest and the brightness was the lowest this proves the A-WPI had the lowest tensile strength and elastic modulus. By the way increasing percent elongation at break can be expressed as emulsion films are not as effective barriers as bilayer films, but they have superior mechanical properties (Fairley P. et al., 1997).

Some of the protein in the film is partially immobilized at the interface of the emulsified lipid droplets. It is important to mention here that besides addition of essential oils, the WPI films contained two plasticizers, such as glycerol and water which make such systems very complex from a structural and physicochemical point of view.

Table 4.2. Brightness and Opacity Values of WPI-based Edible Films

Film Type	Brightness	Opacity
WPI	90.67 ± 2.18	15.67 ± 2.90
C-WPI	71.00 ± 1.21	26.50 ± 1.50
A-WPI	51.67 ± 1.31	34.67 ± 7.21
WPI: whey protein isolate based edible films		
C-WPI: clove essential oil incorporated whey protein isolate based edible films		
A-WPI: apricot essential oil incorporated whey protein isolate based edible films		

The brightness and opacity values of the WPI films were similar to those of WPI-Glycerol film reported in literature.

4.3 Fourier transforms infrared spectroscopy (FTIR)

FTIR spectroscopy was used to study the interaction between film matrix and essential oils. The spectra of the WPI films in presence of glycerol with different

essential oils are presented in Figure 6. It was determined that the addition of essential oils did not change the structure of the WPI films. Similar results were detected by Fazilah et al., 2006. They suggested that the similarities between the films prove that there was no interaction between active compounds of essential oils with the functional groups of the WPI based film. Therefore, the active compounds of the essential oils added to WPI-films were free to prevent growth of microorganisms.

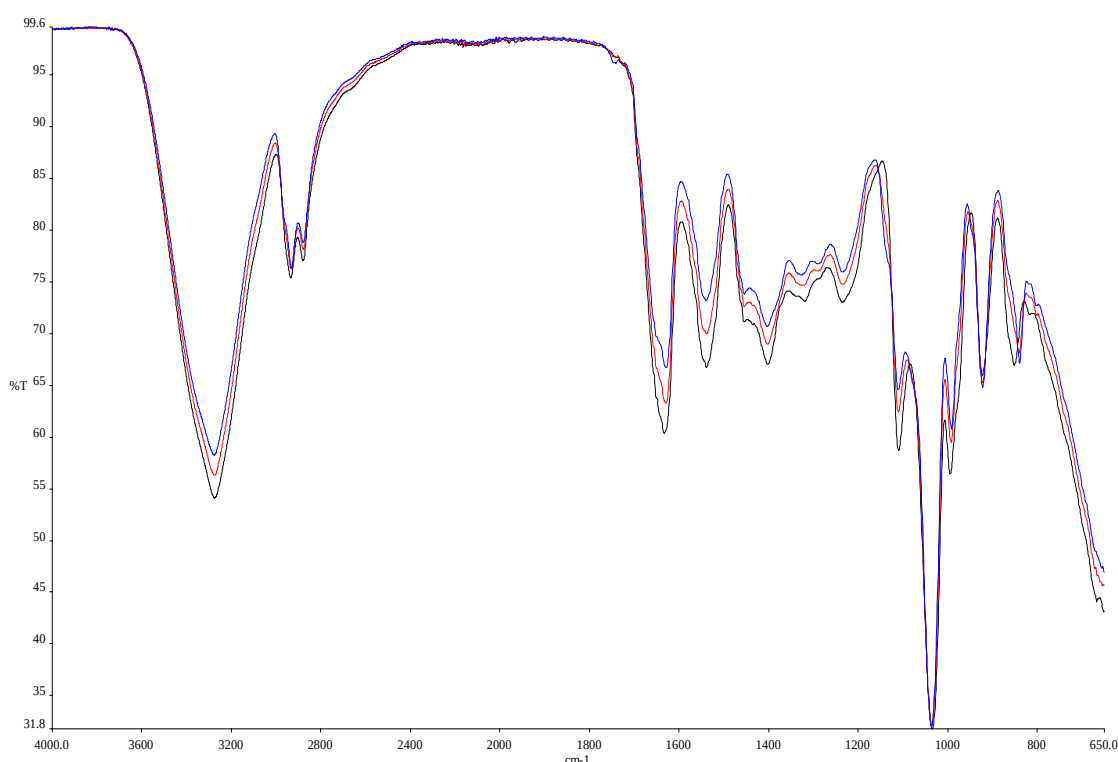


Figure 4.1 Spectra of Fourier Transform Infrared (FTIR) spectroscopy of WPI, C-WPI, A-WPI films: (black line) WPI, (red line) C-WPI, (blue line) A-WPI.

4.4 Thermogravimetric Analysis (TGA)

Thermogravimetric curves of the WPI, C-WPI and A-WPI films measured at a heating rate of 10°C/min were represented in Figure 4.2. All film types showed similar behaviour with two main stages of the mass loss. The first stage, observed up to 200°C, was related to the loss of adsorbed and bound water. The second stage, which was associated with the protein degradation, was analysed to observe behaviour of the edible films with and without the presence of essential oils.

As shown in the results presence of essential oils did not effect the film structure, for all film types the initial temperatures of degradation were in the range 150–200°C, which, associated to the thermogravimetric curves (Figure 4.2).

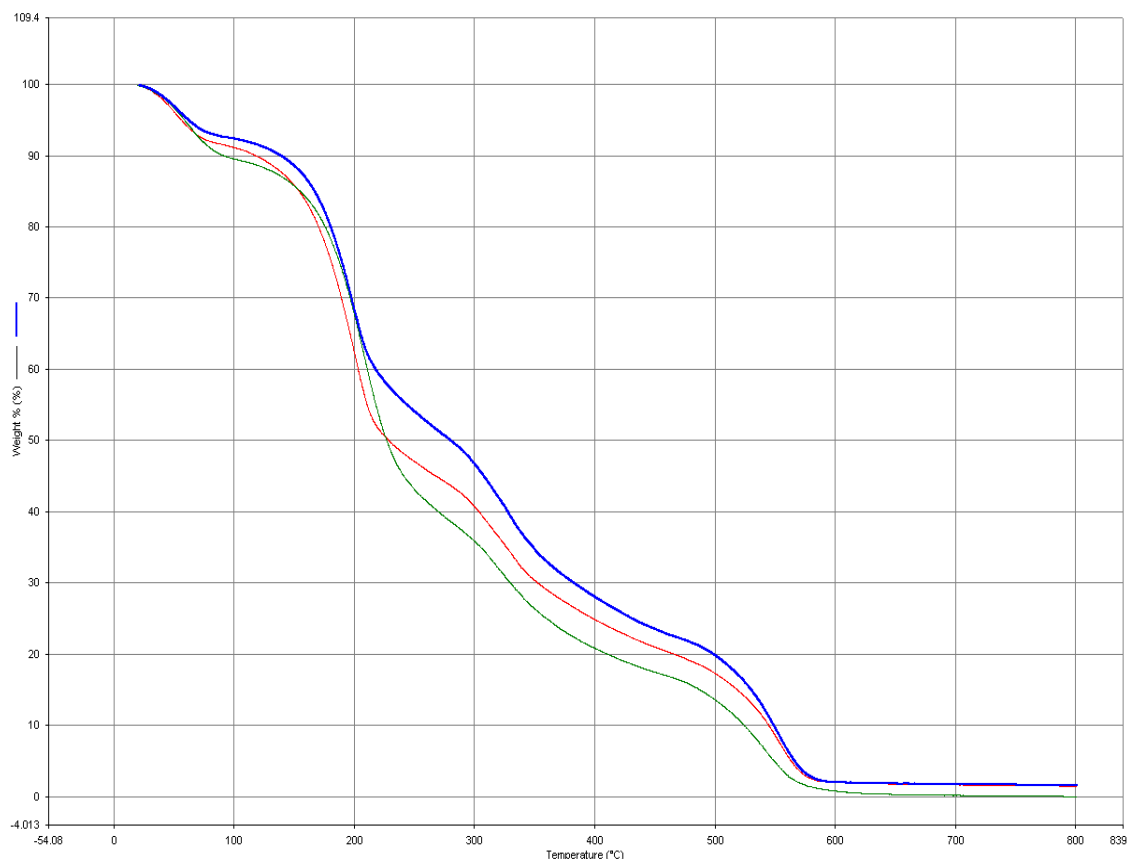


Figure 4.2 Thermogravimetric curves for the WPI, C-WPI and A-WPI films measured at a heating rate of 10°C/min. (WPI; red line, C-WPI, blue line, A-WPI, green line).

4.5 Microbiological Analysis

The number of colonies of A-WPI, C-WPI, and WPI coated and uncoated cheese samples were counted and represented in Figure 4.3. The some of the coated and uncoated samples were used to measure the initial microbial loads and the rest of the samples were stored at 4°C for 5 weeks to determine the effect of possible antimicrobial activity of the essential oils on total microorganisms. It is possible to state that using clove oil was very effective on eliminating microbial growth of the cheese samples. With the comparison of uncoated cheese microbial growth counts, C-WPI and A-WPI coating caused 98% and 37% reduction in growth of

microorganisms. By the way, it is possible to say that each of the essential oils of apricot and clove oil can be used as an antimicrobial growth because it was determined that films containing clove and apricot essential oils has a significant effect to prevent growth of microorganisms ($P < 0.05$; Table A1). Further clove is the best for preventing microbial growth.

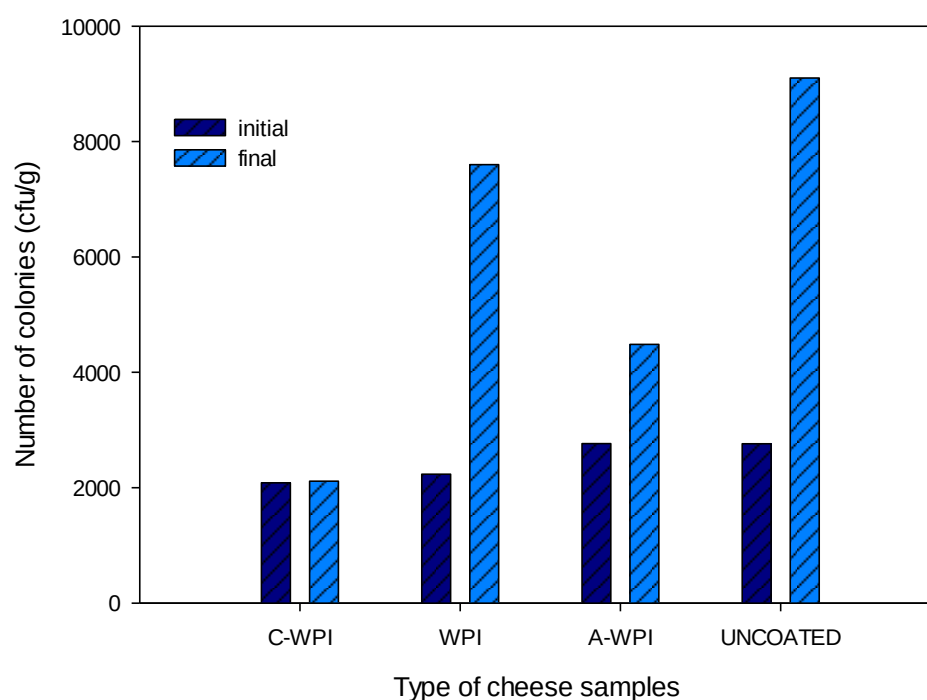


Figure 4.3 Comparison of colony count between initial and final measurements for all type of cheese samples.

There was smaller increasing in colony count of C-WPI and A-WPI coated cheese samples with respect to their initial count. However microbial growth rate of uncoated and WPI film coated cheese were closer to each other.

The effectiveness of clove essential oil against microbial growth when in direct contact with the inoculated culture had already been reported by P. Goñi (2009). This inhibitory effect of clove was due to the relatively high concentration of eugenol.

Erdogan and Kartal, (2010) were reported that the butanol extract obtained from the fruits of *P. armeniaca* had a remarkable antibacterial activity against tested microorganisms with the MIC values varying between 31.25 and 500 $\mu\text{g/mL}$. The butanol extract was more effective in inhibiting the growth of Gram-positive bacteria

(MIC values 31.25–250 µg/mL), with the highest activity exhibited against *Micrococcus luteus* (MIC 31.25 µg/mL).

Seydim and Sarikus (2006) studied antimicrobial activities of whey protein isolate (WPI) films containing 1.0–4.0% (wt/vol) ratios of oregano, rosemary and garlic essential oils. They reported that oregano and garlic essential oil added films exhibited larger inhibitory zones on *S. aureus*, *S. enteritidis*, *L. monocytogenes*, *E. coli* and *L. plantarum* as compared to rosemary essential oil incorporated films.

4.6 Texture Profile Analysis

The TPA test consists of compressing bite size pieces of food two times in a motion that simulates the action of jaw, and extracting from the resulting force time curve a number of textural parameters. These can be divided into the primary parameters of hardness, cohesiveness, springiness and adhesiveness and the secondary parameters of fracturability, chewiness and gumminess (Sozer et al., 2007).

Moisture in the cheeses acts as a plasticizer and is another important factor determining the texture. Hardness value, increased with decreasing moisture contents (Konuklar et al., 2003). For all cheese samples, hardness values obtained from texture profile analysis, were increased as shown in Figure 4.4. Due to the hydrophilic nature of whey protein based edible films, they lost moisture and became harder than initial they are less effective moisture barriers (Hui et al., 2007).

Springiness can be measured using several ways, but most typically, by the distance of the detected height of the product on the second compression as divided by the original compression distance (Floury, 2009). Figure 4.5 shows the change in springiness during storage. There was an increasing trend in springiness for coated cheese samples containing especially essential oil of clove oil at the beginning of storage period however after 14 days springiness declined. Uncoated sample did not show an important change during storage period. This result was in agreement with the texture profile analysis data as all the cheeses exhibited similar springiness values WPI and A-WPI coated cheese samples.

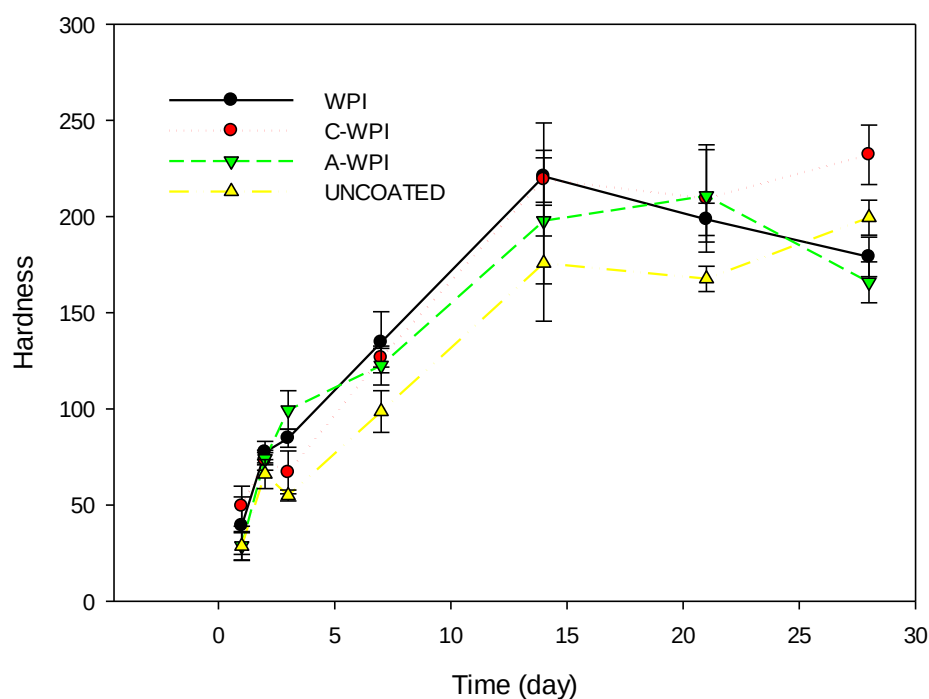


Figure 4.4 Change in hardness for uncoated and WPI, C-WPI, and A-WPI coated cheese samples during storage.

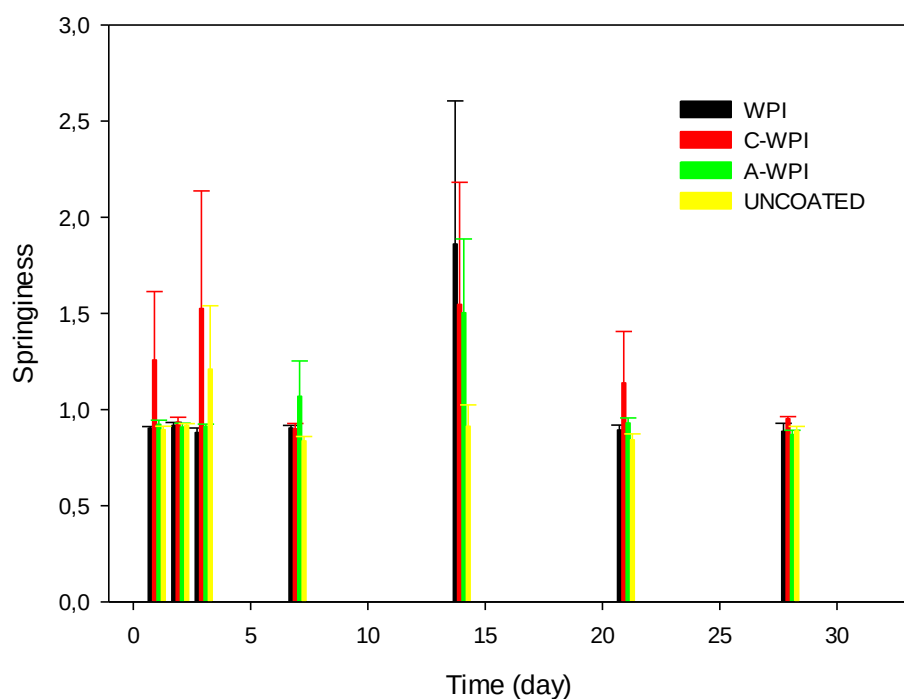


Figure 4.5 Change in springiness values of WPI; C-WPI, A-WPI and uncoated cheese samples during storage.

Similar results has been reported by Kurban, (2007). He studied kaşar cheese stored at 4°C with plastic packaging and he detected that springiness values not changed during storage.

The adhesiveness or ‘adhesive force’ is defined as the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials. It is measured as the negative area of work between the two compressions (Floury, 2009). The adhesiveness of the coated and uncoated kaşar cheese samples were represented in Figure 4.6. As shown in figure C-WPI and A-WPI film coated cheese samples show similar change. This result dedicate that addition of essential oils make film different from WPI film.

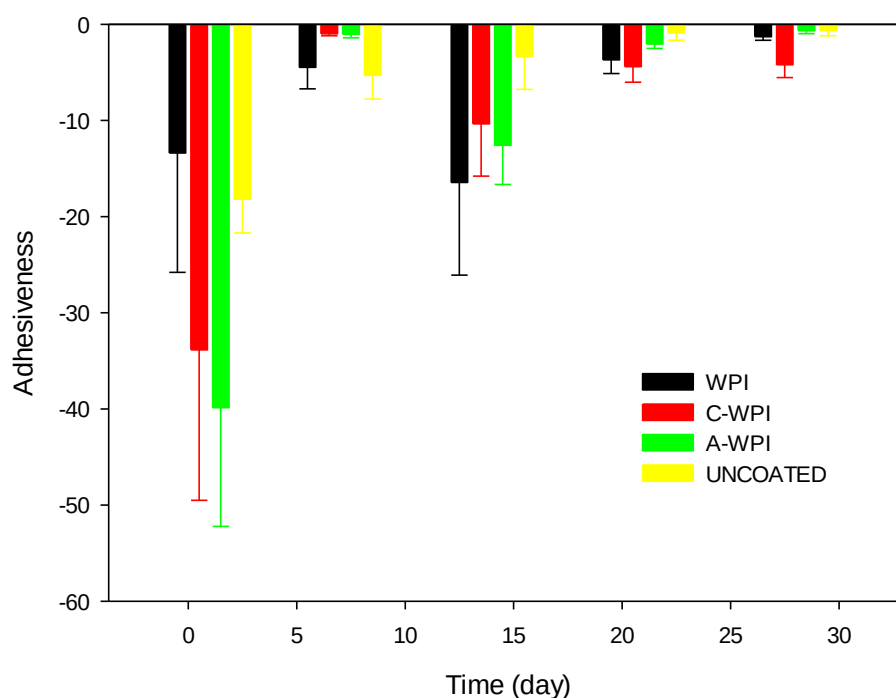


Figure 4.6 Change in adhesiveness values of WPI; C-WPI, A-WPI and uncoated cheese samples during storage.

Chewiness was measured as the number of chews to masticate a known amount of sample at a constant rate of force application to reduce it to a consistency ready for swallowing. Chewiness is defined as the product of hardness times cohesiveness times springiness. There was disconformity between each cheese groups during storage as shown in Figure 4.7.

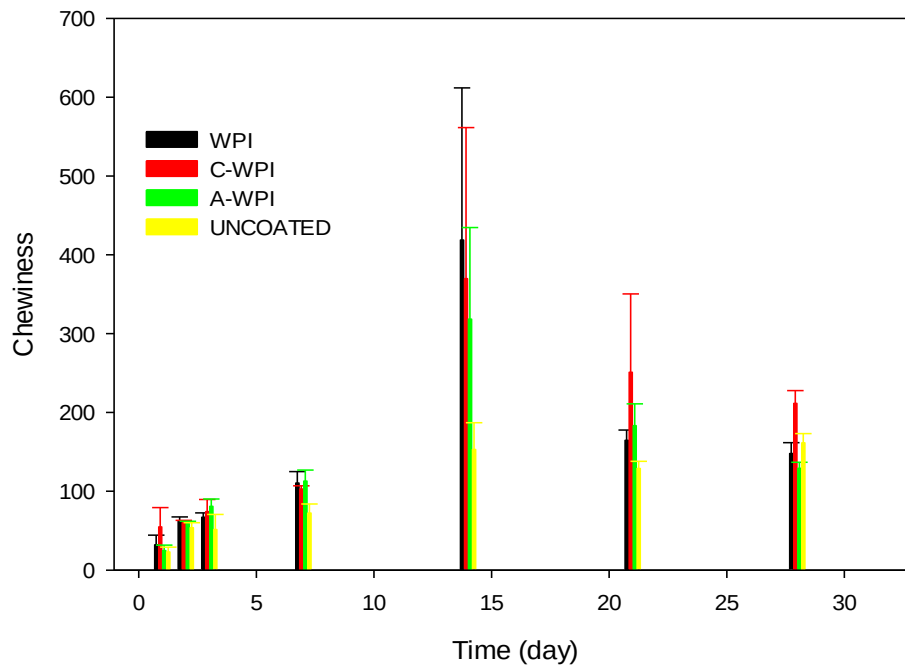


Figure 4.7 Change in chewiness values of WPI; C-WPI, A-WPI and uncoated cheese samples during storage.

Cohesiveness was evaluated by placing a sample between the molar teeth and estimating the amount of deformation before rupture (Kealy, 2005). Figure 4.8 shows change in cohesiveness during storage. In each weeks cohesiveness values of all cheese samples were closer to each other. Changes in cohesiveness, anything that changes the ability of the proteins to interact with water or other proteins can also influence cheese adhesiveness.

Multivariate tests (TableA6) show that type of films were not effect the any texture parameters ($p > 0.05$). However effect of type was determined by using one way anova test for each day and results showed that there is no any difference between samples. But it can be said that the texture parameters were change with time ($p < 0.05$) according to result in TableA6. However the one way anova results (TableA8; a,b,c and d) showed that hardness and chewiness of all samples were effected from time ($p < 0.05$). Furthermore the relationship between chewiness and hardness were explained by pearson correlation. However, adhesiveness of C-WPI and A-WPI coated cheese samples were effected with time this can be caused oily surface of these samples (TableA; a, b).

The Pearson's correlation is used to find a correlation between texture parameters. According to results of pearson correlations there is pretty high correlations between hardness and cohesiveness. This means when cohesiveness values increased hardness values increasing also. However chewiness values increasing with increasing hardness, cohesiveness and springiness values as determined in Pearson correlation test. The Pearson correlation value between hardness and cohesiveness determined as 0.874 then hardness and chewiness correlation value calculated as 0,828. Although it was seen an increasing trend in springiness it was not suggested statistically. Since moisture content of cheese samples decreases they were lost their elasticity and they became hard. The correlations value between hardness and springiness was determined as 0,199 in appendix Table A7.

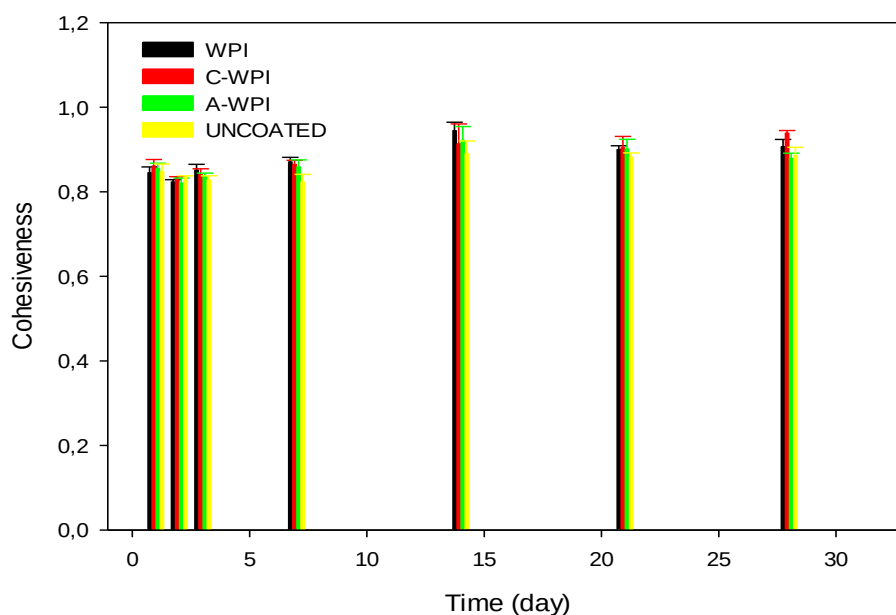


Figure 4.8 Change in cohesiveness values of WPI; C-WPI, A-WPI and uncoated cheese samples during storage.

4.7 Stress Relaxation Test

It was determined that from preliminary studies, films coated cheese samples was hardened in a higher rate than uncoated samples within a few days, so stress relaxation values were determined at initial three days and then a day in a week.

The Maxwell model, consisting of a Hookean spring and a Newtonian dashpot in series, is suitable for understanding stress relaxation data, but does not consider the equilibrium stress. For this reason, the viscoelastic behavior of food can be better described by using a generalized Maxwell model consisting of several elements in parallel with a spring (Del Nobile et al., 2006).

Values of constant of Maxwell model, k_1 and k_2 , was shown in Figure 4.9 and 4.10.

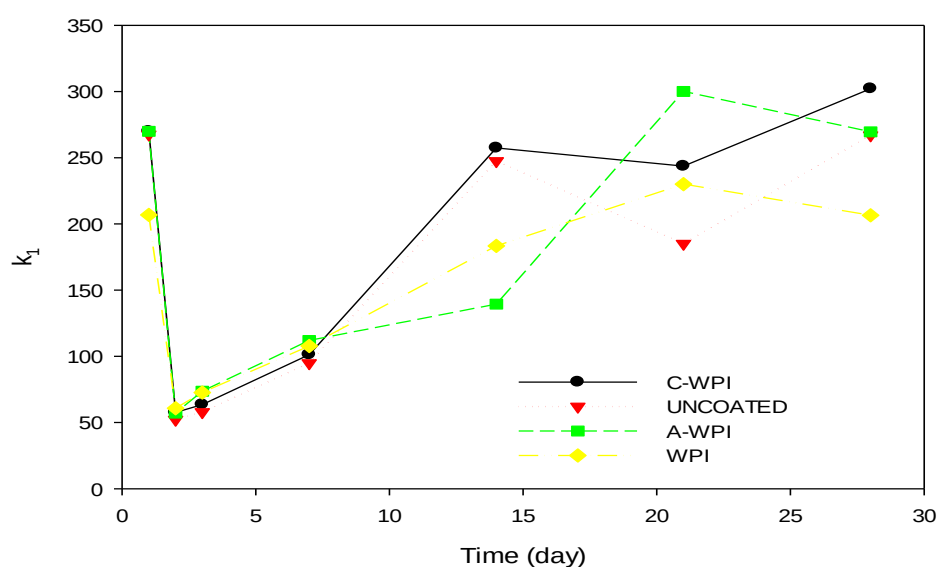


Figure 4.9 Change of Maxwell model constant (k_1) with respect to cheese samples; WPI, C-WPI, A-WPI and uncoated which calculated from stress relaxation results.

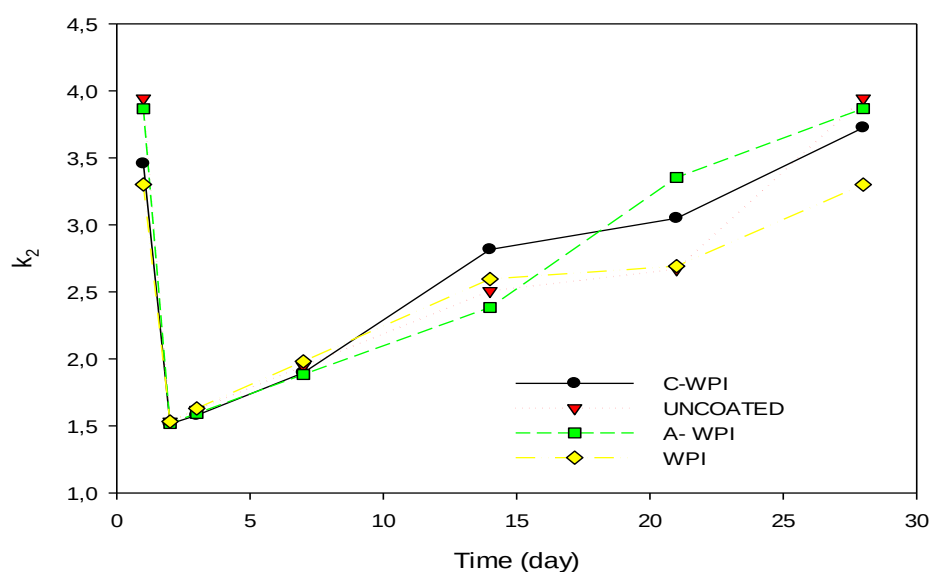


Figure 4.10 Change of Maxwell model constant (k_2) with respect to cheese samples; WPI, C-WPI, A-WPI and uncoated which calculated from stress relaxation results.

Table 4.3. Stress relaxation constant values (k_1 and k_2) of uncoated and coated Kaşar cheese samples stored at 4°C

Sample types	$k_1 \pm SE$	$k_2 \pm SE$
WPI	152.57 $\pm$ 10.05	3.11 $\pm$ 0.10
C-WPI	185.07 $\pm$ 23.34	3.15 $\pm$ 0.15
A-WPI	174.52 $\pm$ 24.96	2.57 $\pm$ 0.20
UNCOATED	167.44 $\pm$ 23.14	3.94 $\pm$ 0.15

WPI: whey protein isolate based edible films

C-WPI: clove essential oil incorporated whey protein isolate based edible films

A-WPI: apricot essential oil incorporated whey protein isolate based edible films

According to results shown in Figure 4.9 and Figure 4.10, k_1 values of cheese samples were closer to each other. Similar results was observed for k_2 values. This indicates that elasticity character of all cheese samples were similar.

4.8 Weight Change

Figure 4.11 shows the weight loss of coated and uncoated cheese samples measured during the storage periods of samples stored at 4°C. Samples were remained either uncoated or coated with WPI, C-WPI and A-WPI films. Coating applications did not significantly prevent moisture loss compared to the control.

Similar results were found by Perez-Gago et al. (2004) who coated fresh apple pieces with the emulsion coatings from whey protein isolate (WPI), whey protein concentrate (WPC) or hydroxypropyl methylcellulose (HPMC) as the hydrophilic phase, and beeswax (BW) or carnauba wax (CarW) as the lipid phase, and weight loss were measured during storage. Their Results showed that coating application did not reduce weight loss in fresh-cut apples.

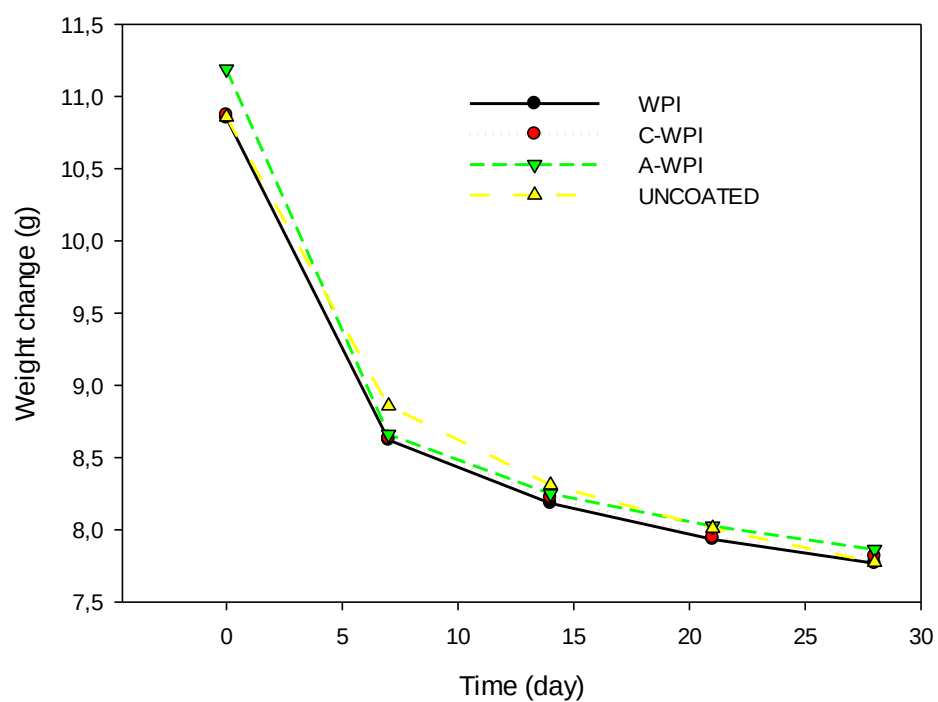


Figure 4.11 Weight change of WPI, C-WPI, A-WPI and uncoated cheese samples during storage.

CHAPTER V

CONCLUSION

The study of properties and application of essential oil incorporated edible films revealed the following conclusions:

1. Addition of essential oils increase the elasticity of edible whey protein isolate based edible films.
2. Addition of essential oils did not effect the structure of whey protein isolate based films with rspect to fourier transform infrared spectroscopy and thermogravimetric analysis result.
3. It is possible to said that esssential oil of clove and apricot can be used as antimicrobial agents. It was significantly determined that essential oil added film coated cheese have lower microbial count.
4. Whey protein isolate based film and essential oil added whey protein isolate based edible films did not effect the texture and rheology of cheese samples with respect to texture analysis tets and stress relaxation test results.
5. Weight change or moisture loss for all coated and uncoated cheese samples was the same. Coating did not effect the moisture loss.

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APPENDIX

Table A1. Tests of effect of different film types on growth of microorganisms.

ANOVA					
NUMBER					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	46367.517	3	15455.839	4.273	0.031
Within Groups	39791.417	11	3617.402		
Total	86158.933	14			

Table A2. Tests of effect of film on weight change of cheese samples.

ANOVA					
Weight					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.050	3	0.017	0.010	0.999
Within Groups	26.306	16	1.644		
Total	26.356	19			

Table A3. Tests of effect of essential oils on mechanical properties (elongation at break %) of films.

ANOVA					
elongation					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	24339.934	2	12169.967	7.258	0.004
Within Groups	35212.566	21	1676.789		
Total	59552.499	23			

Table A4. Tests of effect of essential oils on mechanical properties (tensile strenght) of films.

ANOVA					
tensilestrenght					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3.644	2	1.822	24.315	0.000
Within Groups	1.574	21	0.075		
Total	5.217	23			

Table A5. Tests of effect of essential oils on mechanical properties (elastic modulus) of films.

ANOVA					
elasticmodulus					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	4166.361	2	2083.180	107.021	0.000
Within Groups	389.304	20	19.465		
Total	4555.665	22			

Table A6. Tests of effect of different film types on textural characteristic of cheese samples.

Tests of Between-Subjects Effects

Dependent Variable: hardness

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	383095.910 ^a	27	14188.737	12.993	0.000
Intercept	1424474.237	1	1424474.237	1.304E3	0.000
type	7043.722	3	2347.907	2.150	0.104
time	350678.579	6	58446.430	53.521	0.000
type * time	25373.609	18	1409.645	1.291	0.229
Error	61153.814	56	1092.032		
Total	1868723.960	84			
Corrected Total	444249.724	83			

a. R Squared = ,862 (Adjusted R Squared = ,796)

Hardness

Duncan

time	N	Subset			
		1	2	3	4
1	12	36.0042			
2	12		77.3082		
3	12		78.2040		
7	12			1.2000E2	
21	12				1.9184E2
28	12				2.0072E2
14	12				2.0749E2
Sig.		1.000	0.947	1.000	0.280

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1092,032.

Tests of Between-Subjects Effects

Dependent Variable: adhesiveness

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	10167.083 ^a	27	376.559	3.339	0.000
Intercept	3672.878	1	3672.878	32.569	0.000
type	693.248	3	231.083	2.049	0.118
time	5305.620	6	884.270	7.841	0.000
type * time	3896.839	18	216.491	1.920	0.034
Error	5976.878	53	112.771		
Total	20088.429	81			
Corrected Total	16143.961	80			

a. R Squared = ,630 (Adjusted R Squared = ,441)

Adhesiveness

Duncan

time	N	Subset	
		1	2
1	12	-2.57585E1	
14	12		-9.03917
28	12		-4.52558
2	11		-2.84318
21	12		-2.50733
7	12		-2.36950
3	10		-0.35690
Sig.		1.000	0.090

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error)
=112,771.

Tests of Between-Subjects Effects

Dependent Variable: springiness

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	2.843 ^a	27	0.105	0.882	0.631
Intercept	80.016	1	80.016	670.259	0.000
type	0.092	3	0.031	0.257	0.856
time	0.415	6	0.069	0.579	0.745
type * time	2.336	18	0.130	1.087	0.388
Error	6.685	56	0.119		
Total	89.545	84			
Corrected Total	9.528	83			

a. R Squared = ,298 (Adjusted R Squared = -,040)

springiness

Duncan

time	N	Subset
		1
21	12	0.87633
28	12	0.90700
2	12	0.93000
7	12	0.96567
3	12	1.02983
1	12	1.05367
14	12	1.06950
Sig.		0.245

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean

Square(Error) = ,119.

Tests of Between-Subjects Effects

Dependent Variable: cohesiveness

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	0.113 ^a	27	0.004	2.008	0.014
Intercept	63111	1	63.111	3.024E4	0.000
type	0.009	3	0.003	1.395	0.254
time	0.075	6	0.013	6.018	0.000
type * time	0.029	18	0.002	0.774	0.720
Error	0.117	56	0.002		
Total	63.341	84			
Corrected Total	0.230	83			

a. R Squared = ,492 (Adjusted R Squared = ,247)

Cohesiveness

Duncan

time	N	Subset		
		1	2	3
2	12	0.82933		
3	12	0.83292		
7	12	0.85342	0.85342	
1	12	0.85467	0.85467	
21	12		0.88667	0.88667
28	12			0.90025
14	12			0.91025
Sig.		0.223	0.097	0.239

Means for groups in homogeneous subsets are displayed.

Based on observed means.

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

Tests of Between-Subjects Effects

Dependent Variable:chewiness

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	427418,814 ^a	27	15830,326	3,316	,000
Intercept	1086969,977	1	1086969,977	227,720	,000
type	20009,538	3	6669,846	1,397	,253
time	312043,147	6	52007,191	10,895	,000
type * time	95366,129	18	5298,118	1,110	,368
Error	267303,719	56	4773,281		
Total	1781692,510	84			
Corrected Total	694722,533	83			

a. R Squared = ,615 (Adjusted R Squared = ,430)

Chewiness

Duncan

time	N	Subset			
		1	2	3	4
1	12	38.04			
2	12	59.43			
3	12	64.67			
7	12	99.32	99.32		
21	12		154.04	154.04	
28	12			164.58	164.58
14	12				216.20
Sig.		0.050	0.057	0.710	0.073

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4773,281.

