REPUBLIC OF TURKEY
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY UNIVERSITY

GRADUATE SCHOOL OF INSTITUTE
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PRODUCTION OF EDIBLE FILM FROM BUCKWHEAT STARCH MODIFIED WITH FATTY ACIDS

ESRA KOCA

MASTER OF SCIENCE

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submitted by **Esra KOCA** in partial fulfillment of the requirements for the degree of **Master of Science in Department of Food Engineering, Adana Alparslan Türkeş Science and Technology University** by,

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

Esra KOCA

ABSTRACT

PRODUCTION OF EDIBLE FILM FROM BUCKWHEAT STARCH MODIFIED WITH FATTY ACIDS

Esra KOCA

Department of Food Engineering

Supervisor: Asst. Prof. Dr. Levent Yurdaer AYDEMİR

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Food polymers such as proteins, polysaccharides and lipids in the generally recognized as safe (GRAS) class and various plasticizers capable of increasing the flexibility of the resultant polymeric films are employed in the production of edible films. Among the polysaccharides, starch stands out because of its economical advantages. Starch-based films however are generally hygroscopic nature leading to the poor barrier and mechanical properties. In order to improve these properties, several applications such as cross-linking of starch or forming complexes of starch with other natural polymers can be performed. In this study, the film production parameters in the preparation of edible films from buckwheat starch, and amylose-lipid complexes formed by using different fatty acids were optimized. Capric acid and myristic acid were used as fatty acids and sorbitol was used as the plasticizer. The optimal conditions yielding the buckwheat starch and amylose-lipid complex films with desired mechanical properties were determined via the optimization of three different parameters (sorbitol concentration, pH and the temperature of the film forming solution) important in the film preparation. After optimization, the films having superior mechanical properties were characterized in terms of physical, barrier and optical properties. The ALC films were found to be more elastic yet had lower tensile strength when compared to the BS films. The physical, mechanical, optical and barrier properties of the films prepared at optimal conditions were investigated. The tensile strength, elongation at break and elastic modulus of films produced from natural buckwheat starch and modified starch by capric and myristic acid were found to be 0.339, 0.139, 0.08 MPa, 45.62, 99.44, 127.94 % and 0.062, 0.051, 0.0015 MPa, respectively. When the films were evaluated in terms of water vapour permeability, water solubility and moisture content, the water vapour permeability of the films was determined as 0.312, 0.147, 0.196 g.mm/m^2.h.kPa, while the water solubility and moisture contents of the BS and ALC

films were not significantly different from each other. As a result, the tensile strength, elastic modulus and water vapour permeability of the modified films were lower than the natural buckwheat starch films but the elongation value was higher.

Keywords: Edible Film, Buckwheat Starch, Amylose-Lipid Complex, Fatty acid, Mechanical strength

ÖZET

YAĞ ASİTLERİ İLE MODİFİYE EDİLMİŞ KARABUĞDAY NİŞASTASINