FUNCTIONALIZATION OF A VEGAN SNACK WITH ANTIMICROBIAL EDIBLE COATING

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ABSTRACT

FUNCTIONALIZATION OF A VEGAN SNACK
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Pomegranate peel is usually considered as a waste of fruit by many people, but it has quite high content of antioxidant, phenolic and antimicrobial compounds. The objective of this thesis is to observe the effect of edible film in vegan snack bar containing of dried fruits, nut and to functionalize the snack bar with enrichment of protein and antimicrobial content. The edible film was composed of 8% w/v of lentil protein extract, 15% w/w of pomegranate peel depends on protein content, 2% of pectin as a stabilizer and 0.5% of glycerol as a plasticizer. Application of coating was carried out by dipping method. Then, the coated vegan snack bar was dried at 40^{o}C for 16 hours. The coated and uncoated bars were investigated in different conditions as 30% RH at 25 ^{o}C and 75% RH at 35 ^{o}C for 90 days. Optical properties, texture, total phenolic content, sensory, microbial and water activity analysis were determined to evaluate the impact of edible coating incorporation onto surface of the bar. The results showed that the coated bars have been preserved better than uncoated ones in terms of prevention of color change, microbial and textural properties, phenolic content and moisture loss in both conditions.

ÖZET

VEGAN BİR ATIŞTIRMALIĞIN ANTİMİKROBİYAL FİLM İLE KAPLANARAK FONKSİYONELLEŞTİRİLMESİ

Mercimek, protein ve karbonhidrat içeriği bakımından zengin tahıllardan biridir. Nar kabuğu ise birçok insan tarafından meyve atığı olarak düşünülür. Ancak antioksidan, fenolik ve antimikrobiyal içeriği oldukça fazladır. Çalışmanın amacı vegan atıştırmalık kuru meyve ve fındıktan oluşan bar üzerine kaplanmış yenilebilir filmin etkisini gözlemlemektir. Ayrıca, filmin zenginleştirilmiş protein ve antimikrobiyal içerik sayesinde fonksiyonelleştirilmesini sağlamaktır. Yenilebilir film ağırlıkça %8 mercimekten elde edilmiş protein, protein ağırlığının %15'i kadar nar kabuğu, stabilizatör olarak ağırlıkça %2 oranında pektin ve plastikleştirici olarak ağırlıkça %0.5 oranında gliserolden oluşmuştur. Kaplama uygulaması bandırma metoduyla yapılmıştır. Sonrasında, kaplanmış olan yenilebilir bar 40ºC'de 16 saat boyunca kurutulmuştur. Kaplanmış ve kaplanmamış barlar %30 bağıl nemde 25ºC ve %75 bağıl nemde 35ºC olarak farklı koşullarda 90 gün süresince değerlendirilmiştir. Optik özellikler, bazı tekstür özellikleri, toplam fenolik içerik, duyusal, mikrobiyal içerik ve su aktivitesi ölçüm analizleri yapılarak yenilebilir filmin bara olan etkisi değerlendirilmiştir. Sonuçlar, kaplanmış barın renk, mikrobiyal üreme, bazı tekstür özellikleri, su ve fenolik kaybının kaplı olmayan bara göre her iki ortam koşulunda daha iyi olduğunu göstermiştir.

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CHAPTER 1

INTRODUCTION

1.1. Snack

As said snack food, it comes to mind unhealthy food that are energy dense, nutrient poor, rich content of sodium, sugar and fat (Lipoeto et al., 2013). The definition of snack varies depend on individuals. When some people describe snack is separated specific meals from the breakfast, lunch and dinner, others think that eating alone, short eating periods, small portion of packaged, inexpensive, nutrient poor food (Wansink et al., 2010). Salty snacks, chips (Piernas & Popkin, 2010), crackers, popcorns (Mercille et al., 2010), chocolate (Elena & Maria, 2006), cookies (Bellisle et al., 2003), cakes desserts, candy (Wang et al., 2012) and sweetened beverages (Duffey et al., 2013) are identified as popular snack by the consumers in United States (Wang et al., 2012), Canada (Mercille et al., 2010), Greece (Elena & Maria, 2006). Besides this, sweety grain-based products and sweets described as snack in Mexico (Duffey & Pompkin, 2014), Brazil (Ministry of Health of Brazil, 2014), China (Wang et al., 2009), Oman (Musaiger, 1994), Finland (Ovaskainen et al., 2010) and France (Ancellin et al., 2011).

Snack foods cannot be considered as unhealthy food completely, they have health promoting alternatives food as well. Some health benefits of snacking are controlling of body weight (Debry, 1978), regulating of eating habits (Bellisle et al, 1997), reducing of cholesterol and improving of glucose intolerance (Arnold et al., 1993). The snacks are chosen as a healthy alternative to prevent from the disease, increase in nutritional value and meet consumer requirements (Constantin and Istrati, 2018). In some countries such as Australia (Avustralian Government, 2013), Brazilia (Ministry of Health of Brazil, 2014), Sweeden (Livsmedelsverket, 2015), Greenland (Jeppesen et al., 2011), Switzerland (Suisse Balance, 2012) and France (Ancellin et al., 2011), fruits, vegetables, legumes, grain, nuts, milk, yogurt and seeds are placed among snack food group (Hess etc., 2016).

In today's world, people tend to prepare easily and eat fast foods due to intense working condition and sedentary lifestyle. Snack is the substantial food products instead of traditional diet. Even if it is quick meal to consume, it may substitute in terms of

nutritional value. Snacks has an impact to improve intake of total energy, nutrient (Bellisle et al., 2003). To have long shelf-life duration is another reason for their preference (Constantin and Istrati, 2018).

1.2. Snack Bars

Snack bar is a member of the snack food class. Snack bars can be defined as healthy food product by the consumers who has increased awareness of health and diet (Bower & Whitten, 2000). Snack bars including functional alteration are considered as functional food product. It is also attractive for natural, nutritious ready to eat product and beneficial products from the functional food category. Snack bars can be made up of cereals, fruits and nuts, which is considered as the source of protein, fiber, vitamins and minerals. Thus, they can deliver healthy nutrients, bioactive compounds and dietary fibers into the body (Constantin and Istrati, 2018).

Both the easy portability, storage and health benefits properties of snack bars make more attractive for the consumption as a meal. Moreover, less processed and more nutritious food products become trend in recent years. Some type of snack bars enables to provide energy and micronutrients (Aramouni & Abu-Ghoush, 2018). Snack bars are generally safe, nutritious, palatable, easy to use and distribute (Sheibani et al., 2018). Snack bars are usually consumed to satisfy the need for sweets as a desert meal, save time, using as energy source, using for weight loss, using for protein, fiber and vitamin contents (International Markets Bureau, 2013). Many people prefer to consume cereal bars containing chocolate, but whole grain cereal bars which contains rich sugar, fat, fiber are healthier than these (Boustani and Mitchell, 1990).

Snack bars can be classified as various groups depend on nutrition value, being organic, health and wellness, energetic and nutraceutical bars (Sharanya & Penchalaraju, 2016).

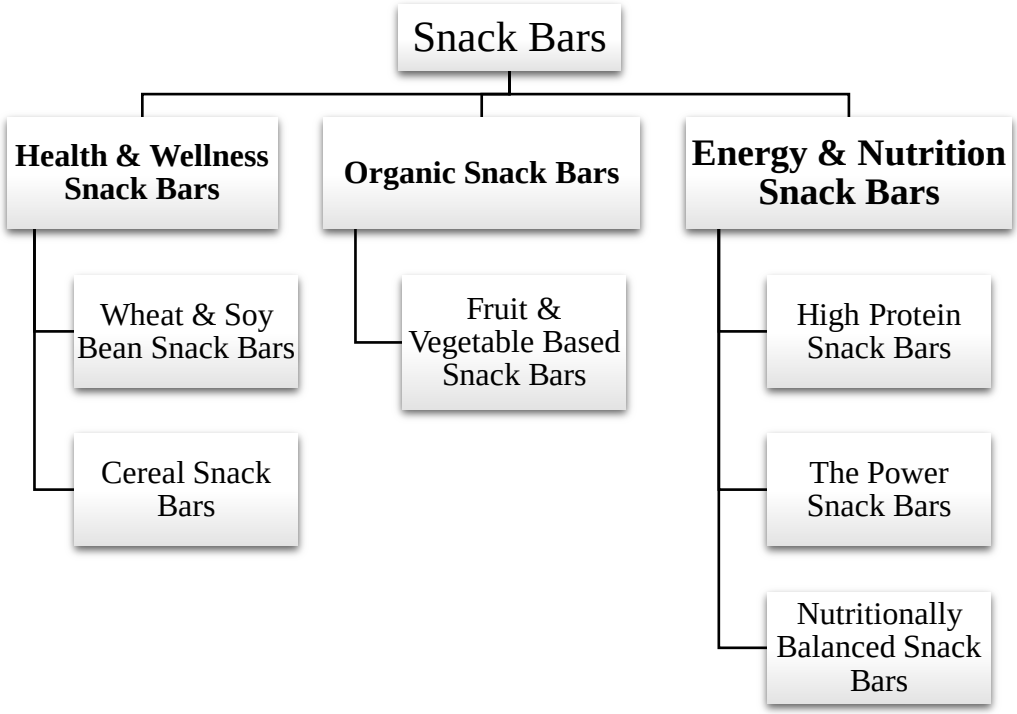

Figure 1: The type of the snack bars (Sharanya & Penchalaraju, 2016)

1.3. Type of Snack Bars

1.3.1. Health and Wellness Snack Bars

1.3.1.1. Wheat and Soy Snack Bars

As a nutritional bar, it provides nutrients the sportive consumers due to its fortified protein, indigestible carbohydrate, fiber, B-complex vitamin content. Wheat and soy bars have an advantage of giving necessary nutrients by fast consuming (Aramouni & Abu-Ghoush, 2011). Moreover, because of its rich source of carbohydrate and fiber, it can prevent some type of cancer (Deen and Margo, 2007).

1.3.1.2. Cereal Bars

Cereals are well-combined food by mixing with a variety ingredient depending on target consumer group (Bower & Whitten, 2000). It makes possible to consume functional food by increasing of the cereal consumption out of the breakfast. The target population

comprises of consumer to eat healthier food and careful to maintain body fitness (Lin et al., 2010). It consists of vitamin A, thiamine, riboflavin, niacin, vitamin B6, vitamin B12, protein, vitamin E, calcium, iron, magnesium, phosphorus, zinc, copper (Nutrition data, 2018) and dietary fiber, vitamin C, minerals and antioxidant activity (Lin et al., 2010). Furthermore, it has a healthy effect on lowering of cholesterol (Ho et al., 2016).

1.3.2. Organic Snack Bars

1.3.2.1. Fruit & Vegetable based Snack Bar

Fruit and vegetable-based snack bars has rich content of polyphenols, dietay fiber (Ferreira et al., 2015), resistant starch, low content of fat and natural antioxidant (Ramirez-Jiménez et al., 2018), protein, minerals and vitamins (Sun-Waterhouse et al., 2011). As a vitamin and minerals, it includes vitamin C (Silva et al., 2014), vitamin D, vitamin B12, potassium, calcium, iron, folic acid and thiamin which provides health benefits. According to recent studies, regular consumption of fruit and vegetables can reduce the risk of chronic disease (Pepsico India, 2015), cancer, obesity, coronary heart disease (Jeanine et al., 2004).

1.3.3. Energy & Nutrition Snack Bars

1.3.3.1. High-Protein Snack Bars

For people engaging in sport activities as a dieting meal, high protein snack bars are good alternative. It includes high content of protein in 15–35% w/w ratio that is sufficient to maintain their caloric needs. The nutritional value is low carbohydrate and low fat (Hogan et al., 2012). The bar can be composed of protein, calcium, iron, magnesium, zinc, copper, manganese, vitamin C, vitamin E, thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid (Nutrition data, 2018), α-lactalbumin, β-lactoglobulin, arginine and glutamine (Malecki et al.,2020). In addition, it has a positive effect to improve post meal and glucose profile in patients with Type 2 diabetes (Gannon et al., 2003).

1.3.3.2. The Power Bars

The power bars are also called as emergency food product (Zoumas et al., 2002). These types of bars are nutritious and consumed to take quick energy source for people doing high physical activity. They boost the energy that can be substitute on a meal with its rich nourished content (Gill & Singh, 2002). It is good source of vitamin C, thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, and manganese (Nutrition data, 2018).

1.3.3.3. Nutritionally Balanced Bars

Nutritionally balanced bars include all main component in other words protein, carbohydrate and fat as an ingredient. It is ideal for consumer to nourish healthy and balanced. An example of this group, it contains 40% protein, 30 % carbohydrate, 30 % fat (Gonzalez & Draganchuk, 2003). They are appropriate to provide energy and micronutrients for the consumers (Aramouni & Abu-Ghoush, 2011).

1.4. Benefits of Snack Bars

The snack bars that consist of good nutritional value can have a significant role in physical and mental health of children, teenager and adults thanks to its ingredients. They may contain fibre, iron, low saturated fat, high protein content, phytochemical, antioxidant and phenolic compound (Larson, 2006), vitamins and minerals (Constantin & Istrati, 2018).

1.4.1. Fibers

The fiber that is found in the bar largely has many positive effects on human health. The fibers are used in bar as a non-caloric partial replacement for fat and sugar to incorporate with functional properties (Spotti and Campanella, 2017). It has a role in prevention of some cancer types (Aramouni & Abu-Ghoush, 2011), obesity, diabetes (Spotti and Campanella, 2017) and cardiovascular disease (Joshipura et al., 2001). It has a positive impact on digestive health and energy balance (Champ et al., 2003).

1.4.2. Essential Fatty Acids

Essential fatty acids are founded in snack bars that contain crop seed and vegetable oils (Saini & Keum, 2018). Even if essential fatty acid is source of high calories, it acts as a biomembrane construction material and vehicle of fat-soluble vitamins (Hansen, 1994). The omega-3 and omega-6 have an important role on human health (Rodriguez et al., 2010). They have a role in the body with antioxidant value by cardiovascular (Dupasquier et al., 2006), anti-arrhytmic (Ander et al., 2004), anti-atherogenic, anti-inflammatory (Dupasquier et al., 2007) and anti-carcinogenic function.

1.4.3. Proteins

Protein sources of the snack bars are usually plant and animals that are egg, soy, meat, fish, milk, whey etc. (Dullius et al., 2018). Proteins might be contained in both protein-based snack bars and other types. There are many beneficial effects to the human body. It has a role as a bioactive factor by modulation of immunity, growth and tissue support, metabolic activity (Shang et al., 2018), energy balance, digestive health (Champ et al., 2003), blood pressure (Lin et al., 2010), anabolic response (Tieland et al., 2012), fat and glucose metabolisms (Wolfe, 2015). It supports the body by improving of muscle strength, physical function (Hartmann & Meisel, 2007), bone health and repairment of tissue (Shang & Chaplot, 2018). Also, the studies show that high content of protein in snack bar that is consumed as post meal enhance diurnal glucose profile in type 2 diabetic and insulin resistant patient (William et al., 2006). It has an impact in inhibition of several disease that are cancer, cardiovascular disease, diabetes (Champ et al., 2003).

1.4.4. Minerals

Minerals that are known as sulfur, iron, copper, zinc, calcium, magnesium are another nutritional content of snack bar (Constantin & Istrati, 2018). Mineral intake is important because it cannot be synthesized in the body, which must be provided by other food suppliers (Rosell, 2007). It participates many important activities of the body. It can activate or inactivate the calcium and magnesium in enzyme structure to functionalize the enzymes (Hathcock, 1997). Diabetes, cardiovascular disease, hypertension, osteoporosis

are the diseases that are reduced the risk by the minerals. For osteoporosis, calcium provides the higher bone mass particularly. It has an effect to control blood pressure, weight and to improve immunity (Ryan-Harshman & Aldoori, 2005).

1.4.5. Vitamins

The other essential food supply enabled by vitamins in snack bar. Snack bars include vitamin A, vitamin B1, vitamin B2, vitamin B5, vitamin B3, vitamin B6, vitamin B9, vitamin B12, vitamin C and vitamin E (Combs & McClung, 2016). They are needed to occur many metabolic functions. These various vitamins can be supplied from cereals, nut, grains, dairy products, oat, soybean, peas, green leaves, fruits, vegetable oils, citrus fruits and vegetables (Constantin & Istrati, 2018). It has a function to enhance digestion, growth, fertility (Gould, 1995), nucleic acid formation (Mahmood, 2014), oxidation-reduction reactions, amino acid and protein metabolism (Saghiri et al., 2017) and maintain nervous tissue (Martel & Franklin, 2017). It enables to protect from obesity, metabolic disorder (Sung et al., 2014), cardiovascular disease, cancer (Kushi et al., 1996) and respiratory distress (Combs & McClung, 2016).

1.5. Functional Food

Functional foods can be defined as food has altered content other than the basic nutrition. This alteration could be distinction or addition of one content beyond their current nutritional value to improve health benefits. Moreover, food with enriched nutritional content and food that is manufactured with modified recipe formula are classified in this class. The benefits may be involved in a certain specific group or all individual person. Prevention from risk of certain disease, decreasing of recruitment time and aging effect, defending of the body (Constantin and Istrati, 2018), promotion of growth, development and enhancement of performance, improvement of children life quality by supporting of learning capability (Sharanya & Penchalaraju, 2016), regulation of energy balance and body weight, defense against oxidative stress, improvement the general physical state and intestinal function (Howlett, 2008) are placed on the benefits of the functional foods. Example of these beneficial effects are reduction of LDL cholesterol effect, prevention of cardiovascular disease (Mishra and Geetha 2009),

improvement of the regular stomach and colon function, anti-carcinogenic effect. Gluten or lactose free products are also known as functional food for the sensitive people (Ma kinen- Aakula, 2006). Besides this, addition of bioactive components such as vitamin, omega 3, mineral and antioxidant make the food functional by rising the consumption of substances (Menrad, 2003).

There are many examples for functional food in various methods and health benefits. Some of them could be found in table below.

Table 1: List of functional food and their health benefits depend on type

Functionality Type	Functional Food	Health Benefits
Fortification	Juices added calcium	Reduces hypertension and osteoporosis (Kotilainen et al., 2006)
Fortification	Eggs enriched with higher omega 3	Decreases heart diseases, birth defects (Kotilainen et al., 2006)
Fortification	Beverage fortified with antioxidants	Support the overall health (Kotilainen et al., 2006)
Fortification	Milk and fruit bar enhanced phytosterol	Support the overall health (Kotilainen et al., 2006) and overall health claim (FDA)
Fortification	Folate enriched foods	Neural tube defects (FDA)
Fortification	High iron or vitamin rice	Provide growth and development (Niba, 2003)
Substitute the ingredient	Sweetened chewing gum with xylitol instead of sugar	Prevention of dental organs (Kotilainen et al., 2006)
A component in whole food	Soluble fiber and beta glucan in oats	Reduces cholesterol, risk of some cancer types and coronary heart disease (Kotilainen et al., 2006)
A component in whole food	Isoflavone in soy protein	Protection of bone heath, improving of cholesterol levels (Ohama et al., 2006)

(cont. on next page)

Table 1 (cont.)

A component in whole food	Beta carotene in orange colored fruit and vegetables	Neutralizes free radicals (Sharanya & Penchalaraju, 2016)
A component in whole food	Lutein, Zeaxanthin in spinach, broccoli, egg and citrus fruits	Protects the eye health (Sharanya & Penchalaraju, 2016)
A component in whole food	Lycopene in tomato, grapefruits, watermelon	Supports prostate health (Sharanya & Penchalaraju, 2016)
A component in whole food	Insoluble fiber in fruit skins, bran of wheat	Maintain the digestive health, reduce some cancer risk (Sharanya & Penchalaraju, 2016)
A component in whole food	Sulforaphane in broccoli, cabbage, cauliflower	Enhance detoxification of undesirable substances (Sharanya & Penchalaraju, 2016)
A component in whole food	Anthocyanin in red fruits	Maintain brain function (Sharanya & Penchalaraju, 2016)
Whole Food	Fruit and Vegetables	Lower risk of cancer (Kotilainen et al., 2006) and heart disease (FDA)
Whole Food	Soy Protein	Prevent coronary heart disease (FDA)

1.6. Edible Coating

Functionalization of the snack bar is made by coating of the edible film in this study. There are many preservation and packing techniques remained in food sector to preserve food in longer shelf life by ensuring the quality (Abbas & Abdul-Rahman, 2020). The preservation means protection from the environment during food production,

distribution, marketing and storage against physical, chemical and biological effect. It enables the product to reach the consumer without changes in sensory characteristics (Selcuk et al., 2017). It also provides the necessary information and attractivity for the consumption (Diaz-Montes & Castro-Munoz, 2021). Necessary information is about daily value intake, ingredients, warnings, production company and other information. Some properties make the attractive the food to be bought from the stores by the consumer, which is lightness, appearance, design, easy opening, biodegradable and eco-friendly plastic etc. Even if traditional packaging provides longer shelf life and attractivity, it has a negative effect in some aspect. The material of this type of packing which are paper, plastic-polymer material and aluminum has risk of chemical migration into the food, which is harmful for human health (Altuntaş, 2014). Also, synthetic packaging materials are not desired by the customers resulted from environmental concern (Hollingworth et al., 2010). People have concern about environmental pollution due to excessive usage of plastic (Soo&Sarbon, 2018) without biodegradable material (Ertugay & Sallan, 2011). People must use renewable active packaging system that is environment friendly (Moghadam et al., 2020). Thus, packaging methods are developed to meet requirement in developing world.

Edible coating is an alternative packaging technique as a new improvement for the food packaging which has many benefits. Edible coating is defined as thin layers placed on surface of food and made up of natural biopolymers (Sadrabat, 2013). It is environment friendly resulted from quickly decomposing properties as biodegradable packing materials that is renewable source (Andrade-Mahecha et al., 2012) and enhances the organoleptic properties-transparency, color, roughness, shininess, stickiness (Valdés et al., 2017). Also, there is no chemical migration risk different from the traditional packaging (Bourtoom, 2008). Besides, it has a positive effect in food to maintain quality, delay ripening, prevent weight loss and prolong the shelf life (Yıldız & Yangınlar, 2016). These are enabled by protection of antioxidant and vitamin components (Pagliarulo et al., 2016), protecting aroma, flavor by protecting color by controlling migration of water soluble solutes (Zhao & Mc Danial, 2005), reducing the browning reactions (Guerreiro et al., 2017) and UV-light protection (Debeaufort et al., 1998), being barrier against mechanical damage (Guimarães et al., 2018), moisture, oxygen, carbohydrate, solute movement (Guilbert, 1986), prevention spoilage changes include unpleasant odors, rancidity, darkening, softening of the texture (Diaz-Montes & Castro-Munoz, 2021), reduction of respiration rate, ethylene production (Ali et al., 2011), lipid peroxidation

(Kumar et al., 2021) and protection of ascorbic acid, total phenolic, flavonoid content and antioxidant activity (Nair et al., 2018).

Moreover, it can be used as functionalization method for the food by supporting or addition of nutritional values. They can carry nutraceutical agents such as anti-browning agent, colorants, flavors (Tharanathan, 2003), antioxidant, antimicrobial agents (Jouki et al., 2014) and high concentration of mineral and vitamins. Because of these agents, edible coating has an impact on reduction of microbial load, enrichment of the food with additive of coating. By releasing active compound, absorption of some components to accelerate deterioration like free radicals, moisture, oxygen (Wrona et al., 2015).

The edible film makes different in sensory parameters of food product. The coating contributes to appearance of food. Whitening, waxiness, shininess and discoloration are observed in edible film applied product. Reason of effect is the oxygen barrier by preventing enzymatic browning and Maillard reaction. Because most active agents give unique properties to the food, when they have interacted with oxygen, their specified flavor, color and aroma can be protected by edible film. The textural quality can affect firmness and crispiness by reducing moisture loss and delaying the ripening process. It also improves the mechanical quality. The edible coating can have an impact even during freezing process and storage to hold liquid and moisture migration (Zhao & Mc Danial, 2005). Edible films that are fruit based have an effect to reduce contamination, undesirable results and improve nutritional value and shelf life (Kumar, 2019).

Therefore, edible films can enhance food stability, quality, functionality and safety. So, the new trend is to use natural polymer as a packaging material instead of synthetic polymers (Shojaee-Aliabadi et al.,2014). The property of the ideal film is being safe to consume, invisible appearance, off-flavor taste, desirable moisture, low water solubility, high thermal stability and gas barrier and having adequate mechanical strength (Zhao & Mc Danial, 2005).

1.6.1. Components of Edible Films

Edible films can be formed by different components. Edible film contents e.g. biopolymers and other additives are dispersed in aqueous media (Bourtoom, 2008).

1.6.1.1. Protein

Protein inside the edible coating has an effect bonding with different positions (Duran, 2013). Proteins can associate with each other closely in parallel dimension by the hydrogen bonding (Bourtoom, 2008). Mechanical properties are highly advanced and increase nutritional value of product when protein is used in content of the film. Because of its hydrophilicity, the film has a weak water barrier but a good gas barrier property (Bourtoom, 2009). As a protein sources, whey protein, casein, gelatin, egg albumin, corn, soy, wheat, rice and lentil could be used (Mellinas et al., 2015). To utilize protein in edible coatings, they usually denaturated by heat, acid, base or solvent (Bourtoom, 2008). The film based on protein has better mechanical and gas barrier properties due to high nutritional value (Gontard & Guilbert, 1994).

1.6.1.2. Polysaccharide

Polysaccharide in the edible coating increases water vapor permeability due to its hydrophilicity, even so the gas permeability is low (Duran, 2013). It is reported that polysaccharides ideal to use as an ingredient of the film as they are nontoxic, antioxidant, antimicrobial, antifungal and nutritional value (Kumar, 2019). Also, it makes the film good gas barrier, excellent transparency and mechanical strength properties. Polysaccharides are used as thickening, gelling, stabilizer agent and encapsulating agent in film (Stephen and Churms, 2006). It can create solid structure in polymer matrix. It can enable to make the film is flexible and tough (Hall, 2012). The source of this component is plant in the ecosystem (Kumar, 2019). Cellulose, starch, pectin, seaweed extracts, pullulan, chitosan, gums, alginate are the additive substance incorporated to edible films (Mellinas et al., 2015).

1.6.1.3. Lipid

Lipid as an ingredient of the film indicates good barrier for the water vapor because of its high hydrophobic and low polarity characteristics. They do not show good bonding properties with each other. Also, it improves appearance properties by giving brightness (Duran, 2013). It forms thicker and more brittle films (Debeaufort et al., 1993).

Lipids used in making an edible film are coconut, peanut, palm, cocoa, butter oils, mint, citrus fruit, lecithin, fatty acids (Mellinas et al., 2015). Most of lipid type used in films are convenient to make polymer structure, that film has good mechanical properties (Bourtoom, 2008).

1.6.1.4. Composite Films

Composite films consisting at least two components meet the expected functionality of the film, which one component could not provide. The functionality is dependent on designing or modification of process and conditions (Okcu et al., 2018). Carbohydrate and protein composite films show good properties for oxygen barrier due to packed hydrogen bonded structure (Bonilla et al, 2012). Lipid and polysaccharide composite films have lower gas blocking and physical properties (Wittaya, 2012).

1.6.1.5. Plasticizer

Plasticizers can be used to decrease intermolecular force and melting temperature in mixture, which provides higher toughness, elongation and flexibility to the film (Valencia et al., 2011). It is used to modify mechanical properties by developing the flexibility with softening the film structure and reducing the cohesion inside the film network. It can associate with the polymer physicochemically, so it can enter the intermolecular chains of the polymer (Maran et al., 2013). If it is absent, the edible film become too brittle and fragileness. When the concentration of plasticizer is higher, mechanical strength of film gets weakened by causing the phase separation (Ramirez-Jiménez et al., 2012). The main plasticizers are glycerol, aloe, resins, sorbitol, polyethylene glycol and sucrose (Quezada-Gallo, 2009). It could affect the flavor and taste of films and the coated product. When the polyethylene glycol has no taste, glycerol has sweet taste.

1.6.1.6. Emulsifiers

Emulsifiers or surfactants might be used as surface-active agent with their amphiphilic nature to interact water-lipid interface and decrease the surface tension, so

the emulsion has higher stability (Han and Gennadios, 2005). It could be used in water soluble films that does not show good water barrier properties. Also, if there is heterogeneous particles in hydrophobic and hydrophilic substances, surfactants could be used to stabilize the surface as a dispersed phase. It enables to make better interaction between food and coating in surface. As a food emulsifier, derivatives of glycerol, fatty acids and polyethylene sorbitan derivatives could be used in film making (Quezada-Gallo, 2009).

1.6.2. Application Method of Edible Films

Difference in edible coating is in liquid form and applied to the product by plugging into solution while edible film is like a sheet in solid form and applied to the product by wrapping (Falguera et al., 2011). These foods can be consumed with or without removal of the edible film or coating (Pavlath & Orts, 2009).

1.6.2.1. Dipping Method

It is made by dipping the food into the coating solution, then the edible coating in the surface of product is dried at room conditions or dryer (Brody & Marsh, 1997). Density, viscosity, surface tension of the film solution are the parameters to specify film thickness (Tavassoli-Kafkani et al., 2014). It is suitable for meat, fish, chicken, fruit and vegetable coating (Lu et al., 2010).

1.6.2.2. Spraying method

It is applied by spraying of liquid form of coating by the high-pressure spray or air blast system to the surface of product (Isık et al., 2013). In this method, coating droplets distributed homogenously in the surface (Badıllı and Tarımcı, 2009). Control of uniformity, thickness, application of multi-layer to the surface of product could be arranged with this technique (Martin-Belloso et al.,2009). Thinner film could be produced because coating is sprayed from one side. This technique is dependent to temperature control, which could result in loss of volatile compounds (Ramos et al., 2012). For this method, film solution having low viscosity should be preferred (Dhanapal et al., 2012).

1.6.2.3. Dripping (Spreading) method

Coating solution is applied by the drops to the moving cylinder and coated the food by the rotating brush and drying with fans, which is called also casting. Therefore, spreading and thickness can be controlled into product surface. Viscosity is major property for this technique whether the coating solution is spread easily or not (Sikalo et al., 2002). This method is appropriate for polysaccharide-protein based films (Mendez-Vilas, 2013).

1.6.2.4. Foaming method

This method is used for emulsion coatings. The foaming agent poured to coating and foamed by compressed air. The emulsion is distributed by brushes in surface of the product. To apply the emulsion uniformly, smooth surfaces should be chosen for this application (Grant & Burns, 1994).

1.6.2.5. Wrapping method

The edible film is poured into petri-plate in requested amount and dried. After that, the film is wrapped into surface of the product. In this method, thickness can be controlled. However, the film should be incorporated into surface of product after wrapping.

1.6.3. Example of Edible coating

There are many application studies of edible coating in various foods that are fruit and vegetables as a plant based, cheese, butter, chicken, fish as an animal base and bread etc. as a bakery product. Some of the examples are found in the table below.

Table 2: List of film substance with the coated material and their health benefits

Coated Food	Film Substance	Benefits	References
Fruit, nut, grain, vegetable	Wheat gluten, whey protein, corn zein, waxes, cellulose derivatives, pectin	O_2, lipid and moisture barrier, antioxidant carrier, binding of salt, reduction of stickiness	(Gennadios& Weller, 1990)
Fresh cut jackfruit	Xanthan, alginate, gellan gum, glycerol	Inhibits microbial growth, increase shelf life	(Vargas-Torres et al., 2017)
Pomegranate arils	Ascorbic acid and chitosan mixture	Protect color, aroma, flavor, prevent microbial growth	(Ozdemir & Gokmen, 2017)
Apples and strawberry	Chitosan films and olive oil waste	Reduce microbial load, inhibits spoiling	(Khalifa et al. 2016)
Kiwi	Cactus pear mucilage, glycerol and tween 20	Maintain quality, flavor, extend shelf life	(Allegra et al., 2016)
Strawberries	Chitosan, acetic acid, canola, cinnamon and roselle extract	Increase antioxidant capacity, shelf life	(Ventura - Aguilar et al., 2018)
Strawberries	Chitosan, beeswax, glycerol, tween 80	Preserve the quality and increase shelf life	(Velickova et al., 2013)
Strawberries	Chitosan, carotene, glycerol and polyvinyl alcohol	Control microbial increase, keep antioxidant activity	(Hajji et al., 2018)
Strawberries	Chitosan, glycerol, acetic acid, propolis extract	Enhance the phenolic content, flavonoid, antioxidant, prevent spoilage and sensory properties	(Martinez-Gonzalez et al., 2020)
Strawberries	Fish gelatin, citrus pectin, glycerol, glycol	Increase shelf life, delay mold growth	(Bermudez-Oria et al., 2017)
Strawberries	Cassava starch, propolis extract	Promotion of vitamin C content	(Thomas et al., 2016)

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Table 2 (cont.)

Fresh cut apple, potato and carrot	Whey protein, pectin and transglutaminase	Inhibit growth of bacteria, protects texture as hardness and chewiness	(Marquez et al., 2016)
Fresh cut apple	Whey protein, glycerol, citric acid	Increase shelf life	(Azevedo et al., 2018)
Fresh cut apple	Carboxymethyl cellulose, glycerol, calcium and ascorbic acid	Maintain vitamin C, antioxidant	(Koushesh & Sogvar, 2016)
Fresh cut apple	Chitosan	Enhance quality	(Shaei et al., 2016)
Fresh cut apple	Alginate, gellan gum, pectin, glycerol, ascorbic acid, inulin	Improve quality and shelf life	(Moreira et al., 2015)
Fresh cut apple	Chocolate, milk butter, glycerol, ascorbic acid	Produce anti-aging properties	(Khan et al., 2014)
Fresh cut apple	Olive oil, sunflower oil, lecithin, ascorbic acid	Produce anti-aging properties	(Khan et al., 2014)
Fresh cut apple	whey protein, soy protein, alginate, carrageenan, glycerol	Control physical change, extend shelf life	(Ghavidel et al., 2013)
Fresh cut apple	Cassava starch, carnauba wax, glycerol, stearic acid	Improve physicochemical properties	(Chiumarelli & Hubinger, 2012)
Fresh cut apple	Soybean gum, jojoba, arabic gum, glycerol and paraffin oil	Maintain quality	(El-anany et al., 2009)
Papaya	Papaya puree, alginate, carrageenan, glycerol and citric acid	Extend the shelf life by delaying ripening	(Hamzah et al., 2013)
Strawberry and plum	Cherry tree resin	Extension of shelf life, keep aroma, appearance	(Ergin, 2015)
Nectarine	Sucrose ester, aloe vera and lecithin	Prevent loss of weight, spoilage by bacteria and fungi, phenolic compound	(Ornek, 2015)

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Table 2 (cont.)

Fresh cut nectarine	Sodium alginate	Protect hardness, delay browning, reduce growth of mold and yeast	(Chiabrando & Giacalone, 2016)
Fresh cut pineapple	Alginate, glycerol, sunflower oil, lemongrass oil, citric acid, ascorbic acid	Enhance shelf life and quality	(Azarakhsh et al., 2014)
Fresh cut mangoes	Alginate, glycerol, sunflower oil, calcium chloride, ascorbic acid, citric acid	Delay browning, extend shelf life	(Robles-Sanchez et al., 2013)
Fresh cut watermelon	Alginate, pectin, calcium lactate, glycerol	Preserve texture, increase shelf life	(Sipahi et al., 2013)
Fig	Polylactic acid	Prolong shelf life, preserve nutraceutical effects	(Palma et al., 2015)
Fig	Chitosan, acetic acid, canola, cinnamon and roselle oil	Preserve the antioxidant capacity, prevent color change and microbial growth	(Contreras Saavedra et al., 2020)
Blueberries	Alginate, chitosan, calcium caseinate, fruit fiber, glycerol, inulin, oligofructose,	Extension of shelf life, protect sensory properties	(Alvarez et al., 2018)
Blueberries	Alginate, chitosan, calcium caseinate,	Maintain flavor, texture, appearance, delay the ripening	(Duan et al., 2011)
Red grape	Gelatin, corn starch, waxy maize starch, glycerol, sorbitol	Improve shelf life and quality	(Fakhouri et al., 2015)
Bell pepper	Chitosan, acetic acid, canola oil, glycerol	Preserve flavonoids, antioxidant, delay microbial growth	(Hernandez-Lopez et al., 2020)
Tomato	Aloe vera gel	Prevent mold growth, increase antioxidant activity	(Chrysargyris et al., 2016)
Tomato	Citrus peel pectin, glycerol, oregano oil	Inhibit the mold growth, protect phenolic and antioxidant activity	(Rodriguez-Garcia et al., 2016)

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Table 2 (cont.)

Tomato	Carnauba wax, mineral oil	Increase antioxidant activity	(Davila et al., 2014)
Tomato	Chitosan, zeolite, Tween 80, lactic acid	Delay the ripening	(Garcia et al., 2014)
Tomato	Soy protein, cellulose oleic acid, glycerol, ascorbic acid, sodium benzoate	Improve physical characteristic and shelf life	(Nandane & Jain, 2011)
Tomato	Cellulose, beeswax, glycerol, Tween 80, oleic acid	Protect physical appearance, inhibit growth of the fungi	(Fagundes et al., 2015)
Potatoes	Locust bean gum, glycerol	Prevent microbial growth, preserve nutritional quality	(Licciardello et al., 2018)
Coated fresh cut carrots	Carrot mash, chitosan, corn starch, gelatin, glycerol and cinnamaldehyde	Delay ripening, protect carotenoids	(Wang et al., 2015)
Broccoli, carrot, cauliflower, zucchini, celery, carrot and chayote	Low methoxylated pectin, wax, glycerol, ascorbic acid	Protect sensory properties	(Hernandez et al., 2014)
Broccoli	Methyl cellulose, polycaprolactone, alginate, glycerol, tween 80, organic acids, essential oil of Italian and Asian spice	Control the microbial growth	(Takala et al., 2013)
Spinach	Agar, K-Carrageenan, konjac, glycerol	Increase the shelf life	(Rhim & Wang, 2013)
Asparagus	Cellulose, whey protein isolate, pullulan, glycerol, sorbitol, stearic acid	Decrease weight loss, maintain quality	(Tzoumaki et al., 2009)
Mushroom	Chitosan, glucose	Protect hardness and ascorbic acid, reduce respiration rate and microbial load	(Jiang et al., 2012)
Mushroom	Malic acid, citric acid and gum arabic	Loss of weight and hardness	(Sedaghat & Zahedi, 2012)

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Table 2 (cont.)

Mushroom	Alginate, silver nitrate, sodium borohydride, polyvinylpyrrolidone	Extend the shelf life	(Jiang et al., 2013)
Onion	Sodium Alginate	Loss of weight	(Rozo et al., 2016)
Poultry, meat, fish	Gelatin, carrageenan, alginate, whey protein, collagen, casein, cellulose derivatives	Prevent mold formation, O_2, lipid and moisture barrier, antioxidant carrier, abuse protection, texture improvement	(Kester & Fennema, 1986)
Sausages	Maltodextrin, alginate, cellulose, glycerol, Terminalia arjuna	Extend the shelf life	(Kalem et al., 2018)
Sausages	Gelatin, carrageenan, glycerol, lard, beeswax	Reduce the weight loss	(Tyburcy & Kozyra, 2010)
Chicken meat	Mango peel powder, cyclodextrin, gelatin, glycerol, polyvinyl alcohol	Prolong shelf life	(Kanatt & Chawla, 2017)
Chicken meat	Gum Arabic, sorbitol, polyvinyl alcohol, plant extract	Enhance bioactive compound and shelf life	(Muppalla & Chawla, 2018)
Chicken meat	Linear low-density polyethylene, cinnamon oil, silver-copper	Extend the shelf life, increase antimicrobial capacity	(Ahmet et al., 2018)
Butter	Low density polyethylene, yerba mate, carotenoid extract	Higher antimicrobial and antioxidant content, increase shelf life	(Moura et al., 2018)
Ham slices	Cassava starch, chitosan, gallic acid, glycerol	Extend shelf life	(Zhao et al., 2018)
Fresh chicken breast	K-Carrageenan, chitosan, glycerol, oriental mustard extract	Reduce growth of microbes and enhance the shelf life	(Olaimat et al., 2014)

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Table 2 (cont.)

Cheese	Galactomannan, chitosan, glycerol, sorbitol, corn oil	Prolong shelf life	(Cerqueira et al., 2010)
Confections	Corn zein, milk and whey proteins, wax	O_2, lipid and moisture barrier, antioxidant carrier	(Debeaufort et al., 1998)
Heterogenous food (Paste, puree, cake, ice cream cones)	MC and palmitic acid composition	Moisture barrier	(Krochta & De Mulder-Johnson, 1997)
Bread	Pectin, alginate, whey protein, glycerol, tween 20	Decrease moisture	(Nallan et al., 2019)
Bread	Starch, glycerol, L-lysine	Extend the shelf life	(Luz et al., 2018)

1.6.4. Functionality of the Edible Films

The edible films can be used to improve quality, shelf life, safety and functionality. Functionality can be applied by coating the food material. Depend on its content, it can make the food functional with antioxidant, antifungal, antimicrobial agents. Also, it can enhance the flavor, color or appearance of the food. The material will be enriched or gained new benefits with coating.

1.6.4.1. Carrying of the Flavor and Colorant

Edible films can be used to give desired flavor or color. Because of the coating material, the taste of the substance might be spicy, sweet, salty or astringent. As an example, pullulan-based films can be placed in this group as it has active compound to stabilize flavor and color. Natural color and flavor also have antioxidant and antimicrobial effect, which gives functionality to the coating and coated product (Ricardo et al., 2018). Flavonoids are very sensitive substances in terms of volatility that are susceptible to loss (Ozdemir et al., 2018).

There are many products that are available in USA markets that are bright orange, cucumber wrapped with carrot-based film, deep red tomato, tuna coated with basil-based

wrap, creamy cheesecake wrapped with strawberry or blueberry, roasted pork coated with pineapple, ginger and apricot based coat, carrot, onion and asparagus encircled by broccoli-based wrap and snack crackers that are wrapped by fruit and vegetable films (The US Department of Agriculture, & Agricultural Research Service, n.d.).

1.6.4.2. Antioxidative Effect

Thanks to antioxidant compound addition to the edible film, the consumers have health benefit from the coated product even if it contains small amounts of compound (Kris-Etherton et al., 2002). These compounds are antioxidant (Valencia et al., 2020), flavonoid (Peterson & Dwyer, 1998), anti-mutagenic, anti-inflammatory (Bravo, 1998), anti-cancer (Diaz-Montes et al., 2020) and anti-cholesterol compounds (Bravo, 1998).

Phenolic compounds are usually extracted from the plant sources (Assis et al., 2018). Citric acid, ascorbic acid, cysteine, glutathione (Son et al., 2001), metal chelating agents, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroxyquinone (TBHQ), propyl gallate, carotenoid, gallic acid, quercetin, anthocyanins (Ricardo et al., 2018) and tocopherols are the example of phenolic content (Nisperos-Carriedo et al. 1990). Antioxidant materials has an effect to delay oxidation, so they prevent free radical reactions (Apak et al., 2007). In this way, it enables to maintain nutritional value and color of the food by protecting from oxidative rancidity, degradation, enzymatic browning (Martin-Belosso et al., 2009).

Table 3 shows examples of antioxidant films with their content and benefits.

Table 3: Examples of antioxidant films with their content and benefits

Name of antioxidant	**Film Content**	**Benefit**	**Reference**
Tricholoma terreum extract	Chitosan, glycerol, acetic acid	Increase elasticity, hydrophobicity	(Koc et al., 2020)
Blackberry extract	Glycerol, Arrow root starch	Improve mechanical properties, color and flavor	(Nogueira et al., 2019)

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Table 3 (cont.)

Coconut protein	Glycerol	Barrier against UV-light	(Rodsamran & Sothornvit, 2018)
Anthocyanin from black carrot	WPC	Higher physical stability	(Ersus et al., 2017)
Thai rice grass extract	Carboxymethyl cellulose, glycerol, olive oil	Increase water barrier and total phenolic content	(Rodsamran & Sothornvit, 2018)
Red pear extract	Carboxymethyl cellulose, glycerol	Increase antioxidant capacity	(Aparicio-Fernandez et al., 2018)
β-carotene	Cassava starch, glycerol, sunflower oil	Maintain the food safety, extend the shelf life	(Assis et al., 2018)
Pomegranate peel	Mung bean extract, glycerol	Protect physiological properties	(Moghadam et al., 2020)
Oregano oil	Quince seed mucilage, glycerol	Improve tensile properties	(Jouki et al., 2013)
Calcium vitamin E	Calcium caseinate, whey protein isolate, gluconate	Enhance tensile properties	(Mei & Zhao, 2013)

1.6.4.3. Antifungal Activity

There are some substances to inhibit the growth of fungi and molds to prolong shelf life. These matters generally have also the antimicrobial effect. Acetic acid, benzoic acid, sodium benzoate, sorbic acids as organic acid, nisin as peptide, lysozyme as enzyme, citrus plants, cinnamon, clove oregano essential oil has both antimicrobial (Quintavalla & Vicini et al., 2002) and antifungal effect (Van Long et al., 2016). The study reported

that presence of cinnamaldehyde in chitosan edible film demonstrate antifungal effect against *Penicillium italicum*, *Rhizopus stolonifera* (Demitri et al., 2015). The antifungal substance also affects mechanical properties of edible film positively. Moreover, Tarazona et al. (2018) shows the antifungal effect of linalool, isoeugenol, citral additives in ethylene vinyl alcohol polymer film against *Aspergillus steynii* and *Aspergillus tubigensis*. Furhermore, grape cane extract in thermoplastic starch demonstrates antifungal activity. Chitosan based edible films mixed with wax makes the antifungal coating (Iverson and Ager, 2003). Locust bean gum prevents the growth of *Penicillium digitarium* and *Penicillium italicum* (Parafati et al., 2016).

1.6.4.4. Antimicrobial Activity

The growth of microorganism in the food is occurred during the shelf life, which is one of the reasons to limit time to consume due to spoilage. The growth of microorganism causes to undesirable flavor, color, texture (Gyawali & Ibrahim, 2014). To prevent the microbial growth, some changes are applied physically on the food by reducing of water activity, storing in low temperature condition or using moisture proof packaging (Torres et al. 1985). Another method to get rid of the growth of microorganism is addition of antimicrobial agent. Antimicrobial edible film could be solution for the growth of microorganism during their production, storage and distribution. The film inhibits or slow down the growth of the microbial creatures, which increases the shelf life. The antimicrobial film shows the barrier properties for moisture, oxygen and other gases that prevent the development of microorganism (Campos et al., 2011). Antimicrobial substances are composed of chitosan, essential oils, bacteriocin, organic acids and their salts (Quintavalla & Vicini, 2002), enzyme systems and natural extracts. Detailed information and examples can be found in Figure 2. Therefore, the foods can be saved without going to the waste. Besides this, people can gain extra healthy compounds thanks to antimicrobial agents (Sung et al., 2012).

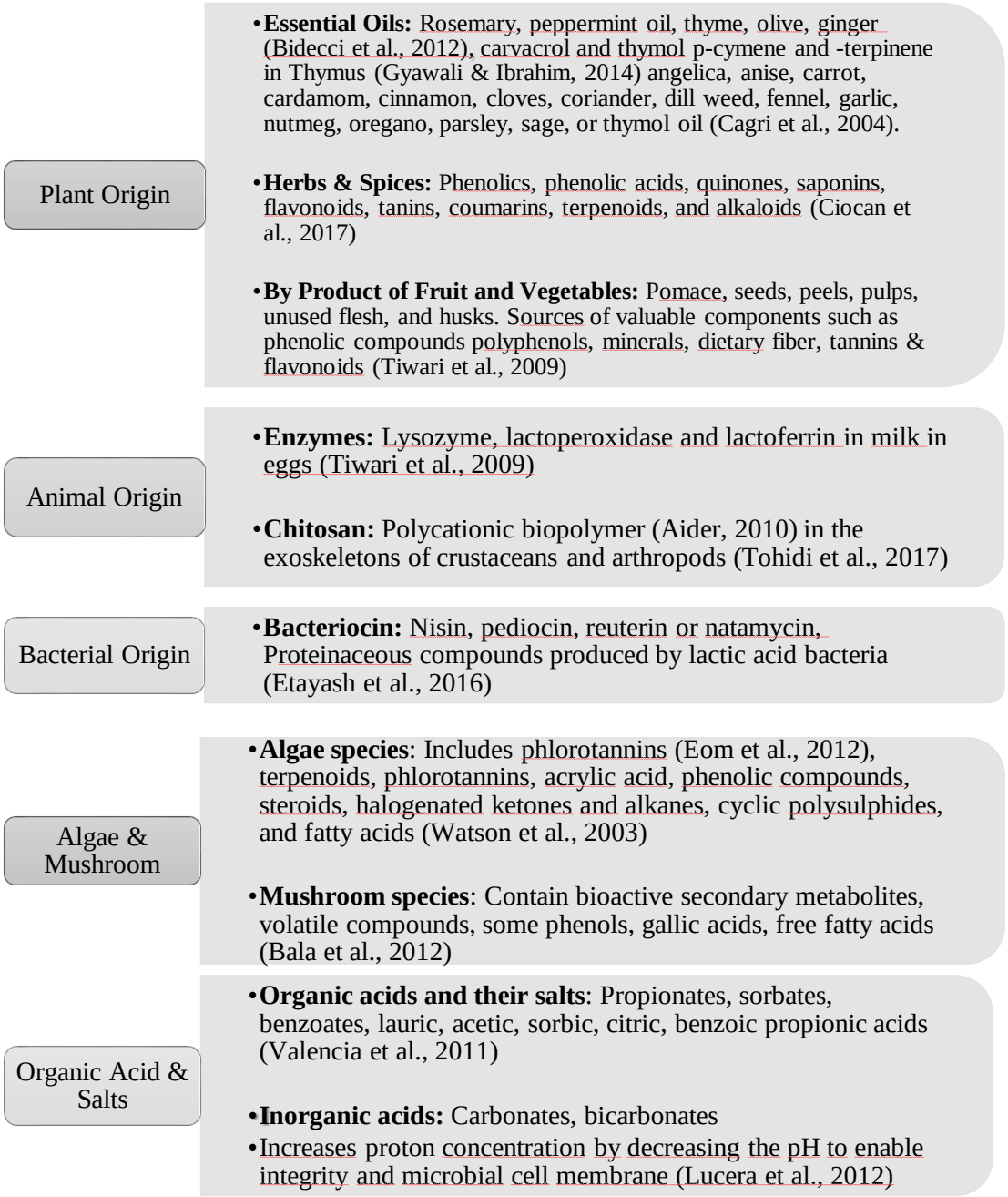

Figure 2: The agent carrying antimicrobial activity depend on sources

Table 4 shows examples of antimicrobial agents used in films and their antimicrobial agents.

Table 4: Examples of antimicrobial agent used in films and their antimicrobial effects

Added Antimicrobial Agent	Film Content	Form of Film	Inhibited Bacteria	Reference
Castor oil	Alginate	Edible Film	*Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Escherichia coli*	(Aziz et al., 2018)
Clove, cumin, caraway, marjoram, cinnamon, and coriander essential oils	Alginate-clay	Edible film	*Escherichia coli, Staphylococcus aureus, Listeria monocytogenes*	(Alboofetileh et al., 2014)
Cinnamaldehyde, garlic oil, rosemary oil	Polypropylene -Polyethylene film	Edible film	*Staphylococcus aureus, Bacillus cereus*	(Gamage, Park, & Kim, 2009)
Oregano, carvacrol and citrus oil	Polypropylene	Edible film	*Escherichia coli, Salmonella enterica*	(Muriel-Galet, 2012)
Oregano oil	Basil seed gum	Edible film	Aerobic mesophilic microorganisms, mold and yeast	(Guerreiro et al., 2016)
Oregano oil	Mucilage	Edible film	*Escherichia coli O157:H7, Staphylococcus aureus, Yersinia enterocolitica, Salmonella typhimurium Listeria monocytogenes*	(Jouki et al., 2014)
Oregano, coriander, basil, anise oil	Chitosan	Pink Salmon Fillets	*Listeria monocytogenes*	(Zivanovic et al. 2005)
Oregano, coriander, basil, anise oil	Chitosan	Pink Salmon Fillets	*Listeria monocytogenes*	(Zivanovic et al. 2005)

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Table 4 (cont.)

Oregano oil	Citrus peel, pectin	Edible Film	*Chromobacterium violaceum, Salmonella choleraesuis, Staphylococcus Aureus, Escherichia coli* O157:H7	(Alvarez et al., 2014)
Oregano oil	Soybean	Edible films	*Staphylococcus Aureus, Escherichia coli* O157:H7	(Emiroglu et al., 2010)
Oregano oil	Whey protein	Edible films	*Staphylococcus Aureus, Escherichia coli* O157:H7, *Salmonella enteritidis, Listeria Monocytogenes*	(Seydim & Sarikus, 2006)
Oregano-thyme	Polyethylene	Edible film	*Escherichia coli, Salmonella typhimurium, Listeria monocytogenes*	(Solano, & Gante, 2010)
Rosemary oil	Whey protein	Edible films	*Staphylococcus Aureus, Escherichia coli* O157:H7, *Salmonella enteritidis, Listeria Monocytogenes*	(Seydim & Sarikus, 2006)
Garlic oil	Alginate	Edible films	*Staphylococcus Aureus, Escherichia coli* O157:H7, *Salmonella typhimurium, Bacillus cereus*	(Yudi et al., 2005)
Garlic oil	Whey protein	Edible films	*Staphylococcus Aureus, Escherichia coli* O157:H7, *Salmonella enteritidis, Listeria monocytogenes*	(Seydim & Sarikus, 2006)
Citral & eugenol oil	Alginate, pectin	Edible film	Aerobic mesophilic microorganisms, mold and yeast	(Hashemi et al., 2017)

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Table 4 (cont.)

Carvacrol	Tomato puree	Edible film	*Escherichia coli* O157:H7	(Du et al., 2008)
Lemon grass oil	Alginate	Edible film	*Escherichia coli*, psychrophilic bacteria, mold and yeast	(Salvia-Trujilo et al., 2015)
Orange essential oil	Gelatin	Shrimp	*Psychrotrophic bacteria, Enterobacteriaceae*	(Alparslan et al., 2016)
Clove oil	Chitosan	Coated lean pork slices	*Staphylococcus Aureus, Escherichia coli*	(Sanchez-Ortega et al., 2016)
Clove oil	Gelatin	Edible film	*Pseudomonas, Enterobacteria*, lactic acid bacteria	(Gomez-Estaca et al., 2010)
Cinnamon bark	Soybean oil, sodium alginate	Canta-lopes	*Listeria monocytogenes, Salmonella enterica, Escherichia coli* O157:H7	(Zhang et al., 2015)
Pomegranate peel	Curry leave powder	Edible film	*Staphylococcus aureus and Micrococcus luteus*	(Kumari et al., 2017)
Grape seed extract	Pea starch	Edible film	*Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecium*	(Corrales et al.,2009)
Chitosan	Agar	Edible film	*Aspergillus Niger*	(Sebti et al. 2005)
Citral & eugenol oil	-	Pre-cooked pizza	*Alternaria sp, Penicillium sp, Aspergillus sp and Cladosporium sp*	(Rodriguez et al. 2003)
Chitosan	Hydroxypropyl methylcellulo-se	Fresh straw-berry	*Cladosporium sp and Rhizopus sp*	(Park et al. 2005)

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Table 4 (cont.)

Chitosan	Pectin, pullulan, sodium benzoate, potassium sorbate	Fresh straw-berries	Total aerobic count, mould and yeast	(Guerreiro et al., 2015)
Chitosan	-	Cooked Ground beef and turkey	*Clostridium perfringens spores*	(Juneja et al. 2006)
Chitosan	Polypropylene	Cheese slices	*Listeria monocytogenes, Staphylococcus Aureus, Escherichia coli* O157:H7	(Torlak& Nizamlioglu , 2011)
Chitosan	Sodium alginate, pectin, calcium lactate	Fresh cut waterme-lon	Psychrotrophic bacteria, mold and yeast, *Coliform*	(Sipahi et al., 2013)
Chitosan	Nisin, Sodium diacetate, sodium lactate, potassium sorbate, sodium benzoate, Plastic material	Coated steaks	*Listeria monocytogenes*	(Ye, Neetoo, & Chen, 2008)
Chitosan	K-Carrageenan, chitosan, acetic acid	Coated Chicken breast	*Camplobacter jejuni*	(Olaimat et al., 2016)
Sakacin, enterocin, pediocin as bacteriocin	Low density polyethylene	Edible film	*Listeria monocytogenes*	(Iseppi et al., 2008)
Nisin	Hydroxypropyl methylcellulo-se	Edible film	*Micrococcus luteus*	(Sebti et al., 2003)
Nisin	Oleic acid, tween 80, glycerol	Persim-mons	*Escherichia coli, Listeria monocytogenes, Salmonella enteritidis*	(Sanchis et al., 2016)

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Table 4 (cont.)

Nisin	Lactic acid, lauric arginate	Coated pork meat	*Listeria monocytogenes, Micrococcus luteus*	(Matiacevich et al., 2015)
Alginate	Glycerol	Ham slices	*Pseudomonas spp, Enterobacteria*, mold and yeast, *Listeria monocytogenes*	(Pavli et al., 2017)
Thyme	Soybean	Edible films	*Escherichia coli, Staphylococcus aureus*	(Emiroglu et al., 2010)
Whey protein Isolate	Lactic acid	Edible films	*Staphylococcus Aureus, Escherichia coli, Yersinia lipolytica*	(Oscar et al., 2012)
Whey protein Isolate	Propionic acid	Edible films	*Staphylococcus Aureus, Escherichia coli, Yersinia lipolytica*	(Oscar et al., 2012)
Whey protein Isolate	Natamycin	Edible films	*Staphylococcus Aureus, Escherichia coli, Yersinia lipolytica*	(Oscar et al., 2012)

1.7. Raw Materials of the Film

1.7.1. Pomegranate

Pomegranate as called *Punica granatum* from the *Lythraceae* family is fruit and grown in Africa, Japan, South Caucasus, South and Central Asia, North and South America and in the Mediterranean region (El-Nemr et al., 1990). It is round berry type fruit with its husk that has reddish appearance but inside of that are white. The fruit is composed of 78% of moisture, 1.6% of protein, 0.1% of fat, 0.7% minerals, 5.1% fiber and 14.5% of carbohydrates (Dahham et al., 2010). Pomegranate is rich source of many phenolic and antioxidant in pomegranate peel, leaf and seeds shown in Table 5.

Table 5: Group and examples of phytochemicals (Source: Wu & Tian, 2017)

Chemical Group	Specific Phytochemicals
Tannin	Ellagitannins, Gallotannins, Punicatannins, Punicalin, Punicalagin
Flavonoids	Catechin, Epicatechin, Gallocatechin, Naringin, Punicaflavanol, Luteolin, Quercetin, Rutin, Cyanin, Gallocatechin
Lignans	Punicatannin, Pomegralignan
Triperpenoids and Phytosterols	Tryptamine, Betulic acid, Maslinic acid, Oleanolic acid, Punicanolic acid
Fatty acid and Lipids	Caprylic acid, Lauric acid, Myristic acid, Palmitic acid, Punicic acid, Linoleic acid, Stearic acid
Organic Acids and Phenolic acids	Ascorbic acid, Citric acid, Fumaric acid, Oxalic acid, Succinic acid, Tartaric acid, Caffeic acid, Cinnamic acid, Quinic acid, Coumaric acid, Cinnamic acid, Gallic acid, Ellagic acid
Alkaloids	Melatonin, Serotonin
Other	Gallagyl ester, Antocyanin, Liganans

Because of its high level of phytochemical content, the fruit is very healthy (Kahramanoglu & Usanmaz, 2016). Some food products can be promoted for health with the addition of pomegranate thanks to its antioxidant and polyphenol content (Carballo et al., 2009). However, if it is used in excessive amount, the food can have an astringent taste due to polyphenol content (Akhavan et al., 2015). Also, it indicates as anti-insecticide, anti-molluscicidal, anthelmintic, anticoccidial, antifungal, antibacterial and antiviral activities (Rosas-Burgos et al., 2016).

It is also broad-spectrum antimicrobial agents to prevent deterioration of the food. Due to high antioxidant activity, it inhibits lipid peroxidation and intrinsic quality (Chen et al., 2020).

1.7.1.1. Benefits of Pomegranate

Scientific studies confirm that pomegranate has many benefits for the human health. It acts as an antioxidative, anti-diabetic, anti-obesity, anti-cancer, anti-inflammatory (Aruna et al., 2016), antiatherogenic, antimutagenic (Xi et al., 2017), anti-proliferative, anti-osteoporotic (Glazier & Bowman, 2001), antihypertensive (Vučić et al., 2019) and antidiarrheal (Ismail et al., 2012) properties.

1.7.1.1.1. Antioxidative and Antimutagenic Effect

Many mutagenic and carcinogenic compounds are activated by reactive oxygen species. Antioxidant has a role in there to prevent interaction of these two compounds (Gegi et al., 2003). Pomegranate can decrease free radicals and oxidative stress due to its bioactive component (Zarfeshany et al., 2014). By reducing of free radicals, it can protect the DNA from the oxidant induced damage (Sierens et al., 2001). Also, by binding of lipid peroxides, it can prevent and reduce atherosclerosis and LDL oxidation (Fuhrman et al., 2010). Mutagenic reactions are occurred in presence of reactive oxygen, it inhibits to form free radical scavenging (Akhtar et al., 2015).

1.7.1.1.2. Antimicrobial Effect

It inhibits to grow pathogenic bacteria that is proven by many studies. The bacteria that are prevented to grow are *Clostridium species, Staphylococcus aureus, Pseudomonas aeruginosa* (Walid et al., 2012) *Shigella dysentriae* (Ahmad & Beg, 2001), *Vibrio cholera, Shigella sonnei, Shigella flexneri, Shigella boydii* (Mathabe et al., 2005), *Escherichia Coli, Salmonella typhi, Staphylococcus aureus, Enterobacter cloacae, Pseudomonas florescens, Proteus vulgaris, Alcaligenes faecalis, Micrococcus luteus, Bacillus cereus, Bacillus coagulans, Bacillus subtilis* and *Candida albicans* (Vasconcelos et al., 2006). This effect makes possible to use pomegranate in antimicrobial coating and packaging material.

1.7.1.1.3. Antiproliferative and Anticarcinogenic Effect

The prevention of cancer for pomegranate examined with tumor cell proliferation, cell cycle, invasion, angiogenesis (Bassiri-Jahromi, 2018). Effects are seen in breast, prostate, lung, colon, skin cancers (Vučić et al., 2019). It is observed that the breast cancer cell is inhibited by pomegranate constituents for angiogenesis (Albrecht et al., 2004), invasiveness (Hiramo et al., 1989), growth (Bowen et al., 1993), induced apoptosis, prostate cancer cells are treated with (Koyama et al., 2010). Thanks to its apoptotic, antioxidant, antiproliferative, anti-inflammatory properties, it has an impact on slowing down and prevention of cancer cell metastasis (Wang et al., 2011). Glazier & Bowman (2001) reported in their study that antiproliferative effect in human breast and endometrial, cervical, ovarian cancer cell lines.

1.7.1.1.4. Anti-osteoporotic Effect

It has an anti-osteoporotic potential. Bone loss is occurred when the bone resorption rate is higher than the bone formation rate (Banu, 2011). The study is made for treatment of post-menopausal osteoporosis (Jordan et al.,1987). For the evaluation of the study, osteoblastic cell population is examined. As a result of the study, it is seen that pomegranate extract can modulate osteoblastic cell differentiation (Sreekumar et al., 2011). Also, anti-osteoporotic effect is detected on skeletal system (Glazier & Bowman, 2001).

1.7.1.1.5. Other Benefits of Pomegranate

The conducted pilot study has demonstrated the cholesterol regulation that decreased cholesterol absorption, increased fecal excretion of cholesterol, reduced LDL cholesterol are observed in type 2 diabetic patients with hyperlipidemia (Esmaillzadeh et al., 2006). It has effect in oxidation of the low-density lipid, which also causes to prevent Alzheimer's disease (Pohanka,2014), cardiovascular diseases (Amarowicz & Pegg, 2017) and cancer (Khanna & Jackson, 2001). For the Alzheimer's disease, it inhibits the activity of AchE enzyme that progress Alzheimer's disease (Morzelle et al., 2019). Moreover,

cardiovascular disease risk is reduced resulted from pomegranate's regulation of blood pressure effect (Vučić et al., 2019).

Pomegranate can be used in the treatment of perimenopausal and menopausal symptom and over hormone therapy (HRT) (Glazier & Bowman, 2001), decreasing of tumor risk (Adlercreutz, 2002) and breast cancer risk (Shang & Brown, 2002). All of these can be treated by phytoestrogen found in pomegranate structure. For menopausal hormone therapy, phytoestrogen has a role to bind estrogen receptor and reduce effect of estrogen hormone (Magee & Rowland, 2004). Also, pomegranate has a preventive effect for breast cancer through estrogen binding mechanism (Shang & Brown, 2002). Phytochemical can inhibit the harmful enzymatic changes in gastrointestinal tract to cause tumor development (Adlercreutz, 2002).

It has a beneficial effect on gut microbiota by connecting gastrointestinal tract. It can heal the chronic gastrointestinal disease (Leone et al.,2013). Thus, pomegranate affects body homeostasis positively. It participates in digestive process, energy regulation, short fatty acid production, vitamin synthesis, modulation of immunologic system (Aziz et al., 2013).

Also, pomegranate consumption is suggested in treatment of many diseases which are rheumatoid arthritis (RA), ulcerative colitis (UC) (Vučić et al., 2019), Crohn's disease, chronic obstructive pulmonary disease, inflammatory bowel disease (IBD) (Aruna et al., 2016), arthritis, diabetes (Sturgeon & Ronnenberg, 2010), coronary disease (Al-Jaralah et al., 2013), neurological disease (Fung et al., 2017), systemic disease (Borre et al., 2014), neurodegenerative disease, inflammation (Fischer et al., 2011). Furthermore, it can protect from ultraviolet radiation and use in dental treatments (Pagliarulo et al., 2016).

1.7.1.2. Components of Pomegranate

The pomegranate fruit has two parts that are %60 of arils as an edible part and %40 of peels as non-edible part (Cam et al., 2014). Detailed information is given in following parts.

1.7.1.2.1. Edible Part of Pomegranate

The edible parts of pomegranate peel are composed of 60% of total fruit. These are 40% arils and %10 seeds (Viuda-Martos et al., 2010).

1.7.1.2.1.1. Arils

Arils are found inside of the peels has red or purple color because of high anthocyanin value. It contains 10% of total sugars (fructose and glucose), 85% of water, % 1,5 pectin, organic acids (ascorbic acid, citric acid, malic acid) and bioactive compounds (phenolic and flavonoids) (Viuda-Martos et al., 2010).

1.7.1.2.1.2. Juice

Juice is formed 45-61% of arils (El-Nemr et al., 1990). It is richer than the seed for vitamin as vitamin B, vitamin C and minerals as iron, cupper, sodium, magnesium and zinc (Akhavan et al., 2015). Pomegranate juice has high antioxidant capacity among other fruit juices (Gill et al., 2000). The content of this is composed of flavonoids, anthocyanin, lignans, organic acids, fatty acids, alkaloids (Wu & Tian, 2017). Also, it is reported that polyphenolic content in pomegranate juice can reduce oxidative stress and atherogenesis by activation of redox-sensitive genes (Nigris et al., 2005).

1.7.1.2.1.3. Seeds

Seed is found in the core of arils in percentage of 9-14 (El-Nemr et al., 1990). 12-20% seed oil can be extracted from the seeds (Viuda-Martos et al., 2010). Seed is composed of 27.2% of lipid, 13.2% of protein, 35,3 % of fibers, 2% of ash 6% of pectin and 4.7% of sugars (El-Nemr et al., 1990). Pomegranate seed is byproduct of the pomegranate juice industry which has sterols, tocopherol, punicic acid (Liu et al., 2009).

1.7.1.2.2. Non-Edible Part of Pomegranate

The waste part of the pomegranate fruit is about %34-52 that is composed of 28-39% peel and 0.1% of central lamella (Veres, 1977).

1.7.1.2.2.1. Pomegranate Peel

Pomegranate peel is not consumed as an edible part, so it is waste as a byproduct of many pomegranate processes despite of its rich content. Pomegranate peel forms 50% of total fruit weight (Ahmad et al., 2015). The pomegranate processes include production of juice, jam, wine, fruit and fortification of formula in cereal bars, beverages, ice cream, and yogurt in order to increase functionality (Viuda-Martos et al., 2010). After these processes, almost 78% of pomegranate peel might be collected as agro-industrial waste (Qu et al., 2009). The pomegranate peel is made up of 16-22% of cellulose, 20-41% of lignin, 14-23% of pectin and some proteins (Hasnaoui et al., 2014).

It is one of the most valuable waste including 48 phenolic compounds found in its structure (Akhtar et al., 2015). In UV spectra measurement, there is 9 anthocyanin, 2 gallotannins, 22 gallotannins, 2 gallyl esters, 4 hydroxybenzoic acids, 7 hydroxycinnamic acid and 1 dihydroflavonol as a phenolic compound detected (Fischer et al., 2011). Polyphenols, flavonoids, alkaloid, tannin groups are included in it. Proanthocyanin, β-carotene, Caffeic acid, Ellagic acid, Cinnamic acid, Pro-Catechuic acid (Kumari et al., 2017), Chlorogenic acid, Syringic acid, Ferulic acid, Vanillic acid, p-Coumaric acid, Cinnamic acid (Elfalleh et al., 2011), Pelleterine, Luteolin, Kaempferol, Quercetin (Sreekumar et al., 2014), Catechins, Lignin, Punicalagin, Punicalin, Gallic acid (Vučić et al., 2019), Ellagitannin, Granatins, Castalagin, Corilagin, gallagyldilactone, tellimagrandin (Abid et al., 2017) are the compounds that are found in pomegranate peel. Pomegranate peel has antioxidant value than pulp and seed of pomegranate fruit (Hanani et al., 2019). Also, it includes many minerals such as potassium, nitrogen, calcium, phosphorus, magnesium, sodium and complex polysaccharide (Viuda-Martos et al., 2010).

Pomegranate peel is used as in dietary supplements, colorants, flavoring agents, edible films and food packaging films (Akhtar et al., 2015). It is used widely in medicine industry for preparation of pharmaceutical preparations as a source of biologically active

source due to its content (Magangana et al., 2020). Pomegranate peel has antibacterial, antioxidant, anti-allergic (Panichayupakaranant et al., 2010), antiulcer activities (Sorrenti et al., 2019), antidiabetic (Demir et al., 2019) and anticarcinogenic (Tew & Gate, 2001).

Antioxidant activity of pomegranate peel can extend the shelf life by retarding lipid peroxidation. Besides, tannins exhibit the antifungal activity and antiviral activity for genital herpes virus (Zhang et al., 2002). Punicic acid in pomegranate peel regulates inflammation in mucosal immune (Shah et al., 2016), epithelial cell and has a role in immunity (Daynes & Jones, 2002), lipid & carbohydrate metabolism (Huang et al., 2017).

Antidiabetic effect of pomegranate peel is detected. Medicine using in treatment of diabetes inhibits the α-amylase and α-glucosidase, limits conversion of glucose from carbohydrate (Dik, 2013) and control insulin level (Middha et al., 2014). Studies show that phenolic compound in pomegranate peel has also activity on absorption of carbohydrate (Jung et al., 2006). It is proved that pomegranate peel inhibits the 50% of the mentioned enzymes activity (Demir et al., 2019).

Anticarcinogenic effect is also proved by lots of studies. Medicines used in treatment of cancer prevent growth and reproduction of cancer cells, but they have side-effect in patients' body (Tew & Gate, 2001). Studies show that pomegranate peel has cytotoxic effect on MACF-7 and MG-63 cells as a cancer cell (Demir et al., 2019). Ellagic acid and gallic acid are impressive in apoptosis of breast cancer (Çağlar et al., 2017) and caffeic acid can repress metastasis of cancer (Hwang et al., 2005) when quercetin has anticancer and apoptosis effect (Yang et al., 2006). Ellagitannins such as punicalagin, punicalin have antiproliferative effect on colon cancer (Adams et al., 2006). Also, epicatechin can inhibit the carcinogenic cells for leucemia and liver cancers (Lea et al., 1993).

There are many studies for the anti-inflammatory effect of pomegranate peel. Inhibition of xsantin oxidase and lipoxygenase that have a role in many inflammatory diseases are investigated for the pomegranate peel extract (Trouillas et al., 2003). Quercetin in pomegranate peel inhibits the 50% of activity of oxidase and lipoxygenase. Moreover, elagic acid (Adams et al., 2006), oleanolic acid, ursolic acid, chlorogenic acid, epicatechin and rutin (Cam et al., 2014) found in pomegranate peel have inhibitory effect for inflammatory disease (Demir et al., 2019).

It can be used in treatment in gastrointestinal disorders, stomach disorders (Ganguly, 2017), diarrheas (Lev & Amar, 2002), intestinal parasites, nose bleeds, gum disease, hemorrhoids (Lasure et al., 2012), colonic inflammation (Fengchun et al., 1997),

inflammatory bowel disease by reducing colitis activity (Kamali et al., 2015). This could be explained that pomegranate peel can act as a prebiotic in gastrointestinal tracts. Prebiotics are the food ingredients that is not digested in the body. Punicalagin and ellagic acid in pomegranate peel hydrolyzed by intestinal microflora that can act as a prebiotic. These can inhibit the pathogens and promote the growth of beneficial microbiota in human guts. Therefore, it helps to regulate pathogens without adverse effect on beneficial bacteria (Bialonska et al., 2009). Also, this situation is related to obesity because gut microbiota is environmental factor for obesity (Neyrinck et al., 2013) and helps to treat obesity by lowering lipid effects (Hontecillas et al., 2009).

It is used in treatment of vaginal white discharges (Ong et al., 1999), dysentery, infection in mastitis, acne, pile, allergic dermatitis (Hu et al., 1997), tympanitis, dysentery, folliculitis, oral diseases (Fengchun et al., 1997), hypertension by decreasing systolic blood pressure (Delgrado et al., 2017).

Studies show that many parts of pomegranate peel have higher potential for antimicrobial activity, which is also higher as compared with many fruits waste besides antioxidant capacity (Agourram et al., 2012). When the antimicrobial effect of seed oil (Schubert et al., 1999), the flower (Kaur et al, 2006), seed extract (Singh et al., 2002) and peel extract are compared, results were indicated the edible parts has lower effect on the growth of the microbes (Nuamsetti et al., 2012). Pomegranate peel inhibits microbial activity for both gram-positive and gram-negative bacteria (Hanani et al., 2019). This property is related to total flavonoid and tannin content. There are many studies to demonstrate antimicrobial effect of pomegranate peel extract in chicken and meat products (Hayratpetyan et al., 2012). Researches have been proved that pomegranate peel has an effect on inhibition of the growth of foodborne pathogens and bacteria that are including *Listeria monocytogenes, Klebsiella pneumoniae*, *Salmonella typhimurium* (Nuamsetti, 2012), *Staphylococcus aureus* and its enterotoxins, *Micrococcus luteus* (Kumari et al., 2017), *Escherichia coli, Bacillus coagulans, Bacillus subtilis, Pseudomonas aeruginosa, Bacillus cereus* (Dahham, 2010), *Yersinia enterocolitica, Salmonella enteritidis* (Al-Zoreky, 2009) and *Latilactobacillus sakeii, Lactococcus lactis, Serretia marcescens, Pseudomonas florescens* (Agourram et al., 2012), *S.cerevisiae, Pseudomonas spp* (Negi & Jayaprakasha, 2003), *Staphylococcus epidermidis, Lactobacillus acidophilus, Streptococcusmutans*, *Streptococcus salivarius* (Abdollahzadeh et al., 2011), *Enterobactererogenes, Serretia marcescens, Brucella spp., and Rhodotarula glutinis* (El Khetabi et al., 2020).

Antifungal activity of pomegranate peel were detected on *Aspergillus niger, Penicillium Citrinum, Rhizopus oryzae, Trichoderma reesei, Mycobacterium İndicus,* (Dahham et al., 2010), *Candida utilis, Saccharomyces cerevisiae, Fusarium verticillioides, Aspergillus parasiticus, Alternaria alternata, Botrytis cinerea, Trichoderma reesel* (Rosas-Burgos et al., 2016), *Aspergillus ochraceus, Penicillium patulum, Penicillium roquefortii* (Negi & Jayaprakasha, 2003), *Stemphylium botyosum, Fusarium spp*. (Glazer et al., 2012), *Fusarium sambucinum* (Elsherbiny et al., 2016), *Peniccillium italicum,* (Tehranifar et al., 2011), *Peniccillium.digitatum* (Kharchoufi et al., 2016), *Penicillium expansum* (Nicosia et al., 2016), *Fusarium oxysprorum* (Rongai et al., 2019*), Botyris cineraea* (Nicosia et al., 2016).

Pomegranate peel has also antiviral activity thanks to ellagitannins, punicalagin in structure of pomegranate peel. This results in precipitation of protein and remove the metal cofactors by their strong affinity for metal ions (Daglia, 2012).

Pomegranate peel is beneficial for edible film structure. As a food-based biopolymer, it shows film-forming capacity (Galus et al., 2020). It improves bio-functionality of edible film (Hanani et al., 2019). Young modulus, tensile strength and stiffness parameters are developed with the addition of it, in fact it improves mechanical properties and flexibility. This affect can be explained by its content. Pomegranate has high complex polysaccharide group to demonstrate good tensile strength by interacting with hydroxyl group of the phenolic compounds. In addition, polyphenols of pomegranate peel form the crosslinks in proteins and polyphenol-protein due to hydrogen bonding, electrostatic interaction and hydrophobic forces (Ebrahimic et al., 2016). Linkage between proteins results to low strength and high flexibility (Moghadam et al., 2020). Also, usage of pomegranate peel makes the edible film as an antimicrobial that can inhibit microbial growth and slower biological activities (Nair et al., 2018). These impacts observed in DPPH and ABST radical scavenging activities when it decreases water solubility (Hanani et al., 2019). However, addition of pomegranate peel increases thickness, water vapor permeability and decreases moisture content, water contact angle due to crystalline structure (Ali et al., 2020). Previous studies are done with the pomegranate peel extract for chicken and meat products. According to Kanatt et al., 2010 chicken meat stored at 4 ^{o}C were obtained 2–3 weeks extended shelf life based on microbial, sensory and oxidative tests in pomegranate peel extract. Microbial analyses were done with *Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium, Pseudomonas florescens.* For meat products, the study is conducted with *Escherichia*

Coli, *Staphylococcus aureus*, *Vibrio cholera* and *Bacillus cereus*. The result is that shelf life could be stored for 46 days at 4^{o}C growth of bacteria (Hayrapetyan et al., 2012).

1.7.2. Lentil Flour

In previous studies, legumes are used in making an edible film. Because of that, they are proper material for this due to rich protein, carbohydrate and even fat nutritional value. Grass peas (Giosafatto et al., 2018), lentils (Aydogdu et al., 2018), chickpeas (Diaz et al., 2019) and mung beans (Bourtoom, 2008) are the legumes that are used in edible film and their results shows the good properties and evaluated (Galus et al., 2020).

Lentil is small legume from *Lens culinaris* belongs to *Leguminosae* family (Samaranayaka, 2017). Lentil is in shape of small purse and changes color of green, red, brown and black (Erskine et al., 2009). Lentil is grown in almost all climatic condition worldwide. Most of lentil producers are in Canada, Turkey, United States, Australia and India (Alexander, 2015). Whole grain of lentil is edible. It might be consumed as split kernel and decorticated form. It is both cheap and nutritious alternative as a feed (Joshi et al., 2017).

It is gluten free, low fat, high content of dietary fiber, protein, vitamin and minerals (Jarpa-Parra et al., 2016). Carbohydrate is one of the highest content that is almost 60% of lentil in weight. Percentage of other components are 23.32% of protein, 1.7% fat, 8.9% moisture and 3% ash (Bamdad et al., 2006). Thus, lentil is nutritional legume in terms of the rich protein content which is ideal vegetarian food alternative for consumers. Lentil can be consumed as a form of soup in the meal, whose portion could meet the half protein requirement a day (Al-Nahry et al., 1980).

Lentils are used in snack bar development, as a dietary fiber, essential amino acid folic acid and iron sources (Ryland et al., 2010). Lentil flour might be used in dough mixture of bread (Turfani et al., 2017), cake (Farooq&Boye, 2011), noodles (Rathod & Annapure, 2017).

Lentil flour is efficient choice for the extraction of protein because of its low price and small quantity of unused residues. This makes it preferable to use for making edible film for direct water contact food products or high moisture containing food (Tapia-Blacido et al., 2005). Lentil proteins are 16% albumins(water-soluble), 70% globulins (salt-soluble), 11% glutenin (base soluble) and 3% prolamins (alcohol-soluble). The main

amino acids in lentil are glutamic acid, aspartic acid, arginine, leucine, lysine (Boye et al., 2010). There are two isolation method to extract protein of lentil. In dry method, roots, stems and seed ground into fine powders, then starch and protein are divided depending on their densities. Wet method is about the application of alkaline solution. Because of alkaline environment, solubilized protein can be separated as a precipitated form (Joshi et al., 2017). Lentil protein is composed of 16% albumins, 70% globulins and 3% prolamins (Boye et al., 2010). Solubility of lentil protein is dependent on thermal stress. Heating of solution decreases the protein solubility. If protein is denatured, the solubility of particles decreases (Kinsella, 1982). Water absorption capacity of lentil protein is affected by polar amino acids (Kunz, 1971). Also, the protein could make a role as emulsifying agent. If there is oil and water interface, protein is adsorbed and result in low tension and cohesive film (Damodaran, 1994). Surface hydrophilicity of the film is resulted from amphibilic structure of protein (McClements, 2005).

Lentil starch is the major component of lentil in the ratio of 47.1% (Bednar et al., 2001) that includes 93-99% of insoluble dietary fiber (Hoover et al., 2010). It has semi-crystalline structure and includes fermentable oligosaccharides, disaccharides, monosaccharides and polyols (Ispiran et al., 2019). Amylose content of lentil is about 29-45 % of weight, the remained part is called amylopectin (Bello-Perez and Paredes-Lopez, 1995). Amylose is linear glucan group that branch with 1-4 linkage. In the absence of OH, group gives hydrophilic properties to polymer. When there is OH group, it states enough closely to make hydrogen binding resulting in less affinity for water. On the other hand, amylopectin is highly branched group with 1-6 linkage. There is 18-25 units interconnected binds to compose amylopectin. Lentil starch has gelatinization ability by heating in excess water and form disorder phase transition by swelling irreversible (Gidley & Cook, 1991).

Lentil has very low-fat content in 1.4%. This limited fat content includes 16.7 % saturated fatty acids, 23.7% monounsaturated fatty acids and 58.8% polyunsaturated fatty acids.

Also, its composition are soluble and insoluble fibers and minerals such as iron, zinc, manganese, calcium, potassium, magnesium, phosphorus, sodium, selenium (Kumar et al., 2013). Iron has role in many metabolic processes like oxygen transport, DNA synthesis and electron transport (Lieu et al., 2001). Zinc is important for nutrient metabolism, gene transcription (Aliasgharpour & Farzami, 2013) when the manganese activate enzymes and metabolism of glucose (Li & Yang, 2018). Calcium is necessary

for bone and tooth health (Beto, 2015). Potassium is significant for intracellular function (Stone et al., 2016). Magnesium has role in biochemical process of metabolic pathway (Schwalfenberg & Genuis, 2017) while phosphorus enables to control homoeostatic balance (Razzaque, 2011). Sodium regulates body water content and electrolyte balance, while selenium is catalyst for thyroid hormone and function of immune system (Rayman, 2000).

The vitamins that are found in lentil are folate, thiamin, riboflavin, niacin (USDA, 2011), pantothenic acid, pyridoxine, tocopherol (Ryan et al., 2007) and phyquinone.

Lentil is good source to benefit for human body. It helps to control body weight due to β-glucans and low glycemic index (Kim et al., 2005). Phytochemicals as phenolic acid, flavanols, saponins, phytic acid, polyphenols, phytosterols, lectin, tannins are included in lentil as an antioxidative compounds (Durazzo et al., 2013). As a health benefit, it reduces cardiovascular disease (Hu, 2003), cancer, diabetes, osteoporosis, hypertension (Tharanathan & Mahadevamma, 2003) and adrenal diseases risk (Bove et al., 2010).

The carcinogenic effect of lentil is studied for colon, breast, prostate cancer (Mollard et al., 2011). Colorectal cancer is related with consumption of high glycemic index and glycemic lipid value. Thus, consumption of lentil with low glycemic index food can prevent the formation of tumor (Nichenametla et al., 2006). Also, many studies show inhibitory effect of various cancer resulted from lectin (Roy et al., 2010), folate (Milner et al., 2001), phytates (Marks et al., 2006), saponin (Guclu & Mazza, 2007) and defensin (Finkina et al., 2008) that are compounds found in lentil.

It has hypocholesterolemic effect. In diabetic patients who consume cooked lentil, total cholesterol level decreases. It is considered that low glycemic index value is effective for this. Amylose has higher content than amylopectin (Kingman, 1991). To evaluate glycemic and lipidemic effect of lentil, the experiment was done in diabetic rats. It is reported that increased HDL cholesterol, but LDL cholesterol has not been changed (Eidi & Eidi, 2009).

It is also used in prevention of diabetes especially in Type-II diabetic people (Venn & Mann, 2004). It is observed that consumption of cooked lentil decreases blood glucose in diabetic patients because of low glycemic index of lentil (Shams et al., 2008). Glycemic index value of lentil is about 26-30 (Foster-Powell et al., 2002). Also, it could be used in treatment of obesity thanks to this low glycemic index value. Moreover, lentil

has a potential for α-glycosidase and pancreatic lipase inhibition that decrease far digestion and absorption in intestine (Balasubramaniam et al., 2013).

Studies show that lentil has Angiotensin I-converting enzyme inhibitor activity. Anti-cholesterol and anti-diabetic effect of lentil has an effect to prevent cardiovascular disease. The enzyme plays role in cardiovascular diseases. Also, low vitamin K in lentil makes safe to consume lentil in cardiovascular patients (Boye et al., 2010).

It has a positive effect on gastrointestinal disease. As lentil has galacto-oligosaccharide and raffinose from the family of oligosaccharides, these are α-galactose derivatives. Because α-galactosidase enzyme is not produced in the body, these compounds could not be digested in intestinal tract. These are fermented by microflora bacteria and produces the gas. Therefore, it is used to treat irritable bowel syndrome (Joehnke et al., 2021). Also, these undigestible carbohydrate promotes beneficial intestinal microorganism (Faris et al., 2013).

The films that are made up by lentil shows good film forming properties due its starch and protein components. The appearance of lentil films is transparent highly. The color of the film is light yellow. The water solubility of lentil film is lower than the other biodegradable films. Also, the mechanical characteristic of the film is improved highly. It enables to have stiffer edible films (Aydogdu et al., 2018). Moreover, barrier properties to oxygen and water are also good (Bonilla et al., 2011). The starch found in content of lentil is composed of amylose-amylopectin group, that is one of the parameters to specify film properties. Besides this, amino acid profile and fiber content are other factor to impact film properties (Tapia-Blacido et al., 2013).

1.7.3. Pectin

Pectin is white, amorphous, colloidal and heavy carbohydrate molecule (Valdés et al., 2015). The chemical formula of pectin is $C_6H_{10}O_7$ that is in heterogenous and acidic structure from group of polysaccharides. Pectin is colloidal carbohydrate found between middle lamellae and cell walls of plant. The component is formed during cell growth and ripening process (Valdés et al., 2015). It is naturally found and produced from the fruit & vegetables and their wastes such as apple juice and citrus peels (Sanchís et al., 2017). Pectin is used in industry as a gelling agent (Brett & Waldron, 1996).

The major component of pectin is α-(1-4)-D-galacturonic acid that is attached by neutral sugars or concentrated in branched long regions. This branched region makes methyl ester forms from a part of carboxylic acid in galacturonic acid. This branched region results to classification of pectin as low methoxyl pectin and high methoxyl pectin. When low methoxyl pectin has less than %50 of carboxyl group methylated, high methoxyl pectin has more than 50 % of methylated carboxylated group with esterification of methyl (Abid et al., 2017). Low methoxy pectin is used in low sugar products because it shows gelling ability in small amount sugar and presence of calcium (Abid et al., 2017). Pectin is used in production of jam and jelly foods, pharmaceuticals and cosmetic products to possess desired structure (May, 1990).

Pectin is known as thickening and emulsifying agent preferred for the edible coatings as their oxygen, oil and aroma resistance. It is good as a carrier for antioxidant and antimicrobial components (Valdés et al., 2015). The only negative effect of pectin for the edible film is poor moisture barrier and hygroscopic properties (Sanchís et al., 2017). Brito et al. (2019) observed the pectin film appearance is homogenous and has yellowish color. The pectin makes the biodegradable film to have functional, good color, mechanical and barrier properties (Galus et al., 2020).

1.7.4. Glycerol

Glycerol is low molecular weight hydroxy compound which is colorless and dense liquid. It is purified form of glycerin (Espitia et al., 2014). It is one of the most common hydrophilic biopolymers as plasticizers used in edible film to improve flexibility and durability (Espitia et al., 2014). In carbohydrate and protein-based films, it shows brittle and stiff characteristics due to its polymer molecules (Gennadios, 2004). Plasticizers develops flexibility of films by interrupting polymer chains and lowering of the glass transition temperature (Guilbert & Gontard, 1995). The interruption is enabled by reducing of internal hydrogen bonds between polymer chains and increasing volume to permit oxygen and water vapor diffusion. However, it increases film permeability and minimize mechanical properties (Yam et al., 2009).

CHAPTER 2

MATERIAL and METHOD

2.1. Materials

Dried pomegranate peel as Hicaz type was acquired from online market E-bahçemiz in Mezitli-Mersin. The drying condition was sun-dried in natural conditions for 15 days. Lentil flour was purchased from the local market named as Bonatelli Gıda Inc. in Balikesir, Turkey. High methoxyl pectin (APC165B) was used as thickening agent supplied from Andre Group Pectner Co. Ltd, China that was extracted from natural sources.

For snack bar preparation, apple juice was infused and dried strawberry was taken from the supplier of Isık Organic Co. Ltd. from Izmir, Turkey. Figs were chosen as naturally sun dried, obtained from farmers in İzmir, Turkey. The pasted figs were also exposed to pasteurization process which has at 102^{o}C for 24 min as a sterilization step. 1-4 mm roasted broken nuts were purchased from Isık Organic Co. Ltd. in Zonguldak, Turkey.

All chemicals including Folin-Ciocalteu reagent, Glycerol, Gallic acid, Methanol, Sodium Hydroxide (NaOH), Hydrochloric acid (HCl), Magnesium Nitrate ($Mg(NO_3)_2$) and others were purchased from Sigma Aldrich Chemie Gmbh with analytical grade (Darmstadt, Germany). Maximum recovery diluent and other medium of microbes was also purchased form Merc KGaA (Darmstadt, Germany).

2.2. Methods

2.2.1. Extraction of Lentil Protein

Lentil flour protein was isolated by wet method depend on Moghadam et al. (2020) method with some modification. Aqueous solution was prepared with 5% w/v lentil flour in deionized water and stirred by magnetic stirrer (UC151, Stuart-Cole Parmer, Staffordshire, England) at 500 rpm for 10 minutes to distribute lentil flour homogenously

in the water. Then, pH was adjusted to 9.0 with 0.1 N NaOH solution and continued to stir at 500 rpm during 1 hour at room temperature. The pH value was measured by pH meter (SevenCompact Duo S213, Mettler Toledo, Zürich, Switzerland). After that, solution was centrifugated (Rotina 380R, Labotech, Midrand, South Africa) at 5000 rpm for 15 min. When the precipitation was removed, the supernatants were collected. The pH of supernatant was arranged to 4.5 by addition of 0.1 N HCl solution. 4.5 is isoelectric point of lentil that is lowest protein solubility point to precipitate more protein (Joshi et al., 2017). Next, it was again centrifuged at 5000 rpm for 15 min. The precipitated matter was collected and washed with distilled water and stored at -20^{o}C for further use.

2.2.2. Film Preparation

The films were prepared with wet method according to Moghadam et al. (2020) with modification. In the beginning, aqueous solution was prepared with 8% w/v protein precipitate in distilled water. It was stirred at 500 rpm for 10 min in magnetic stirrer to solve in a mixture. Then, pH of solution was adjusted to 8.0 by addition of 5.0 N NaOH and stirred for 4 h at 500 rpm. pH was adjusted to 8.0 because isoelectric point of lentil is 4.5 (Bamdad et al., 2009). For maximum protein solubility, pH was adjusted far from the isoelectric point of lentil. Next, 2% w/w high methoxyl pectin depending on water ratio in solution was added to mixture. After that, the solution was put on a hot water bath (SK6210HP, Kudos Machine, Shanghai, China) at 85^{o}C for 30 min in order to denature the protein fraction. And then, it was kept in room temperature until cooling down to 25^{o}C. Before addition, pomegranate peel was powdered by grinder (G1, Yazıcılar, Izmir, Turkey) in 18000 rpm and sieved using 18 mesh size. Then, 15% w/w pomegranate peel powder was added based on lentil flour isolate and stirred for 5 h at 500 rpm. Before the centrifugation was applied at 5000 rpm for 15 min to remove insoluble particles, supernatant of solution was exposed to ultrasonication (SK6210HP, Kudos Machine, Shanghai, China) at 53 kHz for 5 min. Glycerol as a plasticizer was added in 0.2% w/w of lentil protein isolate. Then, ultrasonication was applied at 53 kHz for 30 min to dissolve and distribute glycerol inside the solution. In the final, 20 mL of liquid mixture was cast on LDPE petri dishes (Diameter: 9 cm) and dried at 40^{o}C for 16 h in oven (EN 055/120, Nuve, Ankara, Turkey). Then, film samples were peeled off and kept in a desiccator adjusted to 53% RH at 25^{o}C for 48 h before film characterization experiments.

2.2.3. Preparation of Snack Bars

Snack bar consisted of three ingredients that are 43% dried and pasteurized fig, 37% dried and apple juice infused strawberry and 20% of 1-4 mm broken and roasted nuts. The snack bar was in group from cold formed bar. Both of dried fruits were pasted before mixing with the help of pasting machine (ST 22, Erigi, Izmir, Turkey). After that, strawberry paste, fig paste and broken nut were mixed in a mixing machine (ASM 50, At-Ra, Izmir, Turkey). Then, the dough was shaped with molding machine (MFT0200, Krüger & Salecker, Ostholstein, Germany). The snack bar was 20x20x13 cm^3 square prism without baking steps.

2.2.4. Coating of Snack Bars

The shaped bars were coated with liquid film mixture by dipping method. The bar was dipped into liquid film mixture entirely for 30 seconds. To dry effectively for all dimensions, wood toothpick was sticked in 4 corners of the snack bars. Then, coated bars were dried in oven for 16 h. To observe the coated bar, both control and coated bars were packed with polyethylene material in a horizontal packaging machine (OFW120S, Ozkan Mak. San., Izmir, Turkey).

2.2.5. Film Thickness

Electronic digital micrometer (5900602, CMT, Anhui, China) was used to measure the thickness of the film. It has a sensitivity value of 0.001 mm. Each film was measured from 5 different positions for 3 different edible films.

2.2.6. Bulk Film Density

Aydogdu et al. (2018) method in modified form was used. 20 x 20 mm^2 pieces of film were prepared and dried oven (EN 055/120, Nuve, Ankara, Turkey) at 110^oC 24 h, then they are weighed with analytical scale (OH83021331EU, Ohaus-Explorer, Darmstadt, Germany). The characterization was done with 3 different films and 3

measurements. The density was calculated by division of weight by volume. Volume was calculated by multiplication of area and thickness.

$$\text{Bulk Film Density} = \frac{\text{weight}}{\text{area x thickness}} \qquad (2.1)$$

2.2.7. Water Solubility

The applied method was modified form of Gontard et al. (1992) method to measure the water solubility. Films were cut into 1 cm x 4 cm pieces and dried at 100ºC for 24 h. Later, dried film samples were immersed into 40 mL water. They were stirred for 24 h at room temperature. Then, remaining insoluble particles were collected and dried at 100ºC for 24 h. The final weight was measured. These measurements were used in the formula and replicated as 3 times.

$$\text{WS (\%)} = \frac{\text{initial film weight} - \text{dried final insoluble matters}}{\text{initial film weight}} \text{ x } 100 \qquad (2.2)$$

2.2.8. Degree of Swelling

In degree of swelling, modified method by Domenek et al., 2004 was applied. The film sample were cut in 1x4 cm^2 immersed into 50 mL of distilled water at 25ºC and weighed after 24 h. Then, the samples were dried in oven at 105ºC to see constant weight. Weights were also measured after drying. The experiment was conducted as triplicates. The degree of swelling was calculated the formula below.

$$\text{Degree of swelling} = \frac{\text{dried film weight} + (\text{swollen film weight} - \text{dried final weight})}{\text{dried film weight}} \text{ x} \frac{\text{density of polymer}}{\text{density of solvent}} \qquad (2.3)$$

2.2.9. Contact Angle

By measuring of contact angle, surface wettability of film was observed. Contact angle was an angle between surface of the film and water drop obtained by contact angle meter (DSA100B, Krüss, Hamburg, Germany). The measurement was occurred by dropping of 60 µL of distilled water to the surface of the film depending on sessile drop method by Abdelhedi et al., 2018. The result was read by the device as 5 times for each film.

2.2.10. Water Vapor Permeability

For the investigation of water vapor permeability, Payne permeability cup (Elcometer 5100, England) was used according to ASTM standard E96 (ASTM, 2016). The gravimetric cylindrical test cup has two parts as a metallic cup and a metallic ring attached to the cup. The films were fixed into external diameter of the cup. Cup was filled with 3 g silica beads and 6 cm diameter film was placed under the metallic ring. Total weight of prepared cup and film thickness measured initially. Then, cup and placed film was placed into 75% RH of desiccator and kept in 25 ºC incubator. The changes in the weight of cup were recorded every 1 h as 8 times and final measurement was done after 48 h. Water vapor permeability (g/m s Pa) was calculated the following equation. (ΔP= 2376,39 Pa)

$$\text{WVP}\ (\%) = \ (\text{weight change of cup} - \text{avg film thickness}) \ x \ \frac{1}{\text{time x area of film x pressure difference between both side of film}} \quad (2.4)$$

2.2.11. Mechanical Properties of the Film

Tensile properties were measured by texture analyzer (TA.XT.plus, Stable micro systems, London, England). To observe the strength and flexibility properties, elongation at break and young modulus factors were examined by tensile test. The measurements were taken at room temperature after keeping in 53% RH at 25 ºC for 48 h. 2 replicate

analysis were done with 3 measurements. The changing factors were adjusted according to ASTM 882-02 standards. The films were prepared as 10 x 80 mm^2 and fixed on grips of device. Crosshead speed was 0.83 mm/sec and grip to grip distance was 15 cm.

$$\text{Tensile Stress(Pa)} = \frac{\text{max load}}{\text{original minimum cross} - \text{sectional area}} \quad (2.5)$$

$$\text{Elongation(\%)} = \frac{\text{extension at the moment of specimen rupture}}{\text{initial gage length of specimen}}\ x\ 100 \quad (2.6)$$

2.2.12. Scanning Electron Microscopy

Thanks to scanning electron microscope (250 FEG, Fei Quanta, Oregon, United States); microstructure of the film was imaged for both cross-section and surface. Images were screened with different pressure and magnification settings. Uniformity and non-soluble particles in the film were observed by this analysis. The accelerated voltage was 5.0 kV.

2.2.13. Fourier Transform Infrared Spectroscopy

By this method, chemical composition and probable interaction of film was carried out. The device takes measurement with mid-spectra of sample against air. The result was given as actual graph of two measurement average. 5 mL liquid form of film was poured into Zn-Se crystal as a component of FTIR instrument (Spectrum 100, Perkin Elmer, Massachusetts, USA). Liquid form of film in crystal was dried at 40^{o}C for 16 h. Then, the film was ready to measure with hATR equipment. The FTIR analysis was done with 128 scans and 4000-800 cm^{-1} of wavelength. Scan speed was set for 1 cm/s. Two replicated data were combined in one graph that were analyzed.

2.2.14. Moisture Content

Moisture content of the film was determined by the method of Carpine et al. (2015). The film samples are weighed and put into the aluminum plates was also weighed previously. The samples were dried at 110^{o}C for 24 h in an oven. Then, moisture content

was calculated with initial and dried weight of film by following formula. The analysis of film was made with 3 replicates.

$$\text{MC (\%)} = \frac{\text{initial film weight} - \text{dried film weight}}{\text{initial film weight}} \text{ x } 100 \quad (2.7)$$

Moisture content of the snack bar was made according to AOAC 934.06. After the homogenization of sample, 5 g of sample was spread into pre-weighed aluminum dish (M_0). Before drying process, sample and dish was scaled (M_1). It was dried at 70°C for 6 h in a vacuum oven (VO200, Memmert, Büchenbach, Germany) under 100 mm-Hg. After that, hot sample and dish keep cooling in desiccator filled with silica beads. Then, dish and dried sample were weighed as a last measurement (M_2). To get moisture content, the formula below was applied. The analysis of snack bar was performed as duplicates.

$$\text{MC (\%)} = \frac{\text{M1} - \text{M0}}{\text{M1} - \text{M2}} \text{ x } 100 \quad (2.8)$$

2.2.15. Optical Properties

The color parameters of the film and snack bar were determined by a color reader (CR400, Konica Minolta, Sakai, China). Lightness value (L*), greenness to redness (a*) and blueness to yellowness (b*) values were measured by the color reader and recorded. To calculate overall color change (ΔE) with respect to white, the following formula was used.

$$\Delta\text{E} = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (2.9)$$

Films were placed on white background for measurement. 5 different measurements were taken from 3 replicate films. The color snack bars were measured for 5 different positions for 2 replicates.

The light transmittance of the film was measured by using UV visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) at UV visible range (200-800 nm). To find opacity, absorbance was measured at 600 nm (Dick et al., 2016) and divided

by thickness. A greater opacity value shows lower transparency of film. Three pieces were taken from each film and triplicate measurements were carried out.

2.2.16. Total Phenolic Content

To determine the total phenolic content of film, Folin-Ciocalteu method was used based on the method given in Cemeroglu (2013) with some modifications. For this purpose, 50 mg of films were dipped on 10 mL of distilled water and kept it for 10 h to solve film in water. Then, 0.5 mL of film extract was poured, then 2 mL of Folin-Ciocalteu reagent (10% v/v) and 1 mL of sodium carbonate solution (7.5% w/v) was added to extract. It was kept for 30 min in dark at room temperature. In the final, the absorbance of solution was read at 765 nm with UV-spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan).

Folin-Ciocalteu method was used based on Cemeroglu (2013) with some modifications to measure total phenolic content of bar. For this purpose, 20 g of bar were dipped on 70 mL of methanol solution (20:80) and homogenized with mechanical method by hand-blender (AR1092, Arzum, Uskudar, Istanbul) for 2 min. The extraction method was applied depend on De Ancos (2017) method. Then, it was centrifugated to separate the insoluble particles. The supernatant was taken and kept at -20^{o}C until measurement of total phenolic content. The extract was diluted in ratio of 1:25. Then, 0.5 mL of the solution was poured, then 2 mL of Folin-Ciocalteu reagent (10% v/v) and 1 mL of sodium carbonate solution (7.5% w/v) was added to extract. It was kept for 30 min in dark temperature. In the final, the absorbance of solution was read at 765 nm by spectrophotometer. This analysis was conducted with three experiments.

A standard curve (0-100 µL) was plotted by using gallic acid as a standard. The results were obtained from the curve as unit mg of gallic acid per unit gram weight of film. Then, the result was multiplied with dilution ratio.

2.2.17. Protein Content

The measurement of protein in snack bars were done with Dumas method (AOAC 993.13). Determination of protein content was related with nitrogen compounds. In this method, the food was combusted in combustion furnace at 750^{o}C with high-purity oxygen

to convert all nitrogen found in food to gas form as nitrogen oxide. Then, the gas was degraded to elemental form of nitrogen. These processes were occurred automatically by machine (20-20 IRMS, Sercon Ltd., Crewe, UK). The given result was multiplied with 6.25 for not including the collagen compound as the snack bar.

2.2.18. Microbial Analysis

Microbial analyses were conducted for snack bars with pour plate technique to observe antibacterial effect of film above the snack bar. Total aerobic microbes (ISO 4833-1), mold & yeast (ISO 21527-1), *Escherichia coli* (ISO 16649-2), *Coliform* (ISO 4832), *Enterobacteria* (ISO 21528-2), *Bacillus cereus* (ISO 7932), *Staphylococcus aureus* analysis (ISO 6888-1) were done.

Firstly, peptone water as the growth medium of microbes was prepared. The peptone water called maximum recovery diluent (MRD125) was composed of sodium chloride and pectic digest of animal tissue. The 9.5 g of maximum recovery diluent was added to distilled water and kept in autoclave (HG-80, Hirayama, Saitama, Japan) at 121^{o}C for 15 minutes. The sample was prepared by weighing 10 g of bar and putting 90 mL peptone by automated diluter machine (Dilumat Expert Evo, Biomerieux Inc., Illinois, United States). Then, the mixture was homogenized by pedals for 60 seconds (Smasher, Biomerieux Inc., Illinois, United States).

1 mL of 10^{-1} diluted sample liquid was dispersed to empty Petri Dish (9 cm, Fırat Med, Ankara, Turkey). After that, 15 mL for one layer and 5&5 mL for 2-layer agar suitable molten agar cooled to 47 ^{o}C was poured into the petri dish. The plate count agar (PCA-70152, Merck, Darmstadt, Germany) was used for total aerobic microbes, dichloran rose bengal agar (DRBC-17147, Merck, Darmstadt, Germany) for mould & yeast, tryptone bile X-glucuronide agar (92435-TBX, Merck, Darmstadt, Germany) for *Escherichia coli*, 2-layer violet red bile agar (VRB-70188, Merck, Darmstadt, Germany) for *Coliform*, 2-layer violet red bile glucose agar (VRBD-70189, Merck, Darmstadt, Germany) for *Enterobacteria*, mannitol egg yolk polymyxin agar (MYP-105267, Merck, Darmstadt, Germany) for *Bacillus cereus*, Baird-Parker agar (BPA-105406, Merck, Darmstadt, Germany) *Staphylococcus aureus*. Then, agar solutions poured into the petri dishes were mixed by drawing ‘8’ shape.

For the microbial growth, the suitable conditions were at 30 ^{o}C for 3 days for total aerobic microbes, at 25 ^{o}C for 5 days for mould & yeast, at 44 ^{o}C for 1 day for *Escherichia coli*, at 37^{o}C for 1 day for *Coliform* and *Enterobacteria*, at 30 ^{o}C for 1 day for *Bacillus cereus*, at 30^{o}C for 2 days *Staphylococcus aureus*. The petri dishes stored at specific conditions of all microorganisms. Then, live cells were counted manually. The results were taken by duplicates.

2.2.19. Water Activity and pH

Water activity of snack bar was measured by using water activity device (Aqualab 4TE, Washington, United States). The readings were taken as the value via dew point. The analysis was done according to Aramouni et al. (2010) method. Before the measurement, the sample were homogenized mechanically. The plastic sample cup was filled near to half of the cup almost filled with 2 grams of homogenized sample. The cup was placed into measuring chamber of device, then water activity value was measured automatically. Duplicate measurements were taken.

pH value of snack bar was measured with digital pH meter (SevenCompact Duo S213, Mettler Toledo, Zürich, Switzerland) based on the method of AOAC 02-052. For each measurement, 3 grams of sample was homogenized and dissolved by the addition of 15 mL deionized water mechanically by using hand-blender. Sample was dissolved in 20.0 mL of distilled water. Then, by dipping of pH probe, the pH value of the sample was measured. The analysis was conducted as duplicates.

2.2.20. Texture Properties

Textural properties of snack bar were measured by texture analyzer (TA.XT. plus, Stable micro systems, London, England). Duplicated analyses were done with 4 measurements with quarter part of snack bar in dimension of 16x19x13 mm^3 for both analyses. Texture profile analysis was made with 75 mm diameter aluminum compression cylinder. The blade has ‘V’ slot with 60-degree angle. Two compression was applied to 75% of original thickness of sample. Cell load was applied as 50 kilograms. The distance with plate and platform that sample was placed was adjusted to 15 cm. Plate speed was 0.83 mm/sec.

Shear test was conducted with blade set with knife which 10 mm length and 1 mm thickness. The measurement was taken after the snack bar was entirely cut by the blade. Cell load was 10 kilograms when the distance of knife was 15 cm. The cutting was occurred in a speed of 0.83 mm/sec. By texture analysis, shear force, work force to bite, hardness, springiness, cohesiveness, chewiness and firmness values were evaluated.

2.2.21. Sensory Analysis

Sensory analyses were performed by 30 people contains 5 men and 25 women once in a month for 3 months. The products consisting of coated bar kept at climated and normal room conditions and control (uncoated) bar stored at both conditions. The participants include both of semi-trained and trained members. The average of age was about 28. People that join the survey were filled the prepared questionnaire with hybrid hedonic scale (10 points). When 10 means the liked extremely, 1 point represents disliked extremely (Pimentel et al., 2016). The evaluation included taste, odor, texture and appearance of participants. The questionnaire form is added to Appendix B.

2.2.22. Statistical Analysis

Minitab software in version 21 was used for statistical analysis as descriptive method. Experiments were performed as duplicates. General linear model with three factors generalized model was used for probability value was lower than 0.05. For the comparison, Tukey test was used.

CHAPTER 3

RESULTS and DISCUSSION

In this section, results on the film and coating are presented and discussed. Physical, mechanical and chemical properties of the film were examined. For the snack bars, textural, chemical, microbial, physical and chemical analysis were conducted to evaluate the effect of coating in different conditions.

The prepared film is shown in Figure 3. Film analyses were done in this form of edible films.

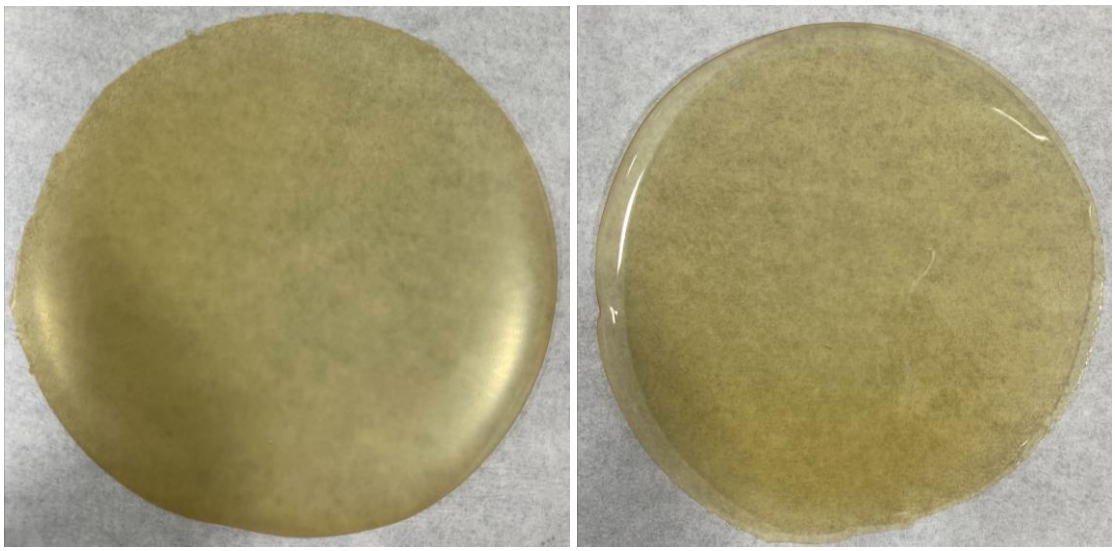


Figure 3: The appearance of prepared edible film (Front and back side)

The edible film was prepared with lentil flour protein, pomegranate peel, glycerol and high methoxyl pectin. The same proportions and ingredients were used to make coating for snack bars by dipping method. To analyze the edible film, film mixture solution was dried at 40^{o}C for 16 h in petri dishes and peeled off. Figure 3 shows the edible film used in film analyses. When the front side was shinier and smoother appearance, the back side was appeared blurrier.

3.1. Film Thickness

The thickness of the edible film was measured as 0.059 ± 0.012 mm. The thickness was directly related with volume of liquid film that was poured to petri dishes before drying. The volume needed for films for proper peeling off from petri dishes at least 18 mL. There have been some studies for the preparation of edible film studied by using less amount of liquid poured (Aydogdu et al., 2018) (Moghadam et al., 2020). Thus, thickness could be reduced by pouring liquid form of film in lower volume, but this application was not suitable for lentil protein and pomegranate peel containing film. In addition to this, the deviation seemed high because of the inconsistency in film thickness which resulted from instabilities in the surface level while placing in drying oven. The way of pouring liquid to petri might variate in height of liquid. Also, drying conditions could be varied, caused to non-homogenous drying of film and differentiated the thickness of dried film.

Moghadam et al., 2020 investigated the characterization of edible film consisted of mung bean protein and pomegranate peel. This study possessed similarity with this thesis regarding film preparation by legume protein and pomegranate peel. Their result on the thickness of film was found as 0.110±0.008 mm for films prepared with 12.5 % pomegranate peel depending on the protein concentration. Also, the thickness of lentil film was found as 0.063 (Aydogdu et al., 2018). Another study showed that the gluten film added to pomegranate peel extract had 0.063±0.008 mm. This value was found close to the thickness value measured in this thesis study, in other words this result was consistent and comparable with the studies as the ingredients were similar.

Studies show that increasing amount of pomegranate peel in edible films increased the thickness due to its rich polyphenol compound (Riaz et al., 2018). Moreover, other antimicrobial compounds had an effect in obtaining thicker films (Emam-Djomeh et al., 2015). Therefore, the thickness value was affected by pomegranate peel content. The ratio of 15% pomegranate peel was used to contribute a good taste and phenolic content.

3.2. Bulk Film Density

Bulk film density was found as 1.14 ± 0.15 g/cm^3. To calculate bulk density, mass and volume were used. To get volume, film area as 4 cm^2 and thickness value as 0.059 mm was used.

In a study, bulk density of the lentil flour film was resulted in the range of 0.97-1.09 g/cm^3 (Aydogdu et al., 2018). Other studies on the banana flour and rice flour, film densities were in the range of 0.94-1.25 g/cm^3 (Pelissari et al., 2013) and around 1.2 g/cm^3 (Dias et al., 2010), respectively. Therefore, the results obtained in this thesis were found in good agreement with the literature results. The density value could be dependent on the glycerol content. Increasing in glycerol concentration made the film thicker (Jouki et al., 2013). Also, the thickness could be affected by differences in film forming and making process. Even if pectin had an effect to form non-homogenous structure, hydroxymethyl pectin did not affect the film density (Giancone et al., 2011).

3.3. Water Solubility

Lower film solubility could be interpreted as good for food protection in coating application (Gontard et al., 1992). On the other hand, higher solubility was related to lower water resistance of film. Higher solubility might be beneficial for many applications (Chana-Thaworn et al., 2011). Water solubility of the film was calculated as 7.94 ± 1.3 among three replicates. The water solubility of the film was found to be lower than many edible films including pomegranate peel powder. For example, the edible film that included gluten and pomegranate peel extract had water solubility value of 34.91 (Kumari et al., 2017). The reason of this could be due to the interaction of phenolic extracts, which caused lower solubility of film because of the phenolic resistance (Jutapom et al., 2011). Furthermore, Moghadam et al. (2020) reported that the moisture value for edible films containing pomegranate peel and mung bean protein contained edible film's water solubility value was 27.60. This value might be affected by pomegranate peel addition. Pomegranate peel caused to rise the pore size and heterogenicity (Hanani et al., 2018).

In this study, lower water solubility value was resulted from the high protein structure. Film solubility directly depended on protein and non-proteinaceous component interaction in the structure of the film (Aguirre et al., 2013). This could be explained with interaction of hydrogen bonds in polyphenols of pomegranate peel and protein molecules, which could limit the formation of protein hydrophilic groups and water molecules (Riaz et al., 2018). Also, lower solubility could be resulted from insolubilized parts of pomegranate peel remained at the end of experiment (Hanani et al., 2019). Moreover,

lentil was related to high level of cohesion in film structure, which caused to more compact structure (Aydogdu et al., 2018). Liu et al. showed that in peanut protein film preparation, temperature used for denaturation of proteins caused to increase hydrophobic interaction. High molecular weight of protein including hydrogen and disulfide bonds formed more crosslinked and compact network by interacting with functional groups, which gets harder to solubilize water. Furthermore, high amylose content found in lentil flour was one of the reasons for obtaining starch-based films with low solubility of these starch-based films (Mehyar and Han, 2006). Moreover, denaturation of protein decreased solubility of particles (Kinsella, 1982). It was proved that glycerol had no effect in film solubility by observation of glycerol film immersed in water for 24 h (Tapia-Blacido et al., 2007). Therefore, the lower solubility of film could be explained with the high protein content.

3.4. Degree of Swelling

Degree of swelling value of the edible film was found as 4.53 ± 0.51 %. In the formula, density of film was taken as 0.114 g/ cm^3. The measured high value could be explained by crosslinking of the lentil protein and pectin content. Pomegranate peel as an antimicrobial agent had no effect on degree of swelling value. Degree of swelling was also the parameter to specify water resistance characteristics (Yu et al., 2014). Swelling degree was measured to find the ratio of the cross-linking of the film. If the edible film had high cross-linkage in its structure when placed into a solvent, it swelled by absorbing of solvents instead of dissolving. When the cross linkage increased in the structure of the film, swelling ratio got lower. In denaturation step, longer denaturation time and higher denaturation temperature resulted in lower degree of swelling value (Perez-Gago & Krochta, 2001). The effect of antimicrobial activity on swelling degree was evaluated by Kumari et al. (2017) and there was no significant effect observed on the degree of swelling by antimicrobial addition. Thus, antimicrobial activity from pomegranate peel had no effect in crosslinking of the film by comparing gluten film and pomegranate peel extract added film. They observed that the swelling degree of antimicrobial film was found as 2.08. This low value obtained for swelling ratio of gluten and pomegranate peel extract might be resulted from gluten-based crosslinking. This low value of swelling degree arised from the higher amount of cross-linking between the gluten and

pomegranate peel ingredient. The different component of the film is gluten that had rich cross-linkage in its structure. Moreover, pectin content could be another parameter effective on swelling degree. When the pectin concentration was increased, degree of swelling was decreased (Matta & Bertola, 2019). In pectin and alginate-based films, the degree of swelling value was between 1.0 and 3.5. In the pH value of 4.5, pectin chains were anionic and protonated free amino groups. The anionic group made the swelling degree higher (Phuong Ngo et al., 2020).

3.5. Contact Angle

This experiment was done to detect wettability of films. It was determined by presence of adhesive and cohesive forces between film surface and water. In literature, if contact angle was less than 90^o, it means that the surface of the film was considered as hydrophilic (Hanani et al., 2018). This value was also related to homogeneity, smoothness and porosity of the film surface, which was dependent on exposure of air during drying process (Basiak et al., 2016).

Contact angle was measured as 22.5 ± 2.75^o that was changing between 18 and 26^o. This showed the film had high wettability and hydrophilicity. According to result of Basiak et al. (2016) study, when the starch film had 43^o contact angle value, the whey protein containing film had 93^o value. The result was also examined with the drawing of sorption isotherms. After 2 minutes, contact angle of starch film was about 30^o while the wettability of protein was very lower contact angle value than 90^o. Also, increasing in protein content caused to get higher contact angle. Protein content increased the polarity and hydrophobicity of the film depending on its hydrophobic amino acid content (Białopiotrowicz and Janczuk, 2002). Thus, the whey protein was considered as a good substance for contact angle measurements. However, this effect was not obtained in lentil flour protein. The result might arise from the possibility of protein denaturation or differences of amino acid compound between the two protein types. Pectin addition caused to the lower contact angle effect by lowering of the hydrophilicity. The contact angle of pectin based edible films was measured as 45.20^o when the nano chitosan film had 73.37^o (Phuong Ngo et al., 2020). McClements (2005) reported that amphiphilic structure of protein causes higher hydrophilicity on film surface. As the glycerol increased hydrophilicity and hygroscopicity of the films, contact angle increased due to

higher water binding capacity. In another study that was conducted by Moghadam et al. (2020) contact angle value of mung bean protein film was found as 44.23^{o}. Fathi et al. (2019) reported that higher concentration of pomegranate peel increased the contact angle by reducing hydrophilic groups. The reduction of hydrophilicity caused to increase in contact angle due to low amount of free hydrophobic groups by forming new interaction. Pomegranate peel addition also resulted in more heterogenicity, larger pore size (Hanani et al., 2018).

Thus, the low value of contact angle in lentil flour protein film enriched with pomegranate peel could be explained by addition of pectin and glycerol and lower amount of pomegranate peel addition. Even pomegranate peel affected the contact angle positively, other ingredients might cause to get lower contact angle values.

3.6. Water Vapor Permeability

Water vapor permeability was determined as 0.455 ± 0.005 ng x m^{-1} s^{-1} Pa^{-1}. The result was calculated from the average value of three replications. Water vapor permeability was measured as the permeability rate of water, which was important for the protection of coated product. Water vapor permeability of lentil flour film was reported as 0.245 and 0.352 ng x m^{-1} s^{-1} Pa^{-1} at different glycerol concentration and denaturation temperature (Aydogdu et al., 2018). These findings were higher than the lentil flour edible film; this might be due to the addition of glycerol, pectin addition and pomegranate peel. Chang & Nickerson (2015) reported that glycerol addition affected the water vapor permeability proportionally. This effect was resulted from hydrophilicity of glycerol due to the reducing intermolecular forces. This free polymer network volume increased water holding capacity, which made easier to pass water vapor into the film (Jouki et al., 2013). The permeability value could be lowered by decreasing of concentration. On the other hand, glycerol in existence of gallic acid could reduce water molecule transfer by interacting with those and provided lower free volume in the film matrix (Rui et al., 2017). For protein containing films, 3D structure of proteins could make the film hydrophobic by enhancement of side chains and increased disulfide bonds based on the denaturation time and temperature. More crosslinking means higher resistance of moisture transfer and decreases water vapor permeability. These values could be higher than synthetic polymers or lower than the biodegradable films. The other study was reported on mung bean protein

and 12.5% of pomegranate peel contained film had water vapor permeability value as 0.361 ng x m^{-1} s^{-1} Pa^{-1}. The permeability increased with the addition of pomegranate peel. It could be resulted from heterogeneous structure of film or presence of void and crack in structure (Matta et al., 2019). The differentiation in water vapor permeability arised from hydrocolloid composition of lentil. Amylose-amylopectin group and amino acid composition had a role for this (Samaranayaka, 2017). Also, water vapor permeability increased with addition of pectin content due to high free hydroxyl group content. (Matta& Bertola, 2019).

Consequently, the higher water vapor permeability could be explained by pomegranate peel and pectin addition. The effect of protein could be reduced by denaturation in different time and temperature values.