Table A7. Effect of film type on texture parameters at different time

a. One Way Anova Results for 1st day

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1486.352	3	495.451	0.844	0.496
Within Groups	7046.349	12	587.196		
Total	8532.701	15			

hardness

Duncan

type	N	Subset for alpha = 0.05
		1
A-WPI	4	34.33250
UNCOATED	4	35.80075
WPI	4	49.96075
C-WPI	4	57.26000
Sig.		0.238

Means for groups in homogeneous subsets
are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4351.633	3	1450.544	2.118	0.151
Within Groups	8219.925	12	684.994		
Total	12571.558	15			

Adhesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
C-WPI	4	-42.20725
A-WPI	4	-39.87325
WPI	4	-16.60450
UNCOATED	4	-2.62700
Sig.		0.070

Means for groups in homogeneous subsets
are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.602	3	0.201	1.017	0.419
Within Groups	2.367	12	0.197		
Total	2.968	15			

springiness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.88200
WPI	4	0.90050
A-WPI	4	0.93475
C-WPI	4	1.35150
Sig.		0.191

Means for groups in homogeneous subsets
are displayed.

ANOVA

chewiness					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	4477.164	3	1492.388	1.561	0.250
Within Groups	11470.125	12	955.844		
Total	15947.289	15			

Chewiness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	27.08450
A-WPI	4	28.35500
WPI	4	38.77650
C-WPI	4	68.58925
Sig.		0.103

Means for groups in homogeneous subsets
are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.002	3	0.001	0.452	0.720
Within Groups	0.016	12	0.001		
Total	0.017	15			

cohesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.83900
WPI	4	0.84925
A-WPI	4	0.85775
C-WPI	4	0.86750
Sig.		0.321

Means for groups in homogeneous subsets
are displayed.

b. One Way Anova Results for 2nd day

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.905	3	4.968	0.040	0.989
Within Groups	1508.133	12	125.678		
Total	1523.038	15			

Hardness

Duncan

type	N	Subset for alpha = 0.05
		1
C-WPI	4	76.15675
UNCOATED	4	76.77250
A-WPI	4	77.21050
WPI	4	78.76775
Sig.		0.765

Means for groups in homogeneous subsets
are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.262	3	0.087	3.333	0.056
Within Groups	0.314	12	0.026		
Total	0.575	15			

adhesiveness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
UNCOATED	4	-0.49050	
C-WPI	4	-0.27712	-0.27712
A-WPI	4		-0.20400
WPI	4		-0.15625
Sig.		0.087	0.335

Means for groups in homogeneous subsets are displayed.

ANOVA

springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.004	3	0.001	0.677	0.583
Within Groups	0.023	12	0.002		
Total	0.026	15			

springiness

Duncan

		Subset for alpha = 0.05
type	N	1
A-WPI	4	0.90625
UNCOATED	4	0.91500
WPI	4	0.91900
C-WPI	4	0.94750
Sig.		0.236

Means for groups in homogeneous subsets
are displayed.

ANOVA					
chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.939	3	5.980	0.084	0.967
Within Groups	852.831	12	71.069		
Total	870.771	15			

chewiness

Duncan

type	N	Subset for alpha = 0.05
		1
A-WPI	4	57.03925
UNCOATED	4	58.82300
C-WPI	4	59.59800
WPI	4	59.66525
Sig.		0.690

Means for groups in homogeneous subsets
are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.001	3	0.000	1.408	0.288
Within Groups	0.003	12	0.000		
Total	0.004	15			

cohesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
A-WPI	4	0.81550
WPI	4	0.82200
C-WPI	4	0.83225
UNCOATED	4	0.83700
Sig.		0.111

Means for groups in homogeneous subsets
are displayed.

c. One Way Anova Results for 3th day

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4186.692	3	1395.564	7.748	0.004
Within Groups	2161.543	12	180.129		
Total	6348.236	15			

hardness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
UNCOATED	4	59.62075	
C-WPI	4	62.08650	
WPI	4		90.50400
A-WPI	4		95.43625
Sig.		0.799	0.613

Means for groups in homogeneous subsets are displayed.

ANOVA

adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.231	3	0.077	2.474	0.116
Within Groups	0.342	11	0.031		
Total	0.573	14			

Adhesiveness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
UNCOATED	4	-0.51800	
C-WPI	4	-0.28550	-0.28550
WPI	4	-0.25100	-0.25100
A-WPI	3		-0.19067
Sig.		0.075	0.501

Means for groups in homogeneous subsets are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.005	3	0.002	0.842	0.497
Within Groups	0.022	12	0.002		
Total	0.027	15			

springiness

Duncan

type	N	Subset for alpha = 0.05
		1
WPI	4	0.87325
UNCOATED	4	0.88200
A-WPI	4	0.90650
C-WPI	4	0.91525
Sig.		0.227

Means for groups in homogeneous subsets
are displayed.

ANOVA					
chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	174.072	3	58.024	0.072	0.974
Within Groups	9625.460	12	802.122		
Total	9799.532	15			

chewiness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	64.21750
WPI	4	67.84950
A-WPI	4	71.89300
C-WPI	4	72.31225
Sig.		0.714

Means for groups in homogeneous subsets
are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.001	3	0.000	0.502	0.688
Within Groups	0.007	12	0.001		
Total	0.008	15			

Cohesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
A-WPI	4	0.83125
C-WPI	4	0.83550
UNCOATED	4	0.83775
WPI	4	0.85125
Sig.		0.303

Means for groups in homogeneous subsets
are displayed.

d. One Way Anova Results for 7th day

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1830.077	3	610.026	0.778	0.528
Within Groups	9406.879	12	783.907		
Total	11236.955	15			

Hardness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	107.83475
A-WPI	4	124.54675
C-WPI	4	127.65700
WPI	4	137.53325
Sig.		0.189

Means for groups in homogeneous subsets
are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.783	3	1.928	3.166	0.064
Within Groups	7.306	12	0.609		
Total	13.089	15			

adhesiveness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
WPI	4	-2.27075	
UNCOATED	4	-1.85575	-1.85575
A-WPI	4	-1.04025	-1.04025
C-WPI	4		-0.78275
Sig.		0.055	0.088

Means for groups in homogeneous subsets are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.215	3	0.072	1.470	0.272
Within Groups	0.585	12	0.049		
Total	0.800	15			

springiness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.83525
WPI	4	0.90375
C-WPI	4	0.92175
A-WPI	4	1.14400
Sig.		0.091

Means for groups in homogeneous subsets
are displayed.

ANOVA

chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3902.021	3	1300.674	1.538	0.255
Within Groups	10147.174	12	845.598		
Total	14049.195	15			

chewiness

Duncan

		Subset for alpha = 0.05
type	N	1
UNCOATED	4	76.49750
C-WPI	4	101.62400
WPI	4	109.72100
A-WPI	4	118.31625
Sig.		0.083

Means for groups in homogeneous subsets
are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.005	3	0.002	1.369	0.299
Within Groups	0.014	12	0.001		
Total	0.019	15			

cohesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.82975
A-WPI	4	0.85625
C-WPI	4	0.86175
WPI	4	0.87775
Sig.		0.089

Means for groups in homogeneous subsets
are displayed.

e. One Way Anova Results for 14th day

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11646.684	3	3882.228	1.225	0.343
Within Groups	38019.280	12	3168.273		
Total	49665.964	15			

hardness

Duncan

		Subset for alpha = 0.05
type	N	1
UNCOATED	4	160.40100
C-WPI	4	218.42450
WPI	4	222.25575
A-WPI	4	226.41675
Sig.		0.150

Means for groups in homogeneous subsets
are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	395.965	3	131.988	0.903	0.468
Within Groups	1753.511	12	146.126		
Total	2149.476	15			

adhesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
WPI	4	-16.44600
A-WPI	4	-12.58850
C-WPI	4	-7.37500
UNCOATED	4	-3.37725
Sig.		0.181

Means for groups in homogeneous subsets
are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.857	3	0.952	0.559	0.652
Within Groups	20.434	12	1.703		
Total	23.291	15			

springiness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.91225
A-WPI	4	1.66150
C-WPI	4	1.69700
WPI	4	2.07825
Sig.		0.264

Means for groups in homogeneous subsets
are displayed.