3.7. Mechanical Properties

Mechanical properties were measured to describe flexibility and strength of the film (Ruan et al., 2019). During the measurement, stress and strength graph was drawn by application. Tensile strength was inferred from the stress by measurement of length when the sample broke in x-axis of the graph. Tensile strength was measured as 7.60 ± 1.22 MPa. Strain gave the elongation at break from the length when the film sample broke in y-axis of the graph. Elongation at break was 4.63 ± 0.90%. Young's modulus was calculated as 2.706 ± 0.71 from the slope of the stress and strain graph. Tensile properties were affected by raw material and plasticizer (Daudt et al., 2016). As compared with another study, tensile strength was higher than mung bean protein and 12.5% of pomegranate peel film that was 5.16 ± 0.08 MPa, but elongation at break value was lower than that the film which was 163.96 ± 7.22 (Moghadam et al., 2020). It means that the pomegranate peel and lentil protein films were stronger and more fragile. Lentil protein instead of mung bean protein made the film stronger and more fragile. It could be resulted that the composition of various protein type depending on different amylose amylopectin ratio (Salmoral et al., 2005). Protein could be acted as emulsifying agent between oil and water interface, which caused to form low tensioned and cohesive film (Damodaran, 1994). It was reported that pomegranate peel addition in starch film decreased the elongation value. For starch film with 14% of pomegranate peel had elongation value as 5.64 ± 1.17 % and 21.90 ± 2.87 MPa. Thus, starch based edible film result was in good

agreement with our findings (Ali et al., 2019). Pomegranate peel particle filled in starch matrix thanks to its rigid particles and good compatibility properties. Pomegranate peel particles had semi-crystalline structure, which could keep rigidity of the film. When the concentration of pomegranate got higher than 8%, particles placed together. It weakened the mechanical properties. This could be explained by the weak elongation at break (Ali et al., 2019). Moreover, the composition of the film might impact mechanical properties of film. Ebrahimi et al. (2016) reported that mechanical properties might differentiate due to the intermolecular forces in composite films. A study was done by Moghadam et al. (2020) found addition of pomegranate peel increased strength and flexibility of the film significantly. The result of this was considered that pomegranate peel rises molecular mobility and free volume. Also, possible interaction between pomegranate peel and mung bean that were hydrogen bond, electrostatic and hydrophobic forces could impact on tensile properties (Emam-Djomeh et al., 2015). Crosslinking between polyphenols of pomegranate peel and protein could improve the strength of film. Soluble fibers, complex polysaccharide of pomegranate peel acted as plasticizer agent that improved flexibility (Hanani et al., 2018). Glycerol influenced mechanical properties by increasing in elongation at break and decreasing the tensile strength. The mechanism of this effect clarified that glycerol molecules placed in polymer chains to decrease cohesive forces (Muscat et al., 2012). Also, the reason for high tensile strength and low elongation value could process temperature (Aydogdu et al., 2018). High process temperature as 85^{o}C promoted the gelatinization of starch and amylose converted to aqueous phase. This case caused polymer chains got closer links with each other. Therefore, tight matrix provided better strength but poor flexibility. Also, high process temperature denaturated more protein fractions and changed the tertiary structure which led to exposition of sulphite bond. Increasing of crosslinking between polymers resulted to have more rigid films (Andrade-Mahecha et al., 2012). pH could be another factor that affected the mechanical property. Isoelectric point of protein was important for hydrophobic interactions which decreased ionic bonds. Hydrophobic bonding enhanced the tensile properties (Hamaguchi et al., 2007). To have well elongated film, higher value of pH could be used in process that was far from the isoelectric point of lentil protein.

The lower elongation value could be explained with pectin addition. Because neither pomegranate peel nor lentil film had that low value mentioned above. Pectin might result in a stiffer edible film (Phuong Ngo et al., 2020). The study (Jouki et al., 2013) showed that glycerol improves the tensile strength and lower elongation value.

3.8. Scanning Electron Microscopy

Figure 4 and Figure 5 includes photos that were taken by Scanning Electron Microscopy. In Figure 4, the edible film was placed in vertical form in the electron microscope and observed in different scales. The related photos could be seen in Figure 4. In Figure 5, the surface of the edible film was screened. There were non-homogenous structures observed that is placed in Figure 5.

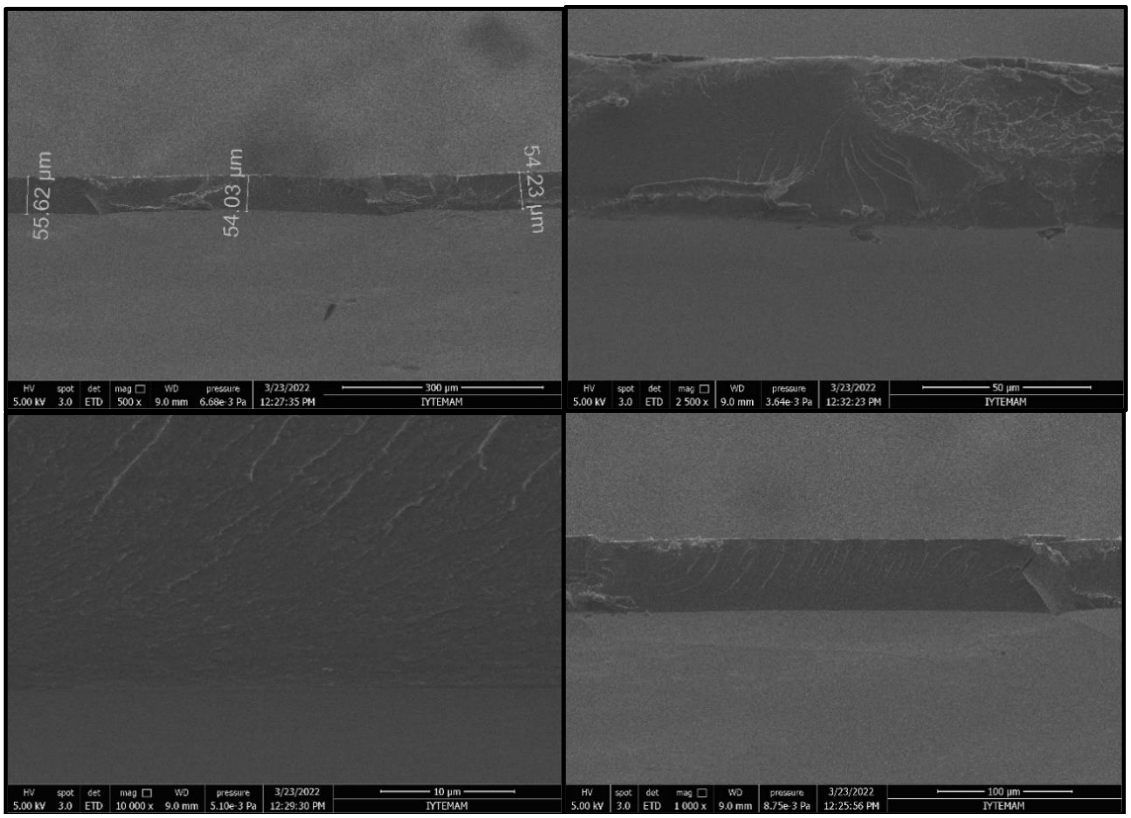


Figure 4: SEM image of cross section of lentil protein films combined with pomegranate peel

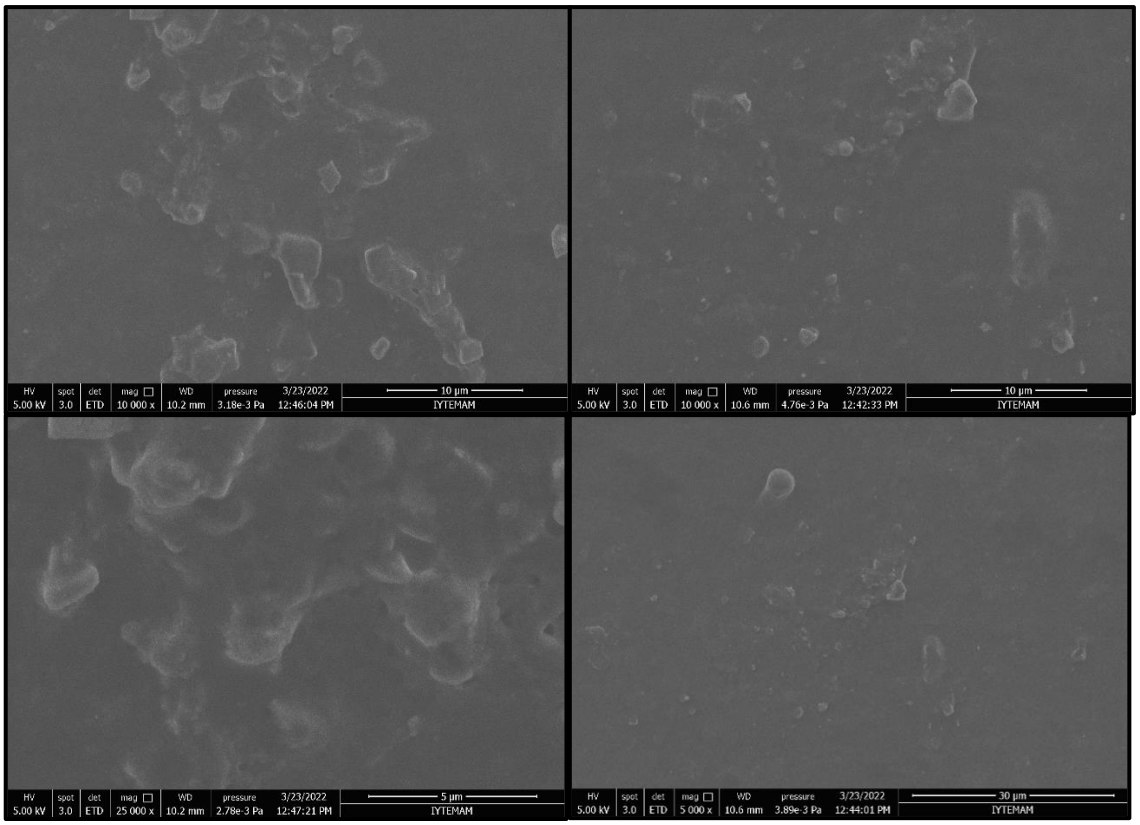


Figure 5: SEM image of surface of lentil protein films combined with pomegranate peel

Scanning with electron microscopy is a method to observe structural morphology to investigate flexibility and fractural behavior of the film. The screened image was observed as similar microstructure in Moghadam et al., 2020.

Cross sectional images with different magnifications can be seen in Figure 4. There was different magnificated images were placed. In the first photo, thickness value could be read from the SEM image. In the second image, there was some layer detected. Its source could be the formation of intermolecular forces between protein and bioactive molecules (Emam-Djomeh et al., 2015). In the third image, there were parallel lines monitored because the cross section was cut by using scissor. To prevent this scene, the film should be cut with the help of liquid nitrogen.

Surface images are found in Figure 5. Surface image of the film was generally smooth and uniform, but small dots in the film surface were observed when the screening was zoomed. There might be incorporated and insoluble part of pomegranate peel in the film. Also, these particles led to higher permeability due to the heterogenous structure. There were some studies carried out with pomegranate peel showing similar results with this thesis (Moghadam et al., 2020) (Hanani et al., 2019).

There was no crack observed and it had a continuous structure without the pores for both dimensional images. Thus, it was deduced that network of lentil protein and pomegranate peel molecules combined well and formed a continuous network.

3.9. Fourier Transform Infrared Spectroscopy

FTIR spectra of lentil protein films incorporated with 15 % of pomegranate peel was determined as seen in Figure 6.

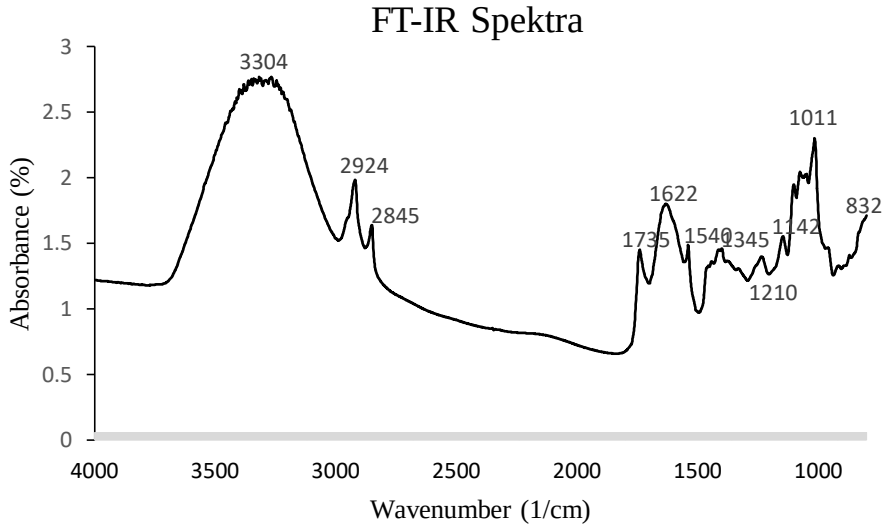


Figure 6: FT-IR spectra of lentil protein films incorporated with 15% of pomegranate peel

FTIR showed the molecular structure by irradiation of the sample. Then, the absorbance of radiation was taken by vibrational motions. (Gomez et al., 2003). Absorption value changed depend on frequencies caused by molecular modes and motions (Sacksteder and Barry, 2001). The wavenumber worked out between 800 and 4000 cm^{-1} is called as mid-IR spectrum. This spectrum was divided into 4 regions. 500-1500 cm^{-1} range was fingerprint region. 1500-2000 cm^{-1} range was double bond region (C=C, C=O, C=N) when 2000-2500 cm^{-1} range showed triple bond region (C≡C, C≡N). Wave number range between 2500 and 4000 cm^{-1} demonstrated single bonds (O-H, N-H, C-H) (Nandiyanto et al., 2019).

Peaks at 832, 1011, 1142 and 1345 cm^{-1} were in fingerprint region. Peak at 832 cm^{-1} could be the marker for aromatic aryl ring with C-H bond (Coates, 2000). These could

be resulted from aromatic amino acid related with phenylalanine, tryptophan and tyrosine from lentil (Samaranayaka, 2017). Also, these could be resulted from sinapic acid, catechin or epicatechin (Xu and Chang, 2010). The peaks at 1011 and 1142 cm^{-1} signed to include secondary alcohol, C-O stretch (Coates, 2000). It was considered that it could be the glycerol as a plasticizer added to the film. In a similar study (Cano et al., 2015), 800-1300 cm^{-1} band indicated the stretching vibrations of C-O in C-C-O, C-C and C-O-H bond in starch and glycerol. The peaks placed on 1540, 1622 and 1735 cm^{-1} in double bond region. Peaks at 1540 and 1622 cm^{-1} were related with primary and secondary amine group (N-H) from lentil protein (Coates, 2000). Also, these peaks showed amide I group by stretching the vibration of C=O and amide II group from N-H and C-N vibration group (Kudre et al., 2013). These peak values were resulted from interaction of protein and phenolic compound. It was proved by Parveen et al. (2019), these two peaks confirmed the incorporation of pomegranate peel and lentil protein due to the interaction of polypeptide chain. The other two peaks at 2924 and 2845 cm^{-1} between 2500 and 3000 cm^{-1}, which indicated CH_2 and C-H bonds, which showed amylose and amylopectin (Cano et al., 2015) and the C-H stretching vibrations (Chentir et al., 2019). In 3000 and 3500 band, there was center point peak at 3304 cm^{-1} which means -OH group stretch from the hydrogen bond in specimen (Pelissari et al., 2013). Peak at 3304 cm^{-1} showed the O-H stretching and overlapping of N-H stretching vibrations (Chentir et al., 2019).

As it was expected, FT-IR analysis gave result of the phenolic compound from pomegranate peel, protein from lentil flour and their interaction products. Many similar results were reported in previous studies given above.

3.10. Protein Content

Protein content of lentil flour and lentil flour extract was measured to determine the efficiency of extraction method. When the protein content of lentil flour was measured as 18.67 %, protein content of the extract was found as 23.15 %. Thus, the protein could be extracted from lentil flour with 81.65 % effectivity from the lentil flour. This result was agreed with the study of Bamdad et al. (2006). In this study, protein content of lentil was measured as 23.32 ± 0.4 % and lentil protein isolate was 68.86 ± 0.33 %. These results were also proven by another study (Aparna et al., 2000) which protein content of legume was reported in between 21-25%. The efficiency of alkaline extraction method

was in yield of 50.3-62.8 % (Boye et al.,2010). pH 9.0 at 30ºC was the optimum condition to get 56.6 % of lentil protein (Jarra-Parra, 2017).

Also, the coated snack bar and control snack bar protein contents were analyzed to observe the effect of coating to protein content regarding to functionality of snack bars. When the coated snack bar had 5.44 % of protein content, the control (uncoated) snack bar had 5.64 % of protein content. Therefore, the extraction of lentil and application method of edible coating was suitable for the functionalization of snack bar by enriching protein content. The enrichment ratio was about 3.68% for each snack bars. Therefore, it could be thought that functionalization of the snack bar was succeeded.

3.11. Moisture Content

Moisture content of the film was found as 16.71 ± 1.61 %. This finding was supported by the study of Aydogdu et al. (2018) as they found the moisture content value of lentil film was 16.77 ± 2.24 %. Moisture content was affected by increasing glycerol concentration because glycerol increased the retention of water and hydrophilic nature. Lower value of moisture content caused to form more stable and water-resistant film (Aydogdu et al., 2018). According to Hanani et al. (2019), addition of pomegranate peel increased the hydrophobicity since pomegranate peel limited the water retention due to its insolubilized parts. Another parameter that had an impact on the moisture content might be hydrophobic bonds between lentil protein and antimicrobial compounds (Emam-Djomeh et al., 2015). In the other study (Moghadam et al., 2020), moisture content was measured as 23.44 ± 0.31% in mung bean and pomegranate peel added film. This change could be resulted from the different protein types in their structure.

Moisture content of the snack bars were measured with two replications during the observation time in every month at 0, 30, 60 and 90th days. The measured values are shown in Table 6. Condition factor presents climated conditions in 70% RH and 35 ºC and normal room conditions in 35% RH and 25ºC.

Table 6: Results of snack bar's moisture content under various parameters

Time (Day)	Coating	Conditions	Moisture Content (%) *
0	Coated	Normal	10.18±0.014 [a]

(cont. on next page)

Table 6 (cont.)

0	Coated	Climated	10.18±0.014 [a]
0	Uncoated	Normal	12.445±0.021 [b]
0	Uncoated	Climated	12.445±0.021 [b]
30	Coated	Normal	11.355±0.021 [c]
30	Coated	Climated	12.415±0.007 [c]
30	Uncoated	Normal	13.15±0.141 [d]
30	Uncoated	Climated	12.8±0.014 [d]
60	Coated	Normal	10.07±0.042 [a]
60	Coated	Climated	10.01±0.056 [a]
60	Uncoated	Normal	11.52±0.099 [b]
60	Uncoated	Climated	12.46±0.028 [b]
90	Coated	Normal	10.24±0.014 [a]
90	Coated	Climated	11.405±0.247 [a]
90	Uncoated	Normal	11.2±0.028 [b]
90	Uncoated	Climated	12.03±0.056 [b]

*Different letters on the same column shows significant difference for $P<0.05$

To evaluate the values measured, chart of moisture was drawn for effects of coating, time and conditions are observed shown below in Figure 7.

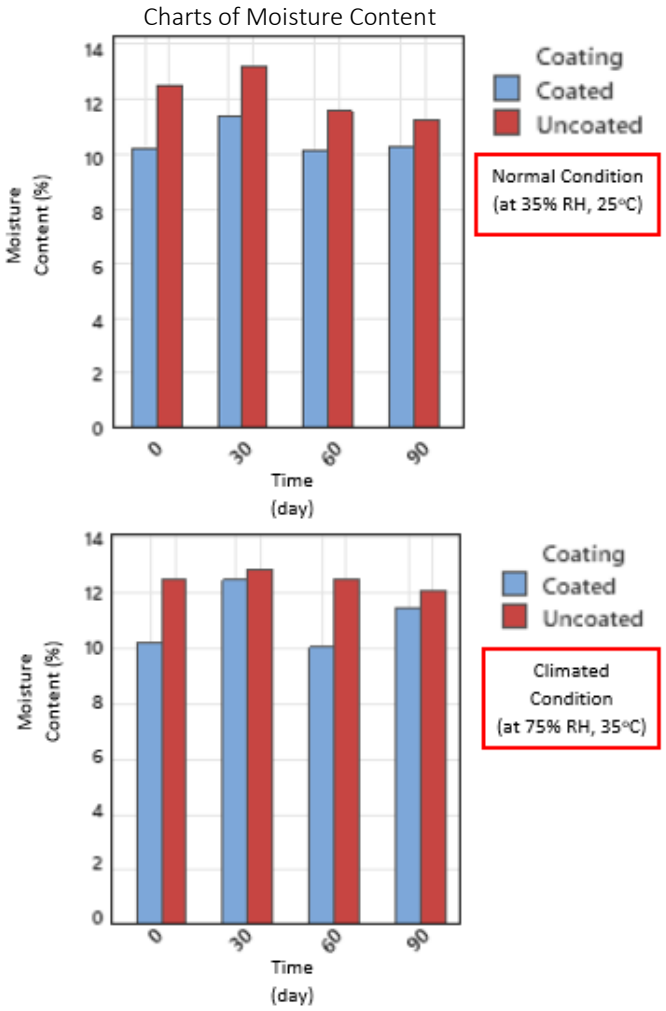


Figure 7: Chart of moisture content for different factors

By looking at the values in Figure 7, moisture content of coated snack bars had lower value than uncoated snack bars. Even the moisture content increased in the first 30 day, it decreased with prolonged shelf-life. The reasons of these changes are discussed as follows. When the statistical analysis was performed, time, conditions and coating factors were evaluated and found as the significant parameters for moisture content by ANOVA table. By controlling of R^2 values, the results were dependable. In this design, coating was the most effective parameter for moisture content change. Figure 8 shows the main effects plot for moisture to see the effect of time, coating and conditions.

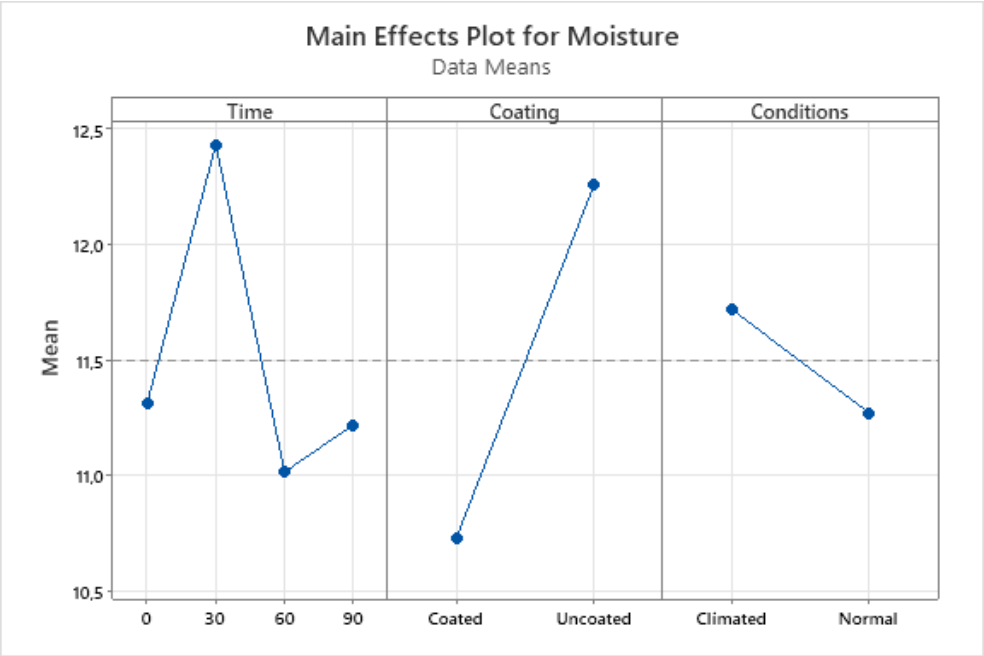


Figure 8: Main effects plot for moisture content for different factors

In time, there was no regular graph, moisture content measurement in 30th day had inconvenient substance. It would be expected that moisture content value decreased during shelf life (Anandito et al., 2017). Except for the 30th day experiment, the data showed the expected result. When the coating effect was observed, the coated bar had lower moisture activity for 90 days. But the expectation was of course in parallel with the optimum achievements for the edible film, which formed protective layer while maintaining the moisture content (Yıldız & Yangınlar, 2016). This lower moisture content value was resulted from the drying process of coating in surface of drying. Therefore, the coated snack bars were exposed to temperature at 40 ^{o}C for 16 hours. This process caused to moisture loss. Moreover, storage conditions were evaluated depend on the value of coated snack bars during storage time. When normal conditions were 35% RH and 25 ^{o}C, the climated conditions were 70% RH and 35 ^{o}C. It was enabled to observe higher moisture content in climatized condition. It was expected because moisture and temperature values were higher than normal conditions. At high temperature and moisture content values of storage conditions, the product tended to lose moisture (Abasi et al., 2009).

3.12. Optical Properties

Color is the significant parameter to attract or distract the customer for the food that is coated because it affects the appearance of food directly (Mehdizadeh et al., 2012). The optical property of edible film was measured. The lightness value (L) was measured as 85 ± 0.38. a value that means greenness to redness was found as 0.18 ± 0.22. The b value which means blueness to yellowness was 18.06 ± 0.39. The results show that the film has lighter and yellowish color. The optical values of mung bean protein and 12% of pomegranate peel added film had L, a, b value results were 72.15, 3.81 and 46.86 respectively. Lentil gave highly yellowish color to the film (Samaranayaka, 2013). The yellowish color was resulted from anthocyanin and carotenoids found in lentil structure (Lee et al., 2017). Glycerol was a colorless compound that was not affected on the film color of the film. In this study, pomegranate peels made the color darker, which resulted to lower L value and higher and b (Moghadam et al., 2020). In comparison of two studies, the color change might be resulted from the mung bean color. This orange or reddish color was caused by anthocyanins found in pomegranate peel. The substance made the fruit's color e.g. orange, red, purple (Hanani et al., 2019). In other study (Emam-Djomeh et al., 2015), an increase in the concentration of pomegranate peel was detected with resulting in higher darkness, redness and yellowness due to the colored nature of pomegranate. Pectin was also translucent and faint yellowish color, which could increase the color intensity of the film (Matta & Bertola, 2020).

The opacity value of the film was measured as 1.08 ± 0.024 absorbance value. Aydogdu et al. (2018) observed the lentil flour films had an opacity value between 3.65 and 5.51. The appearance of lentil flour films was transparent to visible light. The higher process temperature made the film lighter, more transparent and yellower. The lower opacity value could be resulted from the pomegranate or pectin addition. According to Moghadam et al. (2020), brightness was reduced by the addition of pomegranate peel. Their study showed that addition of pomegranate peel gets lowered opacity.

For the snack bars 5 different measurements were taken from different location of 2 replicated snack bars. The average value was evaluated for snack bars. The measured values of color parameters are written in Table 7 below.

Table 7: Results of snack bar's color variables in differing parameters

Time (Day)	Coating	Conditions	L*	a*	b*
0	Coated	Normal	37.27±1.98 [a]	11.51±0.68 [a]	16.69±1.51 [a]
0	Coated	Climated	37.27±1.98 [b]	11.51±0.68 [b]	16.69±1.51 [b]
0	Uncoated	Normal	38.06±1.28 [a]	12.35±1.02 [a]	18.43±1.29 [a]
0	Uncoated	Climated	38.06±1.28 [b]	12.35±1.02 [b]	18.43±1.29 [b]
30	Coated	Normal	33.66±2.37 [c]	9.31±0.87 [c]	12.14±1.85 [c]
30	Coated	Climated	29.85±1.67 [d]	6.17±0.75 [d]	7.38±1.58 [d]
30	Uncoated	Normal	35.42±1.58 [c]	10.06±0.74 [c]	14.22±1.23 [c]
30	Uncoated	Climated	29.77±1.30 [d]	6.22±0.64 [d]	7.62±1.81 [d]
60	Coated	Normal	34.52±1.33 [c]	6.42±0.88 [e]	14.85±1.46 [c]
60	Coated	Climated	29.94±1.47 [d]	3.63±0.55 [f]	9.40±1.67 [d]
60	Uncoated	Normal	35.34±2.89 [c]	6.38±1.65 [e]	15.01±0.96 [c]
60	Uncoated	Climated	27.46±1.10 [d]	2.55±0.41 [f]	7.38±1.04 [d]
90	Coated	Normal	33.01±1.87 [c]	5.34±0.87 [e]	14.46±1.11 [c]
90	Coated	Climated	27.19±0.91 [d]	2.36±0.54 [f]	6.84±1.14 [d]
90	Uncoated	Normal	33.76±2.08 [c]	5.63±0.84 [e]	14.19±1.59 [c]
90	Uncoated	Climated	26.91±0.33 [d]	1.39±0.58 [f]	6.28±0.61 [d]

*Different letters on the same column shows significant difference for $P<0.05$

When all parameters were examined separately, it was found that coating was not significant value as probability values were higher than α value. L, a, b value was affected by time and environmental conditions. L, a, b was not affected by the presence of coating. Thus, comparative test could not be applied for coating. When condition was more significant in b and L value, time was more significant in a value. The effects were observed by checking F values. The models that were made dependable models depend on R^2 values.

The color change (ΔE) was calculated from the measured value by the formula and evaluated statistically for time, condition and coating type. According to ANOVA table, affecting significant factors of color change was detected in time, coating and condition change. Their probability values were lower than α values as 0.05. The data

was normally distributed and reliable with R^2 values higher than 90%. In the Figure 9, calculated color change values are demonstrated.

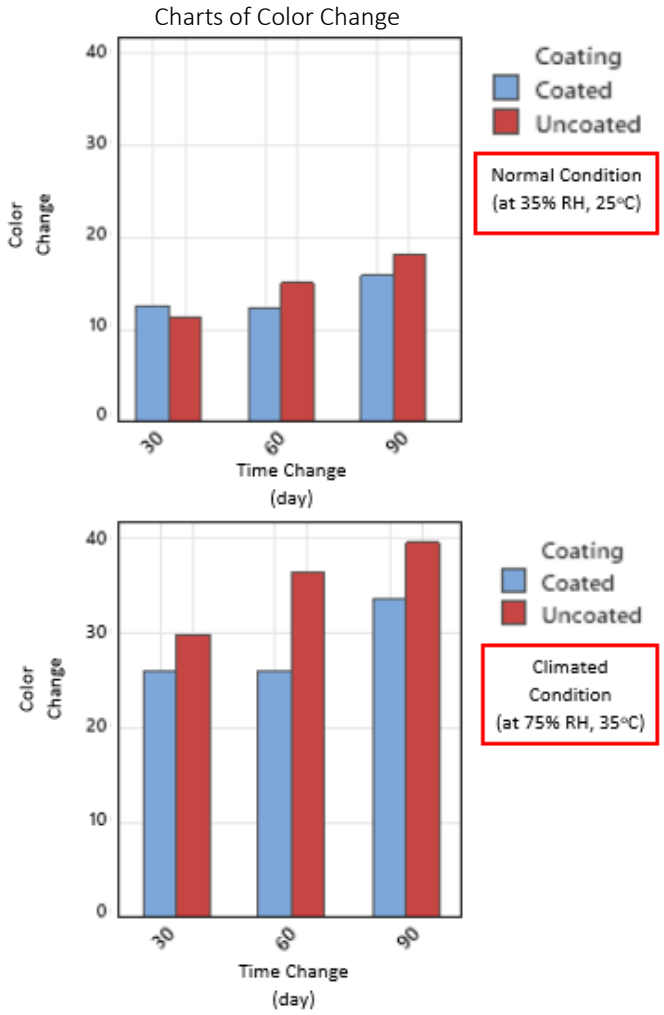


Figure 9: Chart of color change for different factors

There was obvious difference in color of snack bars stored in both conditions as seen in Figure 9. By looking at measured values, in the beginning, the coated bar had slightly darker than uncoated bar because of the drying of coating at the surface boundaries. In the 90th day, the color change in coated bar had lower than uncoated bars, so it could be envisaged that the coating protected color for both conditions. Climate conditions had negative effect on the color of the bars continuously. When coated and uncoated snack bars had darker color under climatized conditions at 70% RH and 35 ^{o}C, other bars stored in normal conditions at 35% RH and 25 ^{o}C which maintained nearly at the starting color value. Even, uncoated bars at the 90th day had lighter color than the

uncoated stack bars the 0^{th} day at the end of observation time. But the coated bar had lower change after and before the storage under normal conditions.