ANOVA					
chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	136180.405	3	45393.468	2.303	0.129
Within Groups	236497.061	12	19708.088		
Total	372677.466	15			

chewiness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
UNCOATED	4	1.35065E2	
C-WPI	4	1.80390E2	1.80390E2
WPI	4	2.26846E2	2.26846E2
A-WPI	4		3.80210E2
Sig.		0.397	0.079

Means for groups in homogeneous subsets are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.014	3	0.005	0.845	0.495
Within Groups	0.068	12	0.006		
Total	0.083	15			

cohesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.87425
C-WPI	4	0.90800
WPI	4	0.94075
A-WPI	4	0.95075
Sig.		0.208

Means for groups in homogeneous subsets
are displayed.

f. One Way Anova Results for 21st day

ANOVA

hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5017.222	3	1672.407	1.097	0.388
Within Groups	18293.810	12	1524.484		
Total	23311.032	15			

hardness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	171.59100
C-WPI	4	191.62950
WPI	4	203.12175
A-WPI	4	220.29875
Sig.		0.127

Means for groups in homogeneous subsets
are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28.617	3	9.539	1.573	0.247
Within Groups	72.773	12	6.064		
Total	101.390	15			

adhesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
WPI	4	-4.27131
C-WPI	4	-4.03800
A-WPI	4	-2.09725
UNCOATED	4	-1.07300
Sig.		0.114

Means for groups in homogeneous subsets
are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.023	3	0.008	1.350	0.305
Within Groups	0.067	12	0.006		
Total	0.090	15			

springiness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.84125
C-WPI	4	0.87375
WPI	4	0.89325
A-WPI	4	0.94525
Sig.		0.093

Means for groups in homogeneous subsets
are displayed.

ANOVA

chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8576.492	3	2858.831	1.207	0.349
Within Groups	28420.753	12	2368.396		
Total	36997.245	15			

chewiness

Duncan

		Subset for alpha = 0.05
type	N	1
UNCOATED	4	127.19525
C-WPI	4	153.83950
WPI	4	164.87325
A-WPI	4	191.74350
Sig.		0.107

Means for groups in homogeneous subsets
are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.002	3	0.001	0.315	0.814
Within Groups	0.024	12	0.002		
Total	0.026	15			

cohesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	0.87625
C-WPI	4	0.89100
WPI	4	0.90050
A-WPI	4	0.90450
Sig.		0.424

Means for groups in homogeneous subsets
are displayed.

g. One Way Anova Results for 28th day

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8091.390	3	2697.130	3.176	0.063
Within Groups	10190.063	12	849.172		
Total	18281.454	15			

hardness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
A-WPI	4	1.75180E2	2.06270E2
WPI	4	1.80856E2	
UNCOATED	4	2.06270E2	
C-WPI	4		2.31777E2
Sig.		0.176	0.239

Means for groups in homogeneous subsets are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	40.965	3	13.655	2.401	0.119
Within Groups	68.255	12	5.688		
Total	109.220	15			

adhesiveness

Duncan

type	N	Subset for alpha = 0.05
		1
UNCOATED	4	-4.38531
C-WPI	4	-3.87900
WPI	4	-1.29900
A-WPI	4	-0.66800
Sig.		0.063

Means for groups in homogeneous subsets
are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.020	3	0.007	1.639	0.233
Within Groups	0.050	12	0.004		
Total	0.070	15			

springiness

Duncan

type	N	Subset for alpha = 0.05
		1
A-WPI	4	0.86700
WPI	4	0.87400
UNCOATED	4	0.87600
C-WPI	4	0.95450
Sig.		0.100

Means for groups in homogeneous subsets
are displayed.

ANOVA

chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13566.146	3	4522.049	4.477	0.025
Within Groups	12120.049	12	1010.004		
Total	25686.195	15			

chewiness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
A-WPI	4	1.32022E2	
WPI	4	1.43212E2	
UNCOATED	4	1.60266E2	1.60266E2
C-WPI	4		2.08274E2
Sig.		0.255	0.054

Means for groups in homogeneous subsets are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.011	3	0.004	2.884	0.080
Within Groups	0.015	12	0.001		
Total	0.026	15			

cohesiveness

Duncan

type	N	Subset for alpha = 0.05	
		1	2
A-WPI	4	0.87025	
UNCOATED	4	0.88125	
WPI	4	0.90050	0.90050
C-WPI	4		0.93900
Sig.		0.275	0.152

Means for groups in homogeneous subsets are displayed.

Table A8. Effect of time on texture parameters of coated and uncoated cheese samples

a. One Way Anova Results for C-WPI film coated cheese samples

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	103148.043	6	17191.340	14.853	0.000
Within Groups	16203.608	14	1157.401		
Total	119351.651	20			

hardness

Duncan

time	N	Subset for alpha = 0.05				
		1	2	3	4	5
1	3	4.79307E1				
3	3	7.01823E1	7.01823E1			
2	3	7.22937E1	7.22937E1			
7	3		1.25357E2	1.25357E2		
21	3			1.76890E2	1.76890E2	
14	3				1.97737E2	1.97737E2
28	3					2.48533E2
Sig.		0.419	0.079	0.085	0.465	0.089

Means for groups in homogeneous subsets are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1652.697	6	275.449	3.046	0.040
Within Groups	1265.842	14	90.417		
Total	2918.539	20			

adhesiveness

Duncan

time	N	Subset for alpha = 0.05	
		1	2
1	3	-27.35700	
14	3		-8.81300
28	3		-5.05367
21	3		-2.02567
3	3		-1.88167
7	3		-0.98733
2	3		-0.42867
Sig.		1.000	0.347

Means for groups in homogeneous subsets are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.872	6	0.145	0.929	0.504
Within Groups	2.190	14	0.156		
Total	3.062	20			

springiness

Duncan

time	N	Subset for alpha = 0.05
		1
21	3	0.83967
14	3	0.90133
7	3	0.91100
3	3	0.91933
2	3	0.95200
28	3	0.95267
1	3	1.48633
Sig.		0.096

Means for groups in homogeneous subsets are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.027	6	0.004	1.275	0.330
Within Groups	0.049	14	0.004		
Total	0.076	20			

cohesiveness

Duncan

time	N	Subset for alpha = 0.05	
		1	2
3	3	0.82533	
2	3	0.83567	0.83567
1	3	0.85167	0.85167
7	3	0.85700	0.85700
21	3	0.87467	0.87467
14	3	0.88267	0.88267
28	3		0.94167
Sig.		0.304	0.069

Means for groups in homogeneous subsets are displayed.

ANOVA					
chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	73305.120	6	12217.520	5.542	0.004
Within Groups	30864.301	14	2204.593		
Total	104169.421	20			

chewiness

Duncan

time	N	Subset for alpha = 0.05		
		1	2	3
3	3	5.31123E1		
2	3	5.70247E1		
1	3	7.04870E1		
7	3	9.80073E1	9.80073E1	
21	3	1.33011E2	1.33011E2	
14	3		1.68412E2	1.68412E2
28	3			2.23613E2
Sig.		0.079	0.102	0.172

Means for groups in homogeneous subsets are displayed.

b. One Way Anova Results for A-WPI film coated cheese samples

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	90581.724	6	15096.954	10.329	0.000
Within Groups	20462.408	14	1461.601		
Total	111044.132	20			

hardness

Duncan

time	N	Subset for alpha = 0.05			
		1	2	3	4
1	3	4.23097E1			
2	3	7.87957E1	7.87957E1		
3	3	9.14377E1	9.14377E1		
7	3		1.17475E2	1.17475E2	
28	3			1.68310E2	1.68310E2
21	3				2.10302E2
14	3				2.32268E2
Sig.		0.157	0.259	0.126	0.071

Means for groups in homogeneous subsets are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6309.622	6	1051.604	58.465	0.000
Within Groups	251.815	14	17.987		
Total	6561.437	20			

adhesiveness

Duncan

time	N	Subset for alpha = 0.05		
		1	2	3
1	3	-5.21233E1		
14	3		-1.02300E1	
3	3		-5.48200	-5.48200
21	3			-2.35667
2	3			-0.99833
28	3			-0.83433
7	3			-0.72700
Sig.		1.000	0.192	0.233

Means for groups in homogeneous subsets are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.787	6	0.131	0.846	0.556
Within Groups	2.170	14	0.155		
Total	2.957	20			

springiness

Duncan

time	N	Subset for alpha = 0.05
		1
21	3	0.85367
28	3	0.87300
3	3	0.91233
2	3	0.92533
1	3	0.94867
7	3	1.22167
14	3	1.40300
Sig.		0.150

Means for groups in homogeneous subsets are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.033	6	0.005	3.481	0.026
Within Groups	0.022	14	0.002		
Total	0.055	20			

cohesiveness

Duncan

time	N	Subset for alpha = 0.05	
		1	2
2	3	0.81633	
3	3	0.83100	
7	3	0.86267	
28	3	0.86567	
1	3	0.87067	
21	3	0.89000	0.89000
14	3		0.94800
Sig.		0.060	0.095

Means for groups in homogeneous subsets are displayed.