The importance of parameters that affected the color change could be ordered as condition, time and coating respectively as could be understood from F-value in ANOVA table for each value. It was also supported by the separated color value, appearance and sensory evaluation. The effect of main factors was shown in Figure 10 apparently.

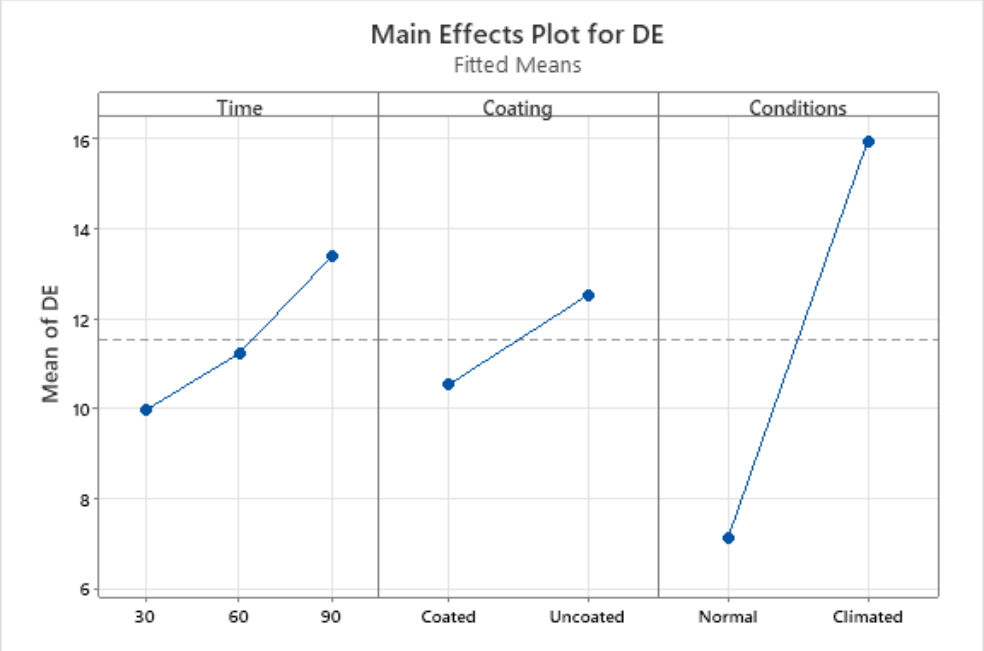


Figure 10: Main effects plot for color change for different factors

In Figure 10, it was observed that the condition was most effective parameter as discussed earlier. Color change was seen in the values between normal and climated condition. The color difference was very high in climated conditions. It was supposed that when stored in higher temperature and moisture conditions resulted with darker color (Agudelo-Laverde et al., 2015). The second affecting parameter was the time; when time was passing, color change increased proportionally. It was expected that dried fruit-based food products especially including fig showed darker color with time (Sen et al., 2015). The lowest effective factor was the coating among these measured parameters. Even if it was the least effective parameter on the color change, it was a significant parameter. With the presence of protein and sugar content in high temperature, there could be Maillard reaction in climated conditions. Thus, the coating might be protected the coated snack bar

(Sen et al., 2015). Therefore, the coating on the surface of the bar could protect the color at different conditions (Valdés et al., 2017).

3.13. Total Phenolic Content

The total phenolic content of the film was reported as 17.22±0.07 mg GAE/g film by Folin-Ciocalteu method. It was proved that phenolic content value of the edible film was improved by pomegranate peel (Moghadam et al., 2020). The total phenolic content of the edible film composed of 12.5% of pomegranate peel and mung bean protein was measured as 7.59 mg GAE/g film, which was lower than this study. This low result could be resulted from other ingredients or process that applied in film. Some amino acids like tyrosine and histidine might react with Folin-reagent (Wu et al., 2019). As it was mentioned in introduction part, pomegranate peel has rich content of phenolic compounds including catechin, punicalagin, gallic and ellagic acid (Smaoui et al., 2019). Another study agreed with the pomegranate peel affected phenolic content value (Kumar et al., 2019). Also, pomegranate peel's phenolic content was found as 186 mg/g, which could differentiate depending on cultivars (Emam-Djomeh et al., 2015). In the lentil, there is 12 mg/g total phenolic content was detected (Han & Baik, 2008). Lentil flour included 6.21 mg GAE/g phenolic content (Ettoumi etal., 2015). The film phenolic content was affected by both lentil flour and pomegranate peel.

For the total phenolic content of snack bar, Folin-Ciocalteu method was used. The analysis was done with triplicates. The calculated values are placed in Table 8 below.

Table 8: Results of snack bar's total phenolic content in different parameters

Time (Day)	Coating	Conditions	Total Phenolic Content* (mg GAE/g)
0	Coated	Normal	916.01±21.12 [a]
0	Coated	Climated	777.7±18.88 [b]
0	Uncoated	Normal	602.08±5.34 [a]
0	Uncoated	Climated	607.83±4.77 [b]
30	Coated	Normal	850.08±51.94 [c]

(cont. on next page)

Table 8 (cont.)

30	Coated	Climated	890.5±64.617 [d]
30	Uncoated	Normal	623.25±10.0 [c]
30	Uncoated	Climated	886.83±12.77 [d]
60	Coated	Normal	793.71±32.41 [e]
60	Coated	Climated	1183.08±101.55 [f]
60	Uncoated	Normal	741.63±64.067 [e]
60	Uncoated	Climated	1216.92±81.117 [f]
90	Coated	Normal	620±73.317 [g]
90	Coated	Climated	1341.58±119.66 [h]
90	Uncoated	Normal	871.08±24.55 [g]
90	Uncoated	Climated	1118.25±131.77 [h]

*Different letters on the same column shows significant difference for $P<0.05$

The measured values were examined in bar chart in comparison to coating, time and conditions, which is shown in Figure 11.

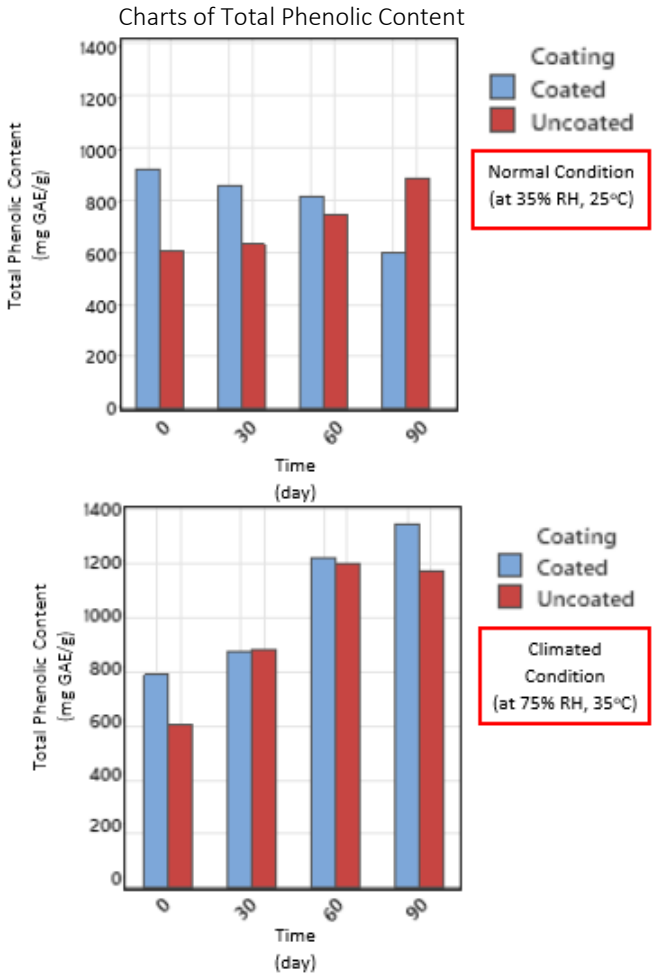


Figure 11: Chart of total phenolic content for different factors

At the beginning of the experiment, it was detected that the coated snack bar had higher value than uncoated snack bar. Effect of coating was observed in total phenolic content as it is expected (Martinez-Gonzalez et al., 2020). Also, the snack bar had phenolic content in it. It could be explained that dried fruit had higher phenolic content than fresh fruit because drying improved the phenolic content (Lutz et al 2015). So, the snack bar had rich total phenolic compound even for uncoated form. It was aimed to increase and protect the phenolic content with the coating. It could not be said that it was effective for this coating depending on the measured values.

For the statistical analysis of total phenolic content, general linear model with three factor was applied. When the condition affected to phenolic content mostly, time was the second affecting parameter for this value depending on F value. Coating was not a significant value depending on α value, but it was very close to being significant. The difference was very low probability and α value, so coating had a slight effect on total

phenolic content. To see the effects, the main effect of phenolic content graph is evaluated in Figure 12.

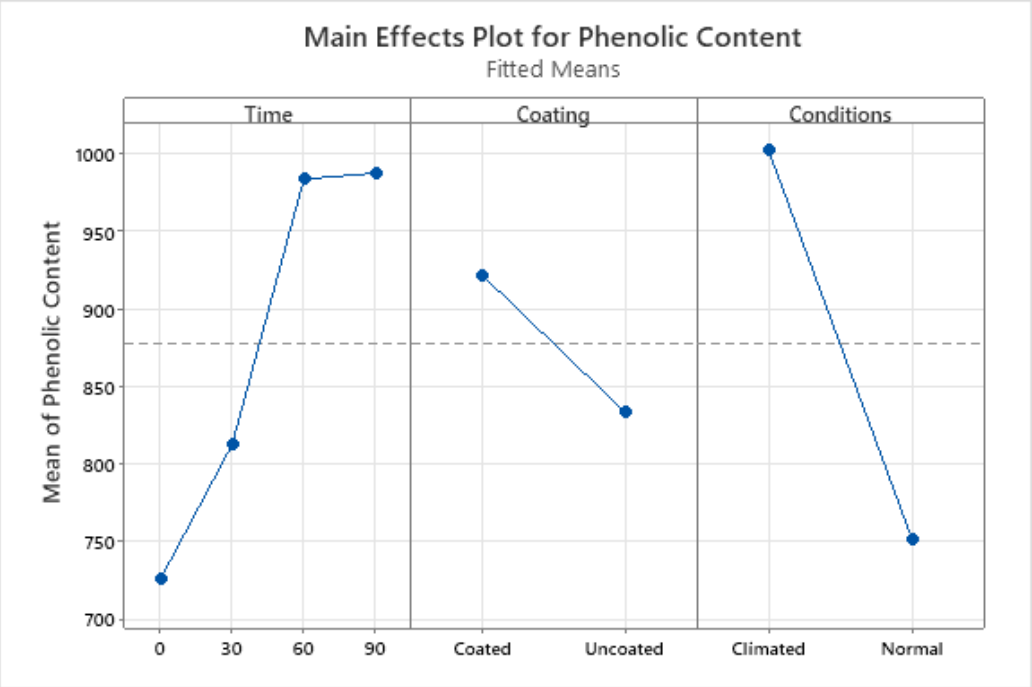


Figure 12: Main effects plot for total phenolic content for different factors

It was observed that phenolic compound increased in time, which was an unexpected result. Even phenolic content prolonged the shelf life, the phenolic content was supposed to be reduced during the shelf life (Deng et al, 2018). The reason of this could be the measurement method by UV-spectroscopy. The UV spectroscopy took measurement affected by color tone with UV light. Consequently, it was proved that the color got darker in shelf life explained in color measurement. The measurement could be affected by the darker color of the product within time, which caused to measure incorrect result (Wilson et al., 2008). This was also supported by the color change results. Also, the unexpected result in 60th day measured could be caused by sensitive phenolic compounds. They were affected in presence of light, temperature etc. The phenolic content might be exposed to these factors (Sotillo et al., 1994). Thus, unsuitable condition might be occurred in this step.

The phenolic content of coated snack bar was higher than the uncoated one as it was expected. Also, there was a good effect of coating to protect phenolic compounds depend on the values as it was expected (Martinez-Gonzalez et al., 2020).

Moreover, total phenolic content of snack bars in climated conditions were measured as higher phenolic content. However, the total phenolic content should decrease more in higher temperature and moisture value (Ghafoor et al., 2019). Therefore, it was resulted from the color change in climated conditions. The method gave incorrect results.

3.14. Microbial Analysis

The microbial analyses for the snack bars were done for total aerobic bacteria count, mould, yeast, *Escherichia coli*, Coliform group, *Enterobacteria*, *Bacillus cereus* and *Staphylococcus aureus*. There was no microbial growth detected in *Escherichia coli*, Coliform group, *Enterobacteria* and *Staphylococcus aureus*. Therefore, there was no observed effect upon changes of these microorganism growth. Statistical analyses for these microorganisms could not be done. Statistical method was conducted for total aerobic bacteria, mould, yeast and *Bacillus cereus*. The measured results of microbial analyses are written on Table 9 below.

Table 9: Results of microbial analysis at different parameters

Time (Day)	**Coating**	**Conditions**	**TABC* (cfu/g)**	**Mould* (cfu/g)**	**Yeast* (cfu/g)**	***B.cereus** (cfu/g)*
0	Coated	Normal	2300 ± 0.0^{a}	93 ± 3.53^{a}	110 ± 0.0^{a}	138 ± 10.6^{f}
0	Coated	Climated	2290 ± 14.14	107 ± 11.31^{b}	99 ± 4.24^{c}	91 ± 3.53^{f}
0	Uncoated	Normal	3675 ± 35.35	103 ± 4.24^{c}	106 ± 2.12^{a}	114 ± 9.19^{d}
0	Uncoated	Climated	3663 ± 53.03	103 ± 3.53^{d}	99 ± 5.65^{c}	183 ± 17.67^{d}
30	Coated	Normal	995 ± 7.07^{c}	84 ± 2.82^{c}	97 ± 2.82^{d}	105 ± 7.07^{e}
30	Coated	Climated	1775 ± 35.35^{c}	80 ± 7.07^{d}	83 ± 1.41^{e}	118 ± 24.74^{e}
30	Uncoated	Normal	2450 ± 176.7^{d}	82 ± 8.48^{g}	80 ± 9.19^{d}	218 ± 25.45^{b}
30	Uncoated	Climated	1485 ± 120.21^{d}	92 ± 6.36^{f}	86 ± 0.0^{e}	179 ± 22.62^{b}
60	Coated	Normal	655 ± 7.07^{c}	77 ± 1.41^{i}	84 ± 2.12^{f}	60 ± 3.53^{e}
60	Coated	Climated	1365 ± 21.21^{c}	61 ± 3.53^{j}	73 ± 0.71^{g}	133 ± 17.67^{e}
60	Uncoated	Normal	2473 ± 180.31^{d}	80 ± 7.07^{k}	91 ± 2.82^{f}	338 ± 10.61^{b}
60	Uncoated	Climated	1223 ± 109.60^{d}	75 ± 1.41^{o}	63 ± 13.43^{g}	197 ± 26.16^{b}

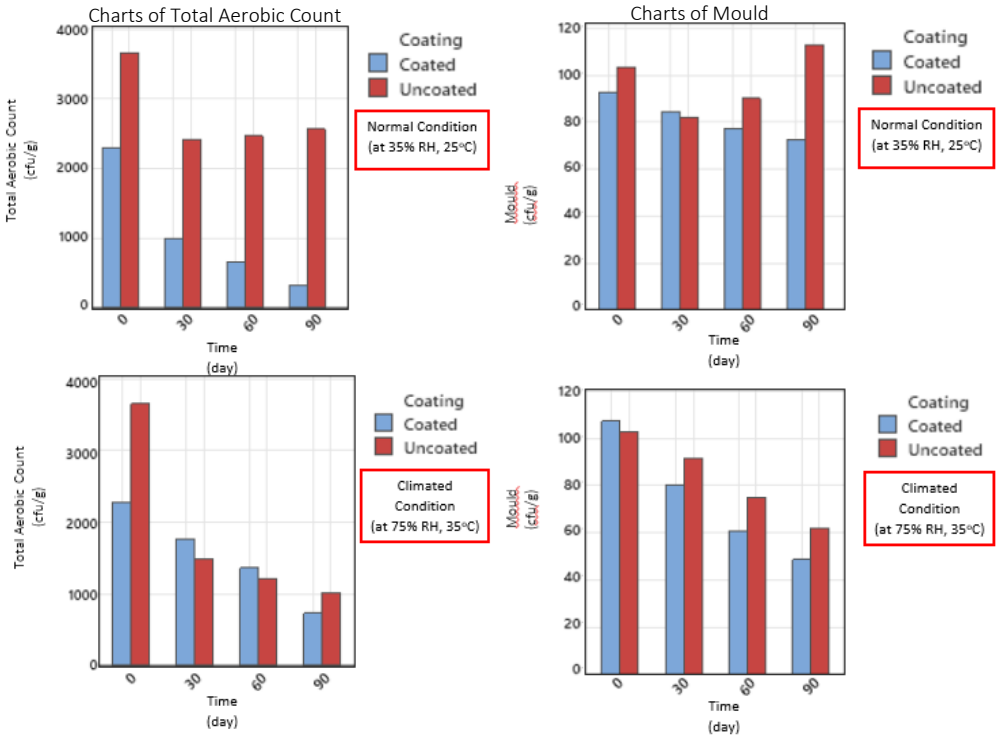
(cont. on next page)

Table 9 (cont.)

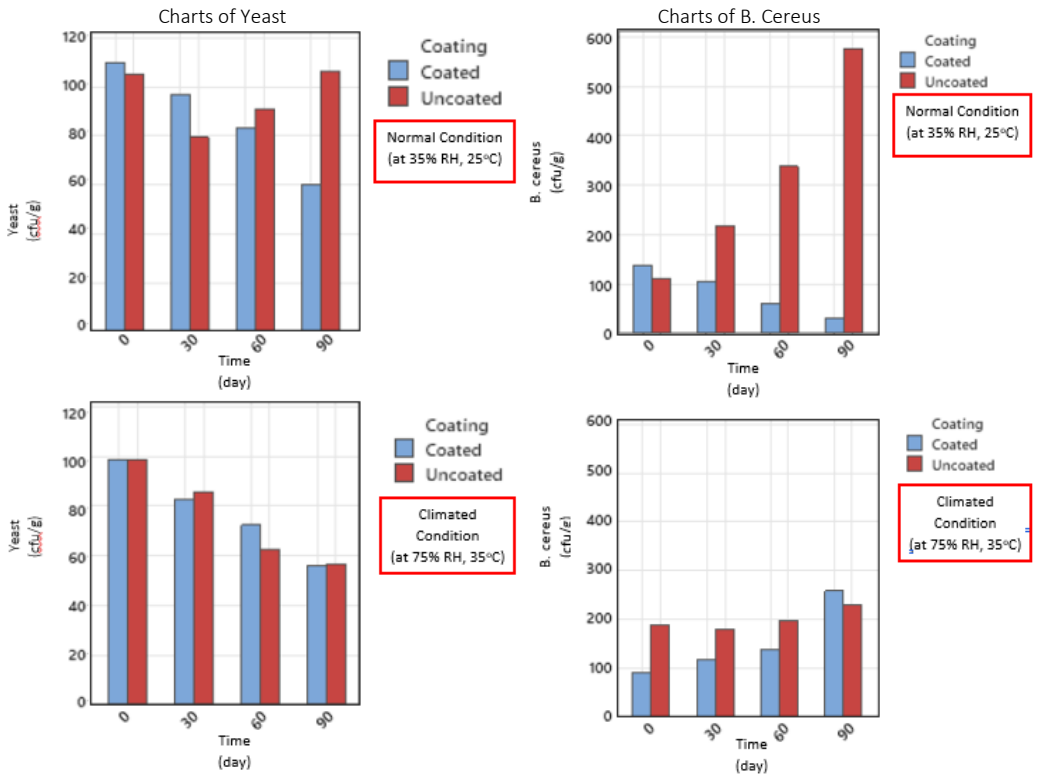
90	Coated	Normal	335±21.21^{c}	73±3.53^{i}	61±3.53^{b}	30±11.31^{a}
90	Coated	Climated	733±45.96^{c}	49±4.94^{j}	56±5.65^{h}	258±10.61^{c}
90	Uncoated	Normal	2575±176.77^{d}	113±3.53^{i}	107±2.12^{b}	575±35.35^{a}
90	Uncoated	Climated	1020±42.42^{d}	62±4,94^{j}	56±12.02^{h}	235±28.28^{c}

*Different letters on the same column shows significant difference for P<0.05

The bar chart below is drawn from the measured values in Figure 13.



(I) (II)



(III) (IV)

Figure 13: Chart of microbial analysis for different factors (I-Total aerobic count, II-Mould, III-Yeast, IV-*B.cereus*)

For all microorganism, coating had an effect to prevent microbial growth except for *Bacillus cereus* in snack bar in climated conditions. This could not be confirmed for uncoated snack bars, which showed irregular design in changing condition and microorganism.

For the total aerobic count, time and coating were the significant factors depending on α value. Coating showed more significant effect than the time. Thus, it can be said that this coating could be a solution against the growth of total aerobic bacteria. For mould growth, all factors were significant by looking at the α value in ANOVA table. In mould formation, significant parameters could be written as time, coating and conditions respectively depending on F-value. Even if time was more significant than the coating, coating was very close to time. Coating was also very effective parameter in mould growth. It could be used to prevent the mould formation. When the ANOVA table was examined for α value of significant parameter, coating was not effective in yeast growth. Except from this, time and condition were significant parameters to inhibit yeast. However, this coating could not be preferred to prevent yeast. For the *Bacillus cereus*, coating was the most significant parameter among all significant factors. Then, time

passing was important parameter for *B.cereus*. The coating could be a good solution to prevent bars from *Bacillus cereus* contamination.

The effect of main factors for total aerobic count, mould, yeast and *Bacillus cereus* are plotted and observed the effects in Figure 14.

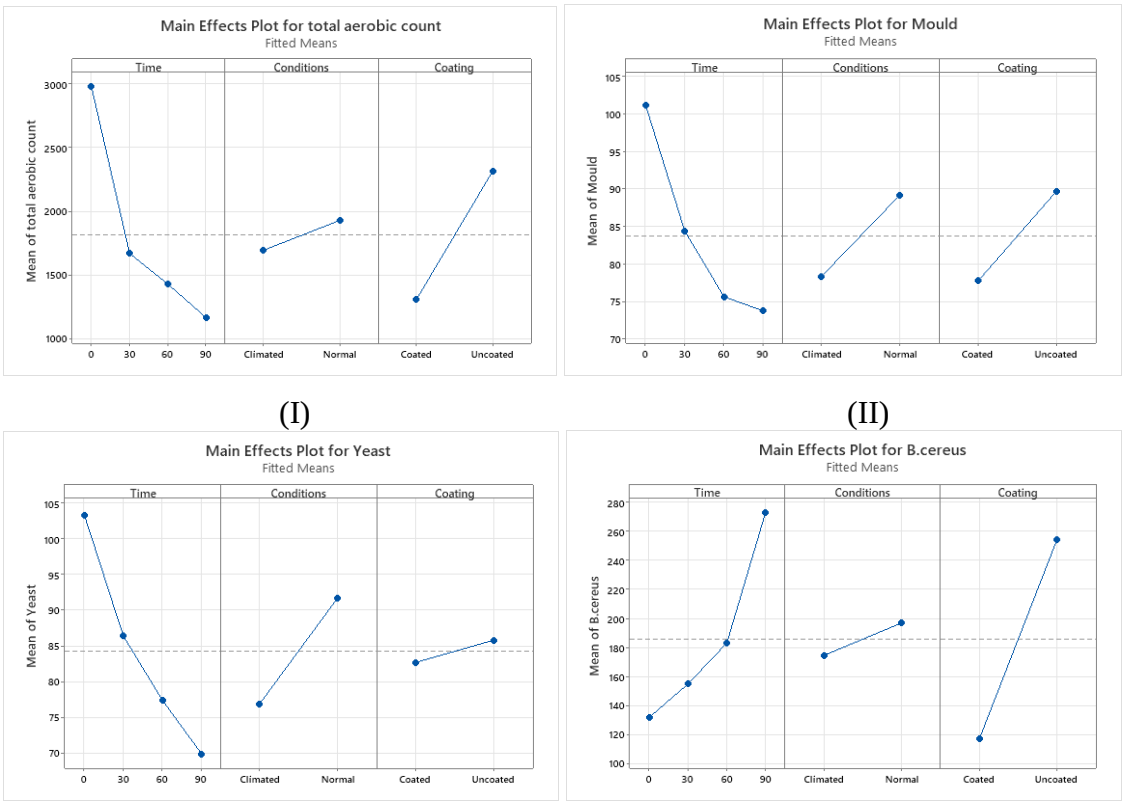


Figure 14: Main effects plot for microbial analysis for different factors (I-Total aerobic count, II-Mould, III-Yeast, IV-B.cereus)

Microbial growth for all microorganism except *Bacillus cereus* was affected inversely despite passing time, which was not expected. In normal conditions, food increased total aerobic count in time. It was resulted from the lower water activity values, these results will be shown in the next title. Lower water activity resulted to limit microbial growth (Tapia et al., 2020). The antimicrobial activity of the film containing pomegranate peel showed the inhibition effect on total aerobic bacteria as it was expected. One of the studies that was conducted for antimicrobial activity of pomegranate peel was done by Moghadam et al. (2020). Snack bars stored at normal conditions showed higher microbial activity than the ones at the climated conditions. However, it should be

observed that the higher microbial value seen in climated storage conditions. This situation also could be explained by lower water activity value in climated conditions. On the other hand, *Bacillus cereus* continued to grow especially in coated snack bar. The reason of this could be enabled by spore forming properties of *Bacillus cereus*, which could be water resistant bacteria (Coroller et al., 2001).

It observed that coating of snack bars demonstrated antimicrobial effect obviously in total aerobic count, mould, yeast and *Bacillus cereus* bacteria. The similar microbial prevention was proved in many studies. *Listeria monocytogenes* and *Escherichia coli* were also inhibited by edible film of mung bean protein incorporated with pomegranate peel. Percentage of pomegranate peel increased in film antibacterial activity of film also increased (Zhang et al., 2019). Moreover, Ali et al. (2018) was reported that pomegranate peel had an antimicrobial effect on *Staphylococcus aureus* and *Salmonella*. The antimicrobial effect was resulted from the presence of tannin and polyphenols, which had also a role in the reduction of membrane fluidity and perforation of membrane (Mushtaq et al., 2018). The other antimicrobial compounds found in pomegranate peels were castalagin, granatin, catechin, kaempferol, quercetin, gallic acid, ellagic acid and punicalagin (Dahham et al., 2010).

According to food standards, total aerobic microbial plate count should be lower than 10^5 to be within the acceptable limits (Institute of Medicine (US) and National Research Council (US), 2003). According to Erkmen & Bozoglu (2016), nut had a growth risk of Salmonella. Also, it was observed that Coliform, *Escherichia coli* (Torres, 2007), Salmonella (Beuchat et al., 2013), Staphylococcus and Clostridium spp. (Witthuhn et al., 2005) in dried fruits. In this study, many bacteria were prevented or decreased for 90 days.

3.15. Water Activity and pH

Water activity and pH values were measured in 0, 30, 60 and 90^{th} day for both coating and condition. The values are given in Table 10.

Table 10: Results of water activity and pH at different parameters

Time (Day)	Coating	Conditions	Water activity*	pH*
0	Coated	Normal	0.495±0.007^{c}	4.535±0.007^{a}
0	Coated	Climated	0.495±0.007^{c}	4.535±0.007^{a}
0	Uncoated	Normal	0.595±0.007^{d}	4.530±0.0^{b}
0	Uncoated	Climated	0.59±0.0^{d}	4.525±0.007^{b}
30	Coated	Normal	0.575±0.007^{b}	4.305±0.078^{d}
30	Coated	Climated	0.59±0.0^{b}	4.34±0.014^{d}
30	Uncoated	Normal	0.635±0.007^{a}	4.355±0.07^{c}
30	Uncoated	Climated	0.605±0.007^{a}	4.45±0.007^{c}
60	Coated	Climated	0.565±0.007^{b}	4.305±0.007^{g}
60	Coated	Normal	0.575±0.007^{b}	4.285±0.049^{g}
60	Uncoated	Normal	0.615±0.007^{a}	4.315±0.021^{e}
60	Uncoated	Climated	0.615±0.007^{a}	4.315±0.007^{e}
90	Coated	Normal	0.585±0.007^{b}	4.245±0.007^{h}
90	Coated	Climated	0.605±0.021^{b}	4.245±0.007^{h}
90	Uncoated	Normal	0.625±0.007^{a}	4.305±0.007^{f}
90	Uncoated	Climated	0.625±0.007^{a}	4.305±0.007^{f}

*Different letters on the same column shows significant difference for $P<0.05$

To see the value apparently, the bar chart is shown in Figure 15 below.

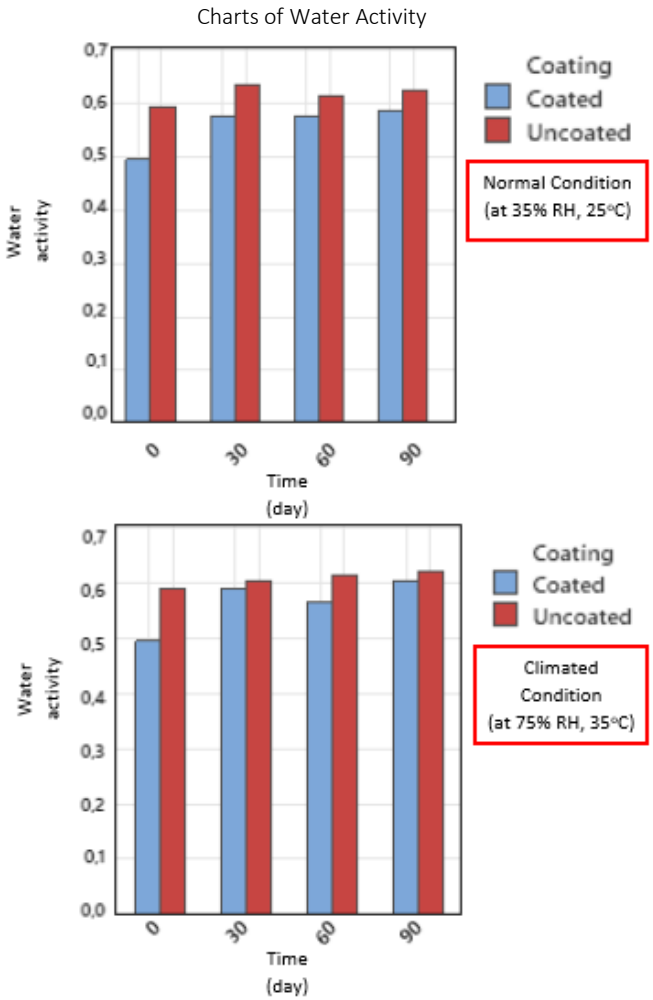


Figure 15: Chart of water activity under different factors

In the beginning of the study, it is detected that coated snack bar had lower water activity value. This was resulted from the drying of coating in the surface, which caused to lose water activity. Other significant effects observed in statistical method done general linear model design with three factors.

From the ANOVA table, the significant factors were coating and time depend on α-value. In this case, there was no effect of condition significantly. For the water activity, coating includes pomegranate peel and lentil flour was main effective factor. More examination is done in the following section.

To see the effect of main factor, the line graph is placed in Figure 16 below.

Figure 16: Main effects plot for water activity for different factors

Coating showed the protection of water activity value inside of snack bar. It was seen that the slope had higher than the other values as the most significant value. This effect was shown as resulted from the beginning water activity value. Snack bar in normal condition had almost no difference in climated conditions. Thus, the snack bar had not been affected during storage. Water activity increased with the time in 60^{th} day measurement, there might be an inconvenient situation during application of the method or in the measurement device. It was an expected result for water activity. During the shelf life, water activity of dried fruit increased with time (Sen et al., 2015).

The results of pH were evaluated by chart of pH dependent on different factors in Figure 17.

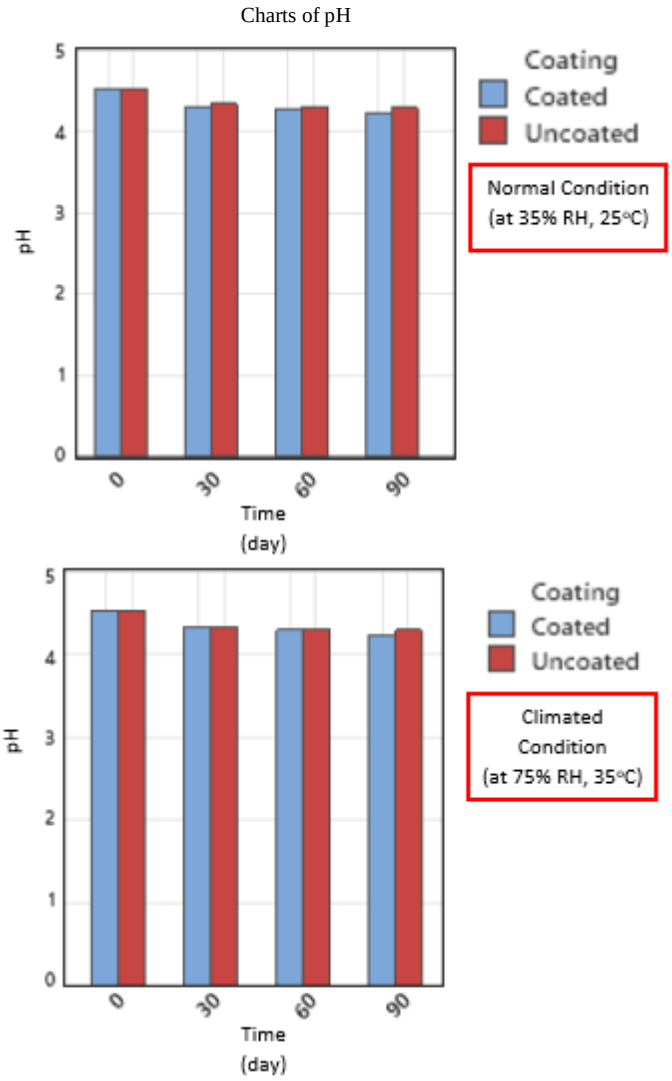


Figure 17: Chart of pH for different factors

The graph was formed from the measured value. For coated and uncoated snack bars, there was slightly decreasing value is observed. There was not much difference in normal and climated conditions.

pH value was mostly affected by the time from ANOVA table. The significant parameters depend on α-value were time and coating. Change in condition were not significant for pH value. The effects of parameters were examined with the main effect plot.

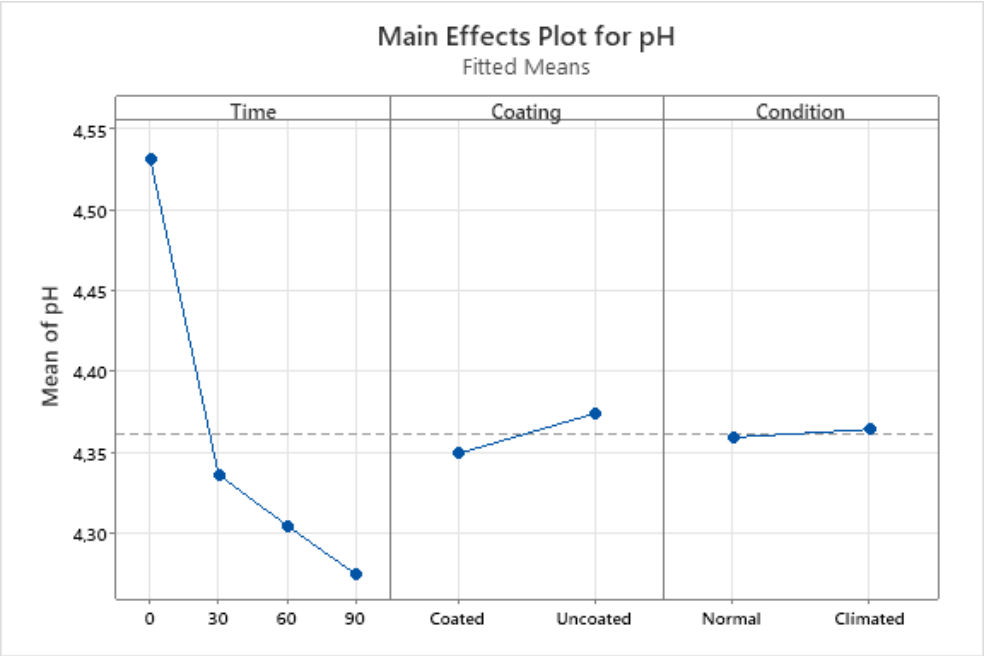


Figure 18: Main effects plot for pH for different factors

pH value decreased with the time. It was observed that this reduction was resulted from rancidity of the snack bar because of the nature of nut, fig and strawberry. The fruit and nuts tended to rancid during its shelf life (Feiner, 2006). In climated and normal condition, pH value did not change obviously. The reason of low pH value in coated snack bar could be because of the pH value of the coating. It had about 4.5 of pH value. This could be the cause of the lower pH value of snack bar.

3.16. Texture Properties

Compression test was conducted with 75 mm diameter aluminum cylinder that shows the variety of viscoelastic products. The texture profile analysis was done with two times compressions. The result of analyses was handed on stress-strain graph. Hardness, cohesiveness, springiness, chewiness and resilience values were calculated from maximum force and area below the lines. The calculated and measured data are given the table below.

Table 11: Results of textural properties in changing parameters

Time (Day)	Coating	Conditions	Hardness	Cohesiveness	Springiness	Chewiness	Resilience
0	Coated	Normal	217.25±12.28^{c}	0.26±0.00^{a}	0.191±0.002^{a}	10.82±0.52^{a}	0.156±0.001^{a}
0	Coated	Climated	217.25±12.28^{d}	0.26±0.00^{a}	0.191±0.002^{a}	10.82±0.52^{b}	0.156±0.001^{b}
0	Uncoated	Normal	200.28±21.26^{c}	0.20±0.00^{b}	0.146±0.004^{d}	6.34±0.57^{c}	0.116±0.009^{e}
0	Uncoated	Climated	200.28±21.2^{d}	0.20±0.00^{b}	0.146±0.004^{d}	6.34±0.57^{e}	0.116±0.009^{f}
30	Coated	Normal	187.91±1.25^{c}	0.19±0.01^{c}	0.138±0.011^{b}	5.92±0.82^{f}	0.111±0.007^{d}
30	Coated	Climated	178.23±5.66^{d}	0.21±0.00^{c}	0.152±0.001^{b}	5.58±0.19^{f}	0.111±0.004^{g}
30	Uncoated	Normal	252.17±16.43^{c}	0.21±0.02^{d}	0.156±0.008^{c}	9.07±1.16^{d}	0.123±0.004^{c}
30	Uncoated	Climated	143.63±7.46^{d}	0.18±0.00^{d}	0.136±0.004^{c}	3.54±0.44^{g}	0.093±0.003^{h}
60	Coated	Normal	384.06±0.31^{a}	0.19±0.00^{c}	0.136±0.003^{b}	10.27±0.48^{a}	0.117±0.004^{d}
60	Coated	Climated	260.75±9.40^{b}	0.19±0.00^{c}	0.142±0.001^{b}	7.39±0.10^{b}	0.095±0.005^{g}
60	Uncoated	Normal	362.72±6.61^{a}	0.19±0.00^{d}	0.136±0.002^{c}	9.48±0.04^{c}	0.109±0.004^{c}
60	Uncoated	Climated	222.81±17.56^{b}	0.16±0.00^{d}	0.136±0.003^{c}	5.59±0.07^{e}	0.08±0.000^{h}
90	Coated	Normal	236.94±8.43^{d}	0.16±0.00^{e}	0.113±0.004^{e}	4.21±0.14^{h}	0.084±0.002^{i}
90	Coated	Climated	134.42±1.27^{c}	0.16±0.00^{e}	0.128±0.008^{e}	2.77±0.21^{j}	0.071±0.001^{j}
90	Uncoated	Normal	223.49±1.36^{d}	0.17±0.00^{f}	0.12±0.001^{f}	4.33±0.81^{i}	0.091±0.002^{i}
90	Uncoated	Climated	104.41±1.18^{c}	0.15±0.00^{f}	0.115±0.001^{f}	1.70±0.19^{k}	0.058±0.001^{j}

*Different letters on the same column shows significant difference for P<0.05

Values were examined in the bar chart drawn for each resulting parameter. From the measured values statistical analyses was done for all results with general linear model with 3 factors. Figure 19 shows the hardness and chewiness value below that is drawn by measured values.

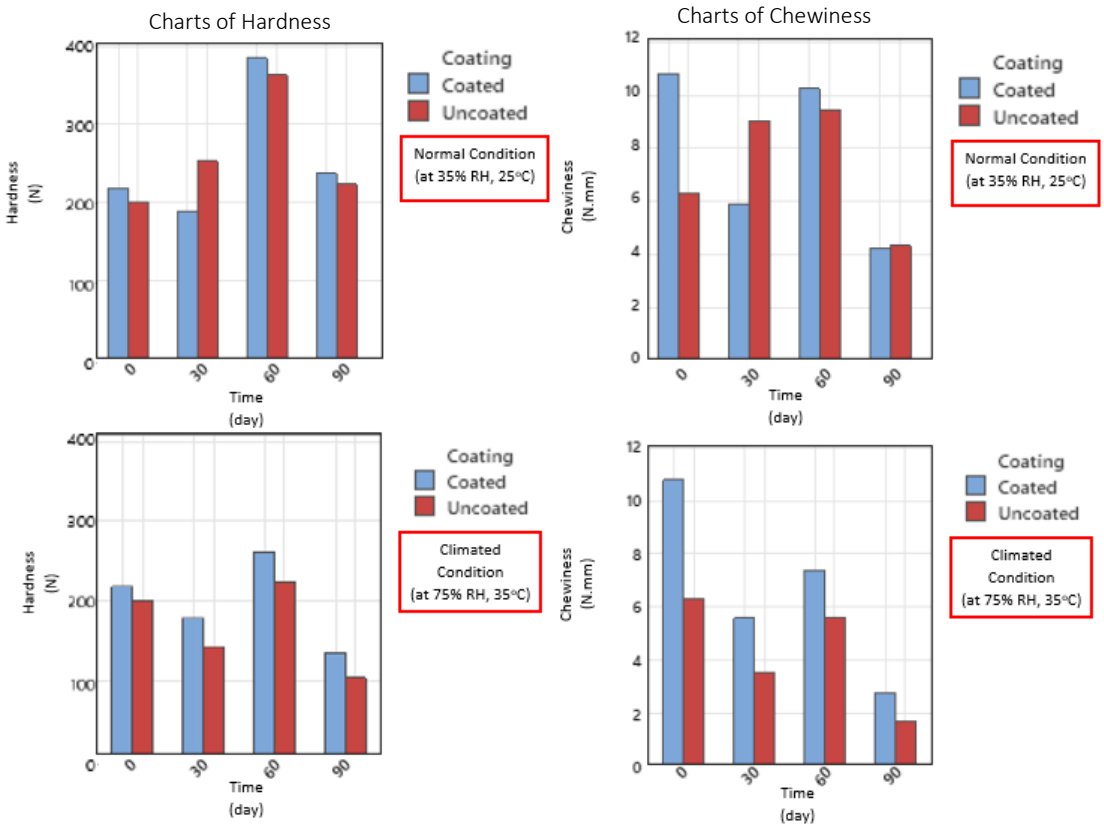


Figure 19: Chart of hardness and chewiness for different factors

Hardness is the value dependent on the force for first bite (Gokus, n.d.). It did not show a regular pattern to comment, but they showed the softer texture 90th day in this study. This irregular pattern could be resulted from non-homogenous structure of the snack bar. For the hardness, time and condition were the significant factors from the ANOVA table. The significance of parameters could be ordered as conditions and time respectively depending on F-value. Chewiness showed the similar pattern with hardness value. Chewiness is the term mastication of food in solid phase that is calculated by multiplication of hardness, cohesiveness and springiness (Gokus, n.d.). Significant affecting factor of chewiness were the entire factors, which affects to chewiness with order of time, conditions and coating.

Figure 20 shows the effect of the main factor to the chewiness and hardness values.

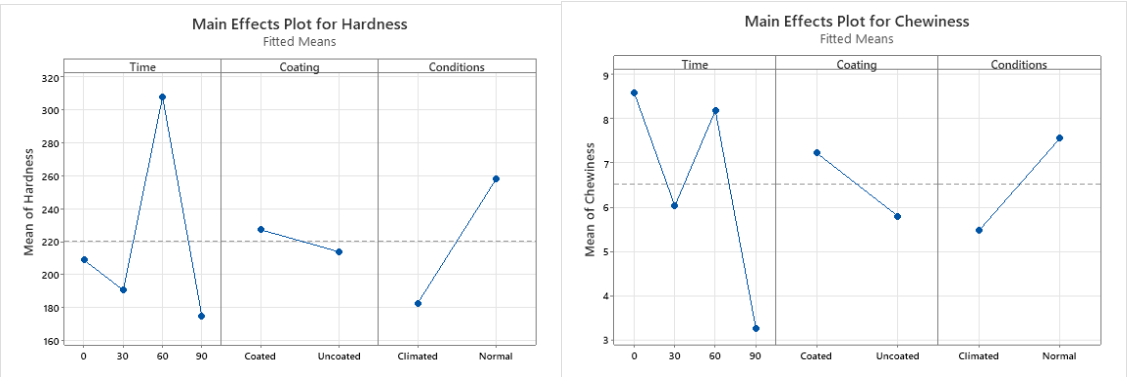


Figure 20: Main effects plot for hardness and chewiness for different factors

In normal conditions, snack bar stored in normal condition was harder than the snack bar in climated conditions. The lower hardness and chewiness value was resulted from the high moisture and a_w result higher than the beginning value (Sen et al., 2015). Also, coating had an effect to protect texture of the snack bar from harsh storage conditions. It was supposed that the hardness increased in time. In 90^{th} day, there was an unexpected result. The reason of this could be non-homogenous structure of the snack bar. It had 1-4 mm broken hazelnut and fig paste in the structure. There could be fig or hazelnut as a hard surface. The chewiness was affected by similar factors with hardness. The same parameters that were mentioned for hardness value. Hardness is related with chewiness properly as chewiness was calculated by using hardness value. However, these changes were not felt by the participants during sensory analysis.

Figure 21 shows the bar charts of measured cohesiveness, springiness and resilience values below.

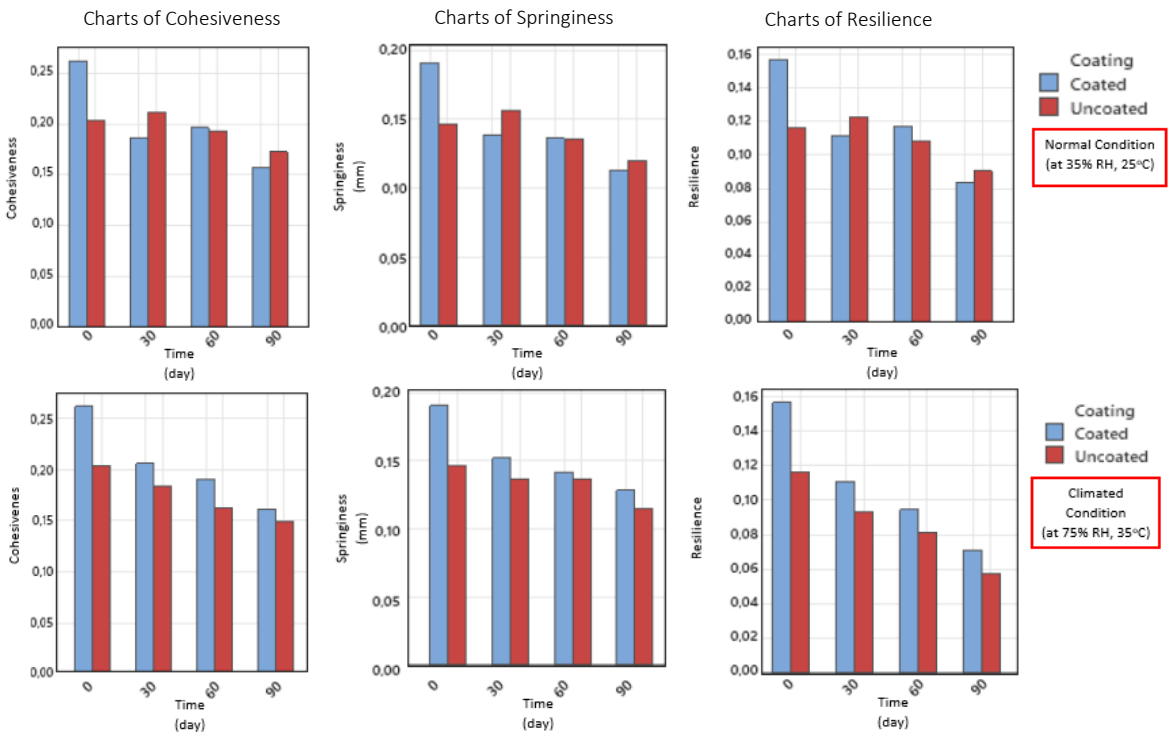


Figure 21: Chart of cohesiveness, springiness and resilience for different factors

Cohesiveness, springiness and resilience values decreased with time. Coated snack bars had higher value than uncoated snack bars. Resilience gives the force resistance of food to bite when springiness shows the elasticity of the food to reverse first form after compression. The stickiness means to disrupt the structure of the food (Gokus, n.d.). Cohesiveness was affected by two factors that are time and storage condition. Springiness was affected by time and coating. For the chewiness, all parameters had effects. Time had more effective parameters for all responses depend on ANOVA table. The effects of factors are discussed in the following section.

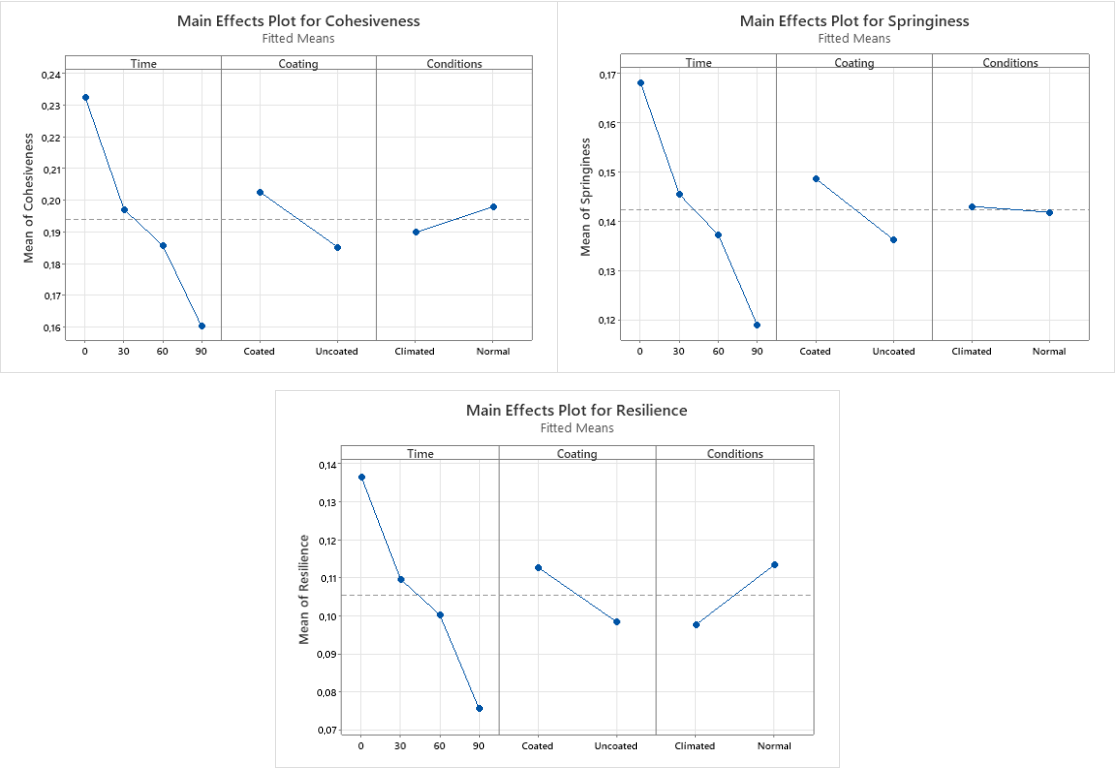


Figure 22: Main effects plot for cohesiveness, springiness and residence for different factors

As cohesiveness, springiness and resilience showed the similar patterns. The reasons of the effects were discussed together. All responses decreased with time. As expected, cohesiveness value decreased because of the reduction of moisture content. Since coating protected the water activity, coating maintained the initial structure. It could remain stickier like 0^{th} day than uncoated snack bar. Depending on the decrease of water activity, climated conditions resulted to decrease in cohesiveness value. For the springiness value as an elasticity of the food, time and coating factors were impacted factors. According to results, the factors affected the response in the same way with cohesiveness. The reason of this was similar with the cohesiveness by reducing of moisture content. This was proved by another study (Serra et al., 2015). During storage, snack bars got harder and decreased the water activity inside in time. It was discussed in the section of moisture content. Thus, force to bite increased with time and coating. As snack bar kept in climated room enabled to maintain structure of bar, necessary force to bite decreased for the snack bar in climated conditions.

Shear test was made with blade set with knife equipment. Shear test was done in snack bars to stimulate the bite and measure the work for bite. It could also stimulate resistance force to cut the product by knife, which indicated toughness value.

Table 12 shows the measured value of textural properties for changing parameters.

Table 12: Results of textural properties of shear test in changing parameters

Time (Day)	Coating	Conditions	Toughness	Work to bite
0	Coated	Normal	20.539 ± 0^{e}	223.38 ± 0^{b}
0	Coated	Climated	23.081 ± 0^{d}	242.734 ± 0^{b}
0	Uncoated	Normal	31.106 ± 0^{e}	336.331 ± 0^{b}
0	Uncoated	Climated	31.62 ± 0^{d}	318.332 ± 0^{d}
30	Coated	Normal	36.723 ± 1.3^{b}	394.408 ± 15.401^{a}
30	Coated	Climated	31.282 ± 0.176^{c}	320.945 ± 7.168^{c}
30	Uncoated	Normal	40.032 ± 0.108^{b}	437.092 ± 3.209^{a}
30	Uncoated	Climated	28.19 ± 1.747^{c}	313.194 ± 15.115^{c}
60	Coated	Normal	43.69 ± 0.646^{a}	485.382 ± 8.633^{a}
60	Coated	Climated	26.403 ± 0.450^{g}	295.736 ± 1.522^{c}
60	Uncoated	Normal	37.943 ± 0.553^{a}	420.322 ± 29.638^{a}
60	Uncoated	Climated	20.161 ± 1.135^{g}	235.197 ± 11.938^{c}
90	Coated	Normal	28.143 ± 1.042^{f}	287.247 ± 2.866^{b}
90	Coated	Climated	23.264 ± 5.085^{h}	187.783 ± 10.102^{d}
90	Uncoated	Normal	26.538 ± 0.456^{f}	278.187 ± 12.251^{b}
90	Uncoated	Climated	14.549 ± 0.103^{h}	158.932 ± 1.102^{d}

*Different letters on the same column shows significant difference for $P<0.05$

The bar charts in Figure 23 were drawn by using the values of Table 12 shown below. For the statistical analysis of toughness and work to bite value, general linear model design with three factor was conducted. Figure 23 shows bar chart drawn by using the measured values of toughness and force to bite.

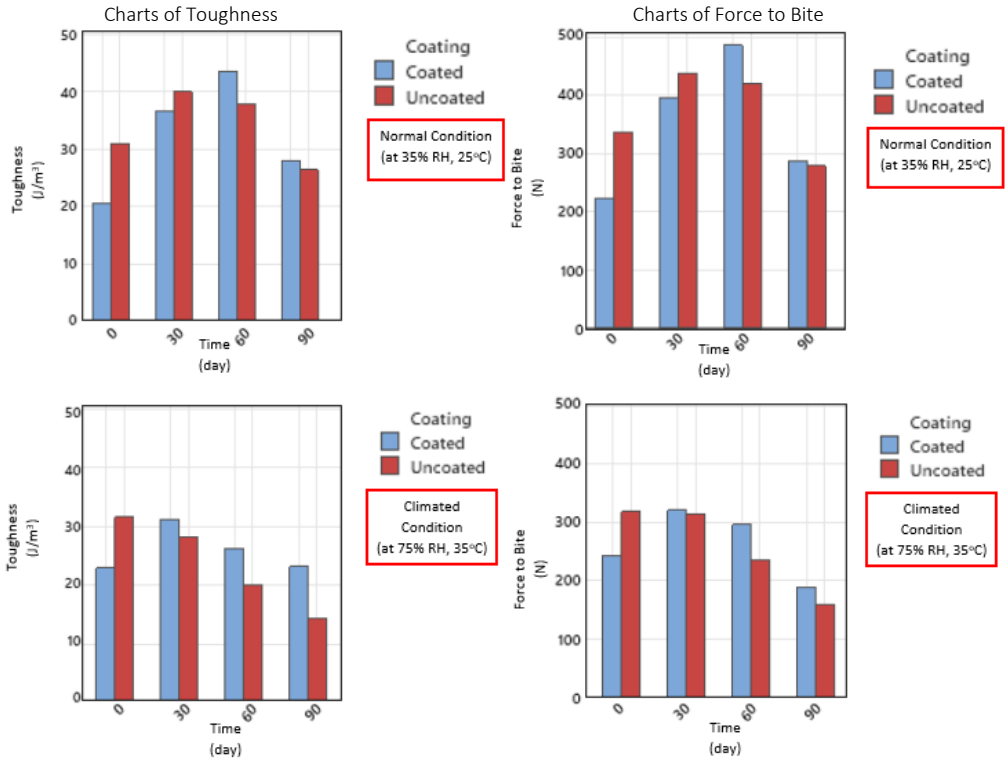


Figure 23: Chart of toughness and force to bite for different factors

For the toughness and work to bite, these parameters increased with time generally in normal conditions for both coated and uncoated snack bars. In climated conditions, values decreased generally. It showed an irregular pattern. Coating was the insignificant value depend on α value in ANOVA table. The affecting parameters for toughness were storage conditions and time respectively, which was same with hardness. This was expected because hardness was directly related with toughness.

Main effects plots for toughness and force to bite depend on changing factors are shown in Figure 24.

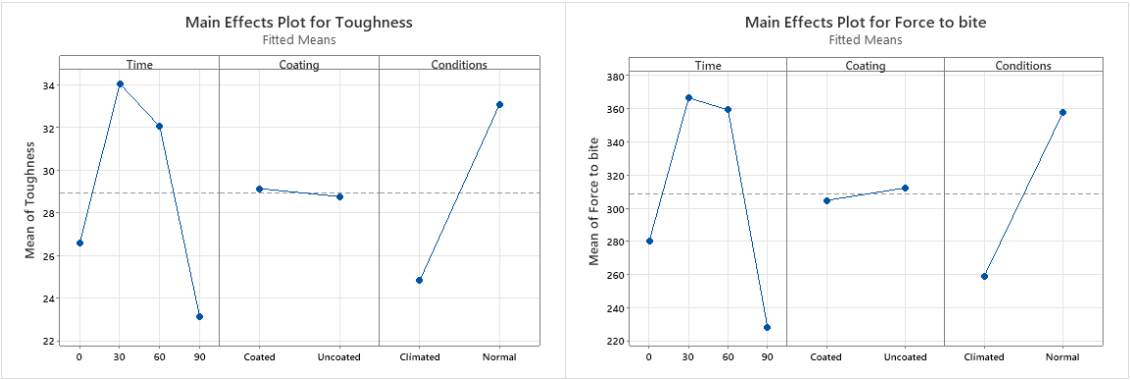


Figure 24: Main effects plot of toughness and force to bite for different factors

According to the main effect plot, the toughness value decreased in time when climated storage conditions made the snack bar softer. This was supposed result similar with Rahman & Al-Farsi (2005). Condition was most effective parameter for both values. Due to temperature and moisture, the texture remained softer other than kept at room conditions (Rahman, 2006). Both toughness and force to bite effects were affected by factors similarly. The unexpected design value in 90th day could be resulted from unsuitable condition in the experiment. It also could be resulted from the textural changes of fig during the shelf life.

3.17. Sensory Analysis

Images of coated and uncoated bars are indicated in Figure 25. The effect of coating, time and conditions could be observed as an appearance.

	Climated Conditions		Normal Conditions	
Time	Coated	Uncoated	Coated	Uncoated
0th day				
30th day				
60th day				
90th day				

Figure 25: Appearance of coated and uncoated snack bars in changing conditions and time

In Table 13 shows the point result of sensory analysis. It was conducted for appearance, odor, taste and texture. The results were determined by sensory evaluation taste done by evaluation of participants.