ANOVA					
chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	197725.475	6	32954.246	2.383	0.085
Within Groups	193633.302	14	13830.950		
Total	391358.778	20			

chewiness

Duncan

time	N	Subset for alpha = 0.05	
		1	2
1	3	35.26967	
2	3	59.39233	
3	3	69.36267	
7	3	1.20724E2	
28	3	1.27000E2	
21	3	1.78699E2	1.78699E2
14	3		3.44226E2
Sig.		0.201	0.107

Means for groups in homogeneous subsets are displayed.

c. One Way Anova Results for WPI film coated cheese samples

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	99425.754	6	16570.959	22.453	0.000
Within Groups	10332.311	14	738.022		
Total	109758.065	20			

hardness

Duncan

time	N	Subset for alpha = 0.05				
		1	2	3	4	5
1	3	3.07163E1				
2	3		8.21807E1			
3	3		8.87523E1			
7	3			1.41329E2		
28	3			1.75696E2	1.75696E2	
21	3				2.11958E2	2.11958E2
14	3					2.33861E2
Sig.		1.000	0.771	0.144	0.124	0.340

Means for groups in homogeneous subsets are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1237.267	6	206.211	0.783	0.597
Within Groups	3688.047	14	263.432		
Total	4925.314	20			

adhesiveness

Duncan

time	N	Subset for alpha = 0.05
		1
1	3	-22.03133
14	3	-14.44733
7	3	-6.11467
21	3	-4.46367
28	3	-1.28733
3	3	-0.33333
2	3	-0.16433
Sig.		0.163

Means for groups in homogeneous subsets are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.195	6	0.033	1.077	0.421
Within Groups	0.423	14	0.030		
Total	0.618	20			

springiness

Duncan

time	N	Subset for alpha = 0.05
		1
3	3	0.85400
1	3	0.90067
7	3	0.92167
28	3	0.93333
2	3	0.93433
21	3	0.95833
14	3	1.17833
Sig.		0.061

Means for groups in homogeneous subsets are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.033	6	0.006	4.123	0.014
Within Groups	0.019	14	0.001		
Total	0.052	20			

cohesiveness

Duncan

time	N	Subset for alpha = 0.05		
		1	2	3
2	3	0.82767		
3	3	0.83667	0.83667	
1	3	0.85667	0.85667	
7	3	0.87867	0.87867	0.87867
28	3		0.90467	0.90467
21	3		0.90667	0.90667
14	3			0.94733
Sig.		0.136	0.051	0.051

Means for groups in homogeneous subsets are displayed.

ANOVA					
chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	92391.976	6	15398.663	11.290	0.000
Within Groups	19094.424	14	1363.887		
Total	111486.400	20			

chewiness

Duncan

time	N	Subset for alpha = 0.05			
		1	2	3	4
1	3	2.45127E1			
2	3	6.35000E1	6.35000E1		
3	3	6.38787E1	6.38787E1		
7	3		1.15006E2	1.15006E2	
28	3			1.44080E2	
21	3			1.77837E2	1.77837E2
14	3				2.27101E2
Sig.		0.235	0.126	0.067	0.125

Means for groups in homogeneous subsets are displayed.

a. One Way Anova Results for uncoated cheese samples

ANOVA					
hardness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	82891.185	6	13815.198	13.663	0.000
Within Groups	14155.869	14	1011.133		
Total	97047.054	20			

hardness

Duncan

Time	N	Subset for alpha = 0.05		
		1	2	3
1	3	2.30603E1		
3	3	6.24437E1	6.24437E1	
2	3	7.59627E1	7.59627E1	
7	3		9.58430E1	
14	3			1.66074E2
21	3			1.68203E2
28	3			2.10324E2
Sig.		0.072	0.242	0.127

Means for groups in homogeneous subsets are displayed.

ANOVA					
adhesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	229.339	6	38.223	1.072	0.424
Within Groups	499.076	14	35.648		
Total	728.416	20			

adhesiveness

Duncan

time	N	Subset for alpha = 0.05
		1
28	3	-10.92867
14	3	-2.67400
3	3	-2.49967
7	3	-1.65033
1	3	-1.53567
21	3	-1.18333
2	3	-0.55867
Sig.		0.079

Means for groups in homogeneous subsets are displayed.

ANOVA					
springiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.897	6	0.150	1.101	.409
Within Groups	1.902	14	0.136		
Total	2.799	20			

springiness

Duncan

time	N	Subset for alpha = 0.05
		1
14	3	0.79533
7	3	0.80833
21	3	0.85367
28	3	0.86900
1	3	0.87900
2	3	0.90833
3	3	1.43367
Sig.		0.080

Means for groups in homogeneous subsets are displayed.

ANOVA					
cohesiveness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.012	6	0.002	1.011	0.457
Within Groups	0.027	14	0.002		
Total	0.039	20			

cohesiveness

Duncan

time	N	Subset for alpha = 0.05
		1
7	3	0.81533
2	3	0.83767
3	3	0.83867
1	3	0.83967
14	3	0.86300
21	3	0.87533
28	3	0.88900
Sig.		0.089

Means for groups in homogeneous subsets are displayed.

ANOVA					
chewiness					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	44180.502	6	7363.417	4.332	0.011
Within Groups	23794.161	14	1699.583		
Total	67974.663	20			

chewiness

Duncan

time	N	Subset for alpha = 0.05		
		1	2	3
1	3	2.19093E1		
2	3	5.78193E1	5.78193E1	
7	3	6.36390E1	6.36390E1	
3	3	7.23640E1	7.23640E1	
14	3		1.25241E2	1.25241E2
21	3		1.26767E2	1.26767E2
28	3			1.63862E2
Sig.		0.188	0.083	0.294

Means for groups in homogeneous subsets are displayed.

Table A9. Correlation between texture parameters

		hardness	adhesiveness	springiness	cohesiveness	gumminess	chewiness	resilience
Hardness	Pearson Correlation	1	0.244	0.199	0.874**	0.998**	0.828**	0.981**
	Sig. (2-tailed)		0.158	0.253	0.000	0.000	0.000	0.000
	N	35	35	35	35	35	35	35
adhesiveness	Pearson Correlation		1	-0.278	-0.104	0.209	-0.033	0.227
	Sig. (2-tailed)			0.106	0.553	0.228	0.852	0.190
	N		35	35	35	35	35	35
Springiness	Pearson Correlation			1	0.325	0.228	0.663**	0.206
	Sig. (2-tailed)				0.057	0.188	0.000	0.236
	N			35	35	35	35	35
cohesiveness	Pearson Correlation				1	0.894**	0.828**	0.907**
	Sig. (2-tailed)					0.000	0.000	0.000
	N				35	35	35	35
Gumminess	Pearson Correlation					1	0.847**	0.983**
	Sig. (2-tailed)						0.000	0.000
	N					35	35	35
Chewiness	Pearson Correlation						1	0.813**
	Sig. (2-tailed)							0.000
	N						35	35
Resilience	Pearson Correlation							1
	Sig. (2-tailed)							
	N							35

** . Correlation is significant at the 0.01 level (2-tailed).