Table 13: Results of sensory analysis in changing parameters

Time (Day)	Coating	Conditions	Appearance*	Odor*	Taste*	Texture*
0	Coated	Normal	9.5^{a}	9.11^{a}	9.04^{a}	9.3^{a}
0	Coated	Climated	8.5^{e}	8.37^{c}	8.49^{a}	8.59^{c}
0	Uncoated	Normal	8.96^{b}	8.57^{a}	8.99^{b}	9.26^{b}
0	Uncoated	Climated	7.3^{h}	7.97^{c}	7.88^{b}	7.98^{f}
30	Coated	Normal	8.8^{c}	8.21^{b}	8.47^{c}	8.13^{e}
30	Coated	Climated	7.3^{h}	7.67^{e}	7.67^{c}	7.69^{j}
30	Uncoated	Normal	8.53^{d}	8.00^{b}	8.27^{d}	8.47^{d}
30	Uncoated	Climated	$6.83^{ı}$	7.6^{e}	7.47^{d}	7.27^{l}
60	Coated	Normal	8.05^{f}	7.93^{d}	7.81^{d}	8.01^{h}
60	Coated	Climated	6.29^{j}	6.99^{g}	7.29^{d}	7.53^{k}
60	Uncoated	Normal	8.03^{g}	7.92^{d}	7.76^{c}	7.86^{g}
60	Uncoated	Climated	6.21^{k}	6.92^{g}	7.19^{c}	7.11^{m}
90	Coated	Normal	7.79^{f}	7.17^{f}	6.83^{f}	8.01^{g}
90	Coated	Climated	5.76^{j}	6.21^{h}	6.41^{f}	7.07^{n}
90	Uncoated	Normal	7.66^{g}	7.59^{f}	7.61^{e}	6.72^{o}
90	Uncoated	Climated	5.38^{k}	5.41^{h}	6.38^{e}	6.55^{p}

*Different letters on the same column shows significant difference for $P<0.05$

There was no replicated data so standard deviation could not be calculated. The values are used to draw bar chart in Figure 26.

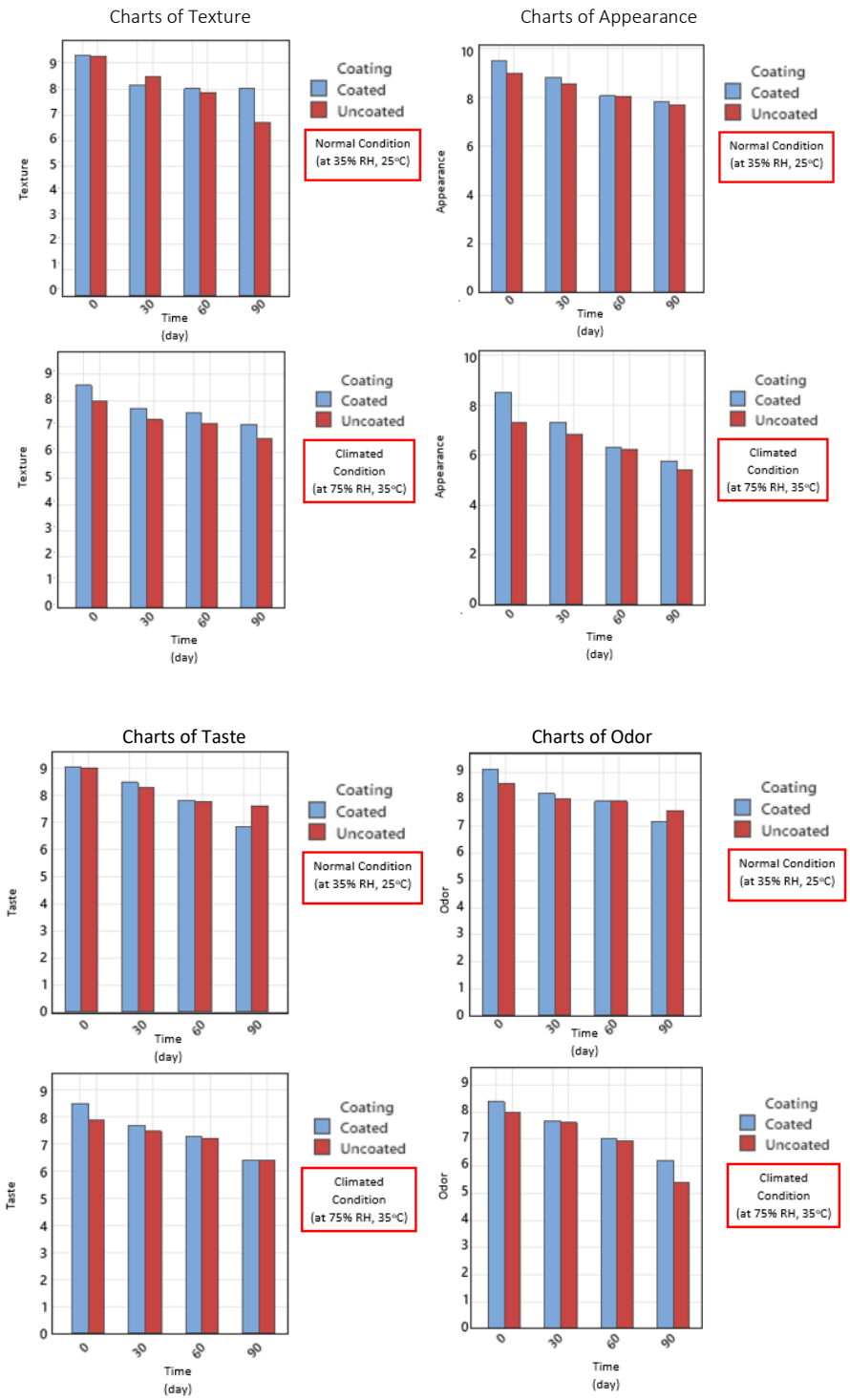


Figure 26: Chart of sensory analysis for different factors

All quality parameters were evaluated by participants. In time, the given points were decreased as expected. Generally, coating could protect snack bars observed from the charts. The snack bars stored in climated condition gained lower point than the one stored in normal conditions. All parameters were significant for appearance and texture

which was expected. Condition was the most critical parameter that affects the quality of snack bars. It was obviously seen that darker color and softer texture in snack bar stored in climated conditions. On the other hand, taste and odor were not affected by coating. Absolutely, it should affect the odor and taste well. However, it was considered the film contains lentil flour that has a dominant odor and taste. Consequently, it was found as a good result not to perceive odor and taste of lentil flour different from the control group. This could be explained that protein denaturation makes better film properties for sensorial and textural properties of food. Denaturation was defined as altered balance between different interactions (Creighton, 1978). Therefore, it can be evaluated as coating shows good result to protect sensory properties.

CHAPTER 4

CONCLUSION

Edible film that prepared from lentil flour protein extract and pomegranate peel were produced. Pectin as thickening agent and glycerol as a plasticizer were also added. Physical and mechanical properties were measured and evaluated in this study. The evaluated results were compared with similar studies. The snack bars composed of dried fig, strawberry and nuts were coated with the edible film. The effects of coating were observed for period of 90 days in room (25^{o}C, RH-35%) and climated conditions (35^{o}C, RH-75%). Treatment of coating was effective for many properties of food. The coating enabled to retain physical, microbiological and organoleptic parameters. The coating was found effective on moisture content, water activity and pH, color change, total phenolic content, microorganism, textural and sensory properties.

In the present study, this content of edible coating could be good alternative to protect quality parameters of snack bars during shelf life up to 90 days. At the outset of this thesis, the aim of study was to functionalize a vegan snack bar by using antimicrobial edible coating by enrichment of protein, protection of moisture content, phenolic content, texture, microbial growth, sensorial properties and color of the study. The protein could be enriched in ratio of 3.68%.

Therefore, it can be concluded that this study fulfills the expected purpose of the study in terms of many properties of snack. It was a suitable sweet food can be alternative snack for vegan, glucose and lactose intolerant, diabetic and all group of consumers.

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APPENDIX A

GALLIC ACID CURVE

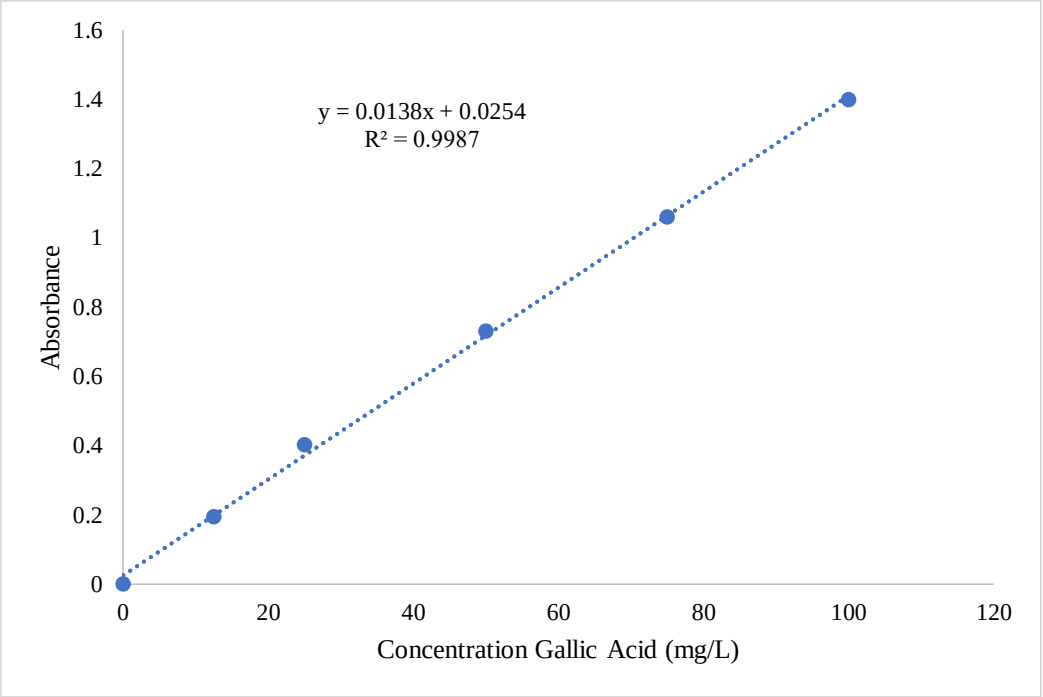
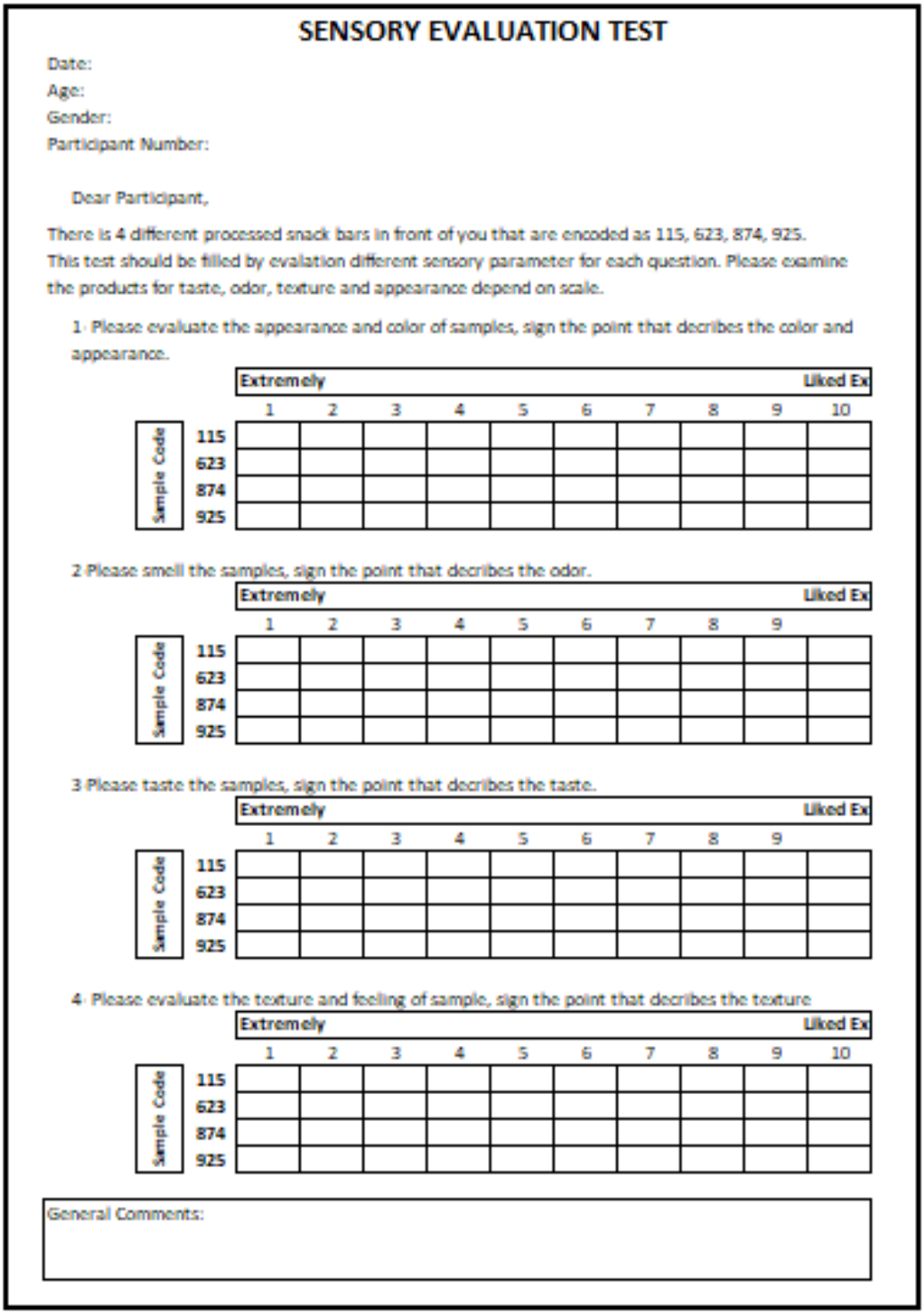


Figure A.1: Gallic acid curve used in calculation of total phenolic content

$$\text{Gallic acid concentration} = \frac{(\text{Absorbance} - 0.0254)}{0.0138} \qquad (2.10)$$

APPENDIX B

SENSORY EVALUATION TEST



SENSORY EVALUATION TEST

Date:
Age:
Gender:
Participant Number:

Dear Participant,

There is 4 different processed snack bars in front of you that are encoded as 115, 623, 874, 925. This test should be filled by evalation different sensory parameter for each question. Please examine the products for taste, odor, texture and appearance depend on scale.

1. Please evaluate the appearance and color of samples, sign the point that decribes the color and appearance.

Sample Code	Extremely									Liked Ex
	1	2	3	4	5	6	7	8	9	10
115										
623										
874										
925										

2 Please smell the samples, sign the point that decribes the odor.

Sample Code	Extremely								Liked Ex
	1	2	3	4	5	6	7	8	9
115									
623									
874									
925									

3 Please taste the samples, sign the point that decribes the taste.

Sample Code	Extremely								Liked Ex
	1	2	3	4	5	6	7	8	9
115									
623									
874									
925									

4. Please evaluate the texture and feeling of sample, sign the point that decribes the texture

Sample Code	Extremely									Liked Ex
	1	2	3	4	5	6	7	8	9	10
115										
623										
874										
925										

General Comments:

Figure B.1: Sensory evaluation form

APPENDIX C

DESIGN OF EXPERIMENT & STATISTICAL TABLES

Response Variable: Moisture content/aw/nem/TPC/L-a-b/ Texture etc. as measured data

Number of Factor: 3

Factor A: Time

Factor Level of A: 4 (0,30,60,90)

Factor B: Conditions

Factor Level of B: 2 (Climated, Normal)

Factor C: Coating

Factor Level of C: 2 (Coated, Uncoated)

α is assumed as 0.05.

General linear model is applied for main factor affect and Tukey's comparison test was applied.

Table C.1: Statistical table for moisture content

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Conditions	Fixed	2	Climated; Normal
Coating	Fixed	2	Coated; Uncoated

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	1	18,5898	18,5898	86,56	0,000
Time	3	9,7139	3,2380	15,08	0,000
Conditions	1	1,6065	1,6065	7,48	0,011
Error	26	5,5840	0,2148		
Lack-of-Fit	10	5,4809	0,5481	85,10	0,000
Pure Error	16	0,1030	0,0064		
Total	31	35,4942			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,463430	84,27%	81,24%	76,17%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	12,2562	A

Coated	16	10,7319	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
30	8	12,4300	A
0	8	11,3125	B
90	8	11,2188	B
60	8	11,0150	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Climated	16	11,7181	A
Normal	16	11,2700	B

Means that do not share a letter are significantly different.

Table C.2: Statistical table for lightness value

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	252,715	84,238	26,22	0,000
Coating	1	0,597	0,597	0,19	0,670
Conditions	1	144,421	144,421	44,96	0,000
Error	26	83,519	3,212		
Lack-of-Fit	10	68,744	6,874	7,44	0,000
Pure Error	16	14,775	0,923		
Total	31	481,253			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,79228	82,65%	79,31%	73,71%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	8	37,6637	A
30	8	32,1453	B
60	8	31,8187	B
90	8	30,2217	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	35,0868	A

Climated	16	30,8380	B

Means that do not share a letter are significantly different

Table C.3: Statistical table for a-value

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	330,079	110,026	152,00	0,000
Coating	1	0,044	0,044	0,06	0,807
Conditions	1	61,224	61,224	84,58	0,000
Error	26	18,820	0,724		
Lack-of-Fit	10	17,234	1,723	17,38	0,000
Pure Error	16	1,586	0,099		
Total	31	410,167			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,850797	95,41%	94,53%	93,05%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
0	8	11,9342	A		
30	8	7,9512		B	
60	8	4,7500			C
90	8	3,6875			C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping	
Normal	16	8,46392	A	
Climated	16	5,69751		B

Means that do not share a letter are significantly different

Table C.4: Statistical table for b-value

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

Time	3	281,462	93,821	29,99	0,000
Coating	1	1,192	1,192	0,38	0,543
Conditions	1	209,168	209,168	66,86	0,000
Error	26	81,345	3,129		
Lack-of-Fit	10	75,325	7,532	20,02	0,000
Pure Error	16	6,021	0,376		
Total	31	573,166			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,76881	85,81%	83,08%	78,50%

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	15,0592	A
Climated	16	9,9459	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	8	17,5608	A
60	8	11,6583	B
90	8	10,4465	B
30	8	10,3447	B

Means that do not share a letter are significantly different.

Table C.5: Statistical table for color change

Factor Information

Factor	Type	Levels	Values
Time	Fixed	3	30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	2	48,24	24,119	13,35	0,000
Coating	1	23,84	23,840	13,19	0,002
Conditions	1	469,58	469,581	259,85	0,000
Error	19	34,34	1,807		
Lack-of-Fit	7	22,37	3,196	3,21	0,037
Pure Error	12	11,96	0,997		
Total	23	575,99			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,34430	94,04%	92,78%	90,49%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
90	8	13,4100	A
60	8	11,2313	B
30	8	9,9787	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	12	12,5367	A
Coated	12	10,5433	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Climated	12	15,9633	A
Normal	12	7,1167	B

Means that do not share a letter are significantly different.

Table C.6: Statistical table for total phenolic content

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	607733	202578	7,88	0,000
Coating	1	93126	93126	3,62	0,064
Conditions	1	753630	753630	29,33	0,000
Error	42	1079180	25695		
Lack-of-Fit	10	943782	94378	22,31	0,000
Pure Error	32	135398	4231		
Total	47	2533668			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
160,296	57,41%	52,34%	44,37%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	24	921,578	A
Uncoated	24	833,484	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Climated	24	1002,83	A
Normal	24	752,23	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
90	12	987,729	A		
60	12	983,833	A	B	
30	12	812,667		B	C
0	12	725,896			C

Means that do not share a letter are significantly different.

Table C.7: Statistical table for total aerobic count

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Conditions	Fixed	2	Climated; Normal
Coating	Fixed	2	Coated; Uncoated

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	1	8181013	8181013	31,91	0,000
Time	3	15627616	5209205	20,32	0,000
Conditions	1	441800	441800	1,72	0,201
Error	26	6665094	256350		
Lack-of-Fit	10	6533194	653319	79,25	0,000
Pure Error	16	131900	8244		
Total	31	30915522			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
506,310	78,44%	74,29%	67,34%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	2317,19	A
Coated	16	1305,94	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	8	2981,88	A
30	8	1670,00	B
60	8	1428,75	B
90	8	1165,63	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	1929,06	A
Climated	16	1694,06	A

Means that do not share a letter are significantly different

Table C.8: Statistical table for mould

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Conditions	Fixed	2	Climated; Normal
Coating	Fixed	2	Coated; Uncoated

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	1	1152,0	1152,00	7,10	0,013
Time	3	3781,2	1260,42	7,76	0,001
Conditions	1	946,1	946,12	5,83	0,023
Error	26	4220,6	162,33		
Lack-of-Fit	10	3738,6	373,86	12,41	0,000
Pure Error	16	482,0	30,13		
Total	31	10100,0			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
12,7409	58,21%	50,18%	36,70%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	89,75	A
Coated	16	77,75	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
0	8	101,250	A	
30	8	84,375	A	B
60	8	75,625		B
90	8	73,750		B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	89,1875	A
Climated	16	78,3125	B

Means that do not share a letter are significantly different.

Table C.9: Statistical table for yeast

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Conditions	Fixed	2	Climated; Normal
Coating	Fixed	2	Coated; Uncoated

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	1	78,1	78,13	0,54	0,468
Time	3	4993,5	1664,50	11,56	0,000
Conditions	1	1770,1	1770,12	12,30	0,002
Error	26	3742,3	143,93		
Lack-of-Fit	10	3206,3	320,63	9,57	0,000
Pure Error	16	536,0	33,50		
Total	31	10584,0			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
11,9972	64,64%	57,84%	46,44%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	85,8125	A
Coated	16	82,6875	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
0	8	103,375	A	
30	8	86,375	B	
60	8	77,375	B	C
90	8	69,875		C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	91,6875	A
Climated	16	76,8125	B

Means that do not share a letter are significantly different.

Table C.10: Statistical table for *B. cereus*

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Conditions	Fixed	2	Climated; Normal
Coating	Fixed	2	Coated; Uncoated

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	1	150975	150975	14,81	0,001
Time	3	92090	30697	3,01	0,048
Conditions	1	4005	4005	0,39	0,536
Error	26	265127	10197		
Lack-of-Fit	10	259370	25937	72,08	0,000
Pure Error	16	5757	360		
Total	31	512197			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
100,981	48,24%	38,28%	21,59%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	254,375	A
Coated	16	117,000	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
90	8	273,125	A	
60	8	183,000	A	B
30	8	154,875	A	B
0	8	131,750		B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	196,875	A
Climated	16	174,500	A

Means that do not share a letter are significantly different.

Table C.11: Statistical table for water activity

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Conditions	Fixed	2	Climated; Normal
Coating	Fixed	2	Coated; Uncoated

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	1	0,021938	0,021938	68,12	0,000
Time	3	0,020973	0,006991	21,71	0,000
Conditions	1	0,000002	0,000002	0,01	0,941
Error	26	0,008373	0,000322		
Lack-of-Fit	10	0,007307	0,000731	10,96	0,000

Pure Error	16	0,001067	0,000067
Total	31	0,051488	

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0179457	83,74%	80,61%	75,18%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	0,613558	A
Coated	16	0,560625	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
90	8	0,609940	A
30	8	0,601250	A
60	8	0,592500	A
0	8	0,543750	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	0,587099	A
Climated	16	0,586621	A

Means that do not share a letter are significantly different.

Table C.12: Statistical table for pH

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Conditions	Fixed	2	Climated; Normal
Coating	Fixed	2	Coated; Uncoated

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Coating	1	0,005036	0,005036	8,16	0,008
Time	3	0,319679	0,106560	172,77	0,000
Conditions	1	0,000039	0,000039	0,06	0,803
Error	26	0,016036	0,000617		
Lack-of-Fit	10	0,006369	0,000637	1,05	0,446
Pure Error	16	0,009667	0,000604		
Total	31	0,342088			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0248348	95,31%	94,41%	92,86%

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	4,37451	A
Coated	16	4,34937	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
0	8	4,53125	A	
30	8	4,33625	B	
60	8	4,30500	B	C
90	7	4,27528		C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Climated	15	4,36306	A
Normal	16	4,36083	A

Means that do not share a letter are significantly different

Table C.13: Statistical table for Hardness

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	85673	28557,7	24,54	0,000
Coating	1	1431	1431,4	1,23	0,278
Conditions	1	45455	45454,8	39,06	0,000
Error	26	30255	1163,7		
Lack-of-Fit	10	28174	2817,4	21,66	0,000
Pure Error	16	2081	130,1		
Total	31	162814			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
34,1125	81,42%	77,84%	71,85%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
60	8	307,583	A	
0	8	208,762		B
30	8	190,486		B
90	8	174,816		B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	16	227,100	A
Uncoated	16	213,724	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	258,101	A
Climated	16	182,723	B

Means that do not share a letter are significantly different.

Table C.14: Statistical table for Cohesiveness

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0,021593	0,007198	24,39	0,000
Coating	1	0,002433	0,002433	8,24	0,008
Conditions	1	0,000520	0,000520	1,76	0,196
Error	26	0,007671	0,000295		
Lack-of-Fit	10	0,006935	0,000693	15,07	0,000
Pure Error	16	0,000736	0,000046		
Total	31	0,032217			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0171770	76,19%	71,61%	63,93%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	8	0,232500	A
30	8	0,197125	B
60	8	0,185750	B
90	8	0,160250	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	16	0,202625	A
Uncoated	16	0,185188	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	0,197937	A
Climated	16	0,189875	A

Means that do not share a letter are significantly different.

Table C.15: Statistical table for Springiness

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0,010062	0,003354	21,73	0,000
Coating	1	0,001238	0,001238	8,02	0,009
Conditions	1	0,000011	0,000011	0,07	0,789
Error	26	0,004013	0,000154		
Lack-of-Fit	10	0,003635	0,000363	15,36	0,000
Pure Error	16	0,000379	0,000024		
Total	31	0,015324			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0124237	73,81%	68,78%	60,33%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	8	0,168250	A
30	8	0,145500	B
60	8	0,137250	B
90	8	0,118875	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	16	0,148687	A
Uncoated	16	0,136250	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Climated	16	0,143062	A
Normal	16	0,141875	A

Means that do not share a letter are significantly different.

Table C.16: Statistical table for Chewiness

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	143,329	47,7763	20,37	0,000
Coating	1	16,226	16,2264	6,92	0,014
Conditions	1	34,839	34,8392	14,85	0,001
Error	26	60,981	2,3454		
Lack-of-Fit	10	56,560	5,6560	20,47	0,000
Pure Error	16	4,421	0,2763		
Total	31	255,376			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,53148	76,12%	71,53%	63,83%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	8	8,57824	A
60	8	8,18617	A
30	8	6,02798	B
90	8	3,25476	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	16	7,22388	A
Uncoated	16	5,79970	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	7,55521	A
Climated	16	5,46837	B

Means that do not share a letter are significantly different.

Table C.17: Statistical table for Resilience

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	0,015185	0,005062	36,60	0,000
Coating	1	0,001653	0,001653	11,95	0,002
Conditions	1	0,001984	0,001984	14,35	0,001
Error	26	0,003596	0,000138		
Lack-of-Fit	10	0,003255	0,000325	15,27	0,000
Pure Error	16	0,000341	0,000021		
Total	31	0,022418			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0117598	83,96%	80,88%	75,70%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	8	0,136500	A
30	8	0,109625	B
60	8	0,100250	B
90	8	0,075625	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Coating

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	16	0,112687	A
Uncoated	16	0,098312	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	0,113375	A
Climated	16	0,097625	B

Means that do not share a letter are significantly different.

Table C.18: Statistical table for Toughness

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	601,97	200,656	6,93	0,001
Coating	1	1,11	1,111	0,04	0,846
Conditions	1	547,10	547,096	18,89	0,000
Error	26	752,91	28,958		

Lack-of-Fit	10	718,74	71,874	33,66	0,000
Pure Error	16	34,17	2,135		
Total	31	1903,08			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5,38127	60,44%	52,83%	40,07%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping		
30	8	34,0591	A		
60	8	32,0503	A	B	
0	8	26,5874		B	C
90	8	23,1234			C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	16	29,1413	A
Uncoated	16	28,7687	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	33,0899	A
Climated	16	24,8202	B

Means that do not share a letter are significantly different.

Table C.19: Statistical table for Work to Bite

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	105563	35187,7	13,80	0,000
Coating	1	449	449,5	0,18	0,678
Conditions	1	77913	77913,2	30,55	0,000
Error	26	66312	2550,5		
Lack-of-Fit	10	64425	6442,5	54,64	0,000
Pure Error	16	1887	117,9		
Total	31	250238			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
50,5020	73,50%	68,40%	59,86%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
30	8	366,411	A
60	8	359,159	A
0	8	280,195	B
90	8	228,038	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Uncoated	16	312,199	A
Coated	16	304,703	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	16	357,794	A
Climated	16	259,107	B

Means that do not share a letter are significantly different.

Table C.20: Statistical table for Appearance

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	8,4314	2,8105	32,66	0,000
Coating	1	0,5968	0,5968	6,93	0,025
Conditions	1	11,8164	11,8164	137,30	0,000
Error	10	0,8606	0,0861		
Total	15	21,7052			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,293362	96,03%	94,05%	89,85%

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	8	8,41500	A
Climated	8	6,69625	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	8	7,74875	A
Uncoated	8	7,36250	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	4	8,5650	A
30	4	7,8650	B
60	4	7,1450	C
90	4	6,6475	C

Means that do not share a letter are significantly different.

Table C.21: Statistical table for Odor

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	7,7101	2,5700	21,88	0,000
Coating	1	0,1764	0,1764	1,50	0,248
Conditions	1	3,3856	3,3856	28,82	0,000
Error	10	1,1746	0,1175		
Total	15	12,4467			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,342724	90,56%	85,84%	75,84%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	4	8,505	A
30	4	7,870	A B
60	4	7,440	B
90	4	6,595	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	8	7,7075	A
Uncoated	8	7,4975	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	8	8,0625	A
Climated	8	7,1425	B

Means that do not share a letter are significantly different.

Table C.22: Statistical table for Taste

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90
Coating	Fixed	2	Coated; Uncoated
Conditions	Fixed	2	Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	6,85035	2,28345	39,27	0,000
Coating	1	0,01322	0,01322	0,23	0,644
Conditions	1	2,25000	2,25000	38,69	0,000
Error	10	0,58153	0,05815		
Total	15	9,69510			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,241148	94,00%	91,00%	84,64%

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
0	4	8,6000	A
30	4	7,9700	B
60	4	7,5125	B
90	4	6,8075	C

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	8	7,75125	A
Uncoated	8	7,69375	A

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	8	8,0975	A
Climated	8	7,3475	B

Means that do not share a letter are significantly different.

Table C.23: Statistical table for Texture

Factor Information

Factor	Type	Levels	Values
Time	Fixed	4	0; 30; 60; 90

Coating	Fixed	2 Coated; Uncoated
Conditions	Fixed	2 Climated; Normal

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Time	3	6,0081	2,00271	21,86	0,000
Coating	1	0,6045	0,60451	6,60	0,028
Conditions	1	2,2276	2,22756	24,31	0,001
Error	10	0,9162	0,09162		
Total	15	9,7563			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,302682	90,61%	85,91%	75,96%

Grouping Information Using the Tukey Method and 95% Confidence

Conditions	N	Mean	Grouping
Normal	8	8,22000	A
Climated	8	7,47375	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Coating	N	Mean	Grouping
Coated	8	8,04125	A
Uncoated	8	7,65250	B

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
0	4	8,7825	A	
30	4	7,8900	B	
60	4	7,6275	B	C
90	4	7,0875		C

Means that do not share a letter are significantly different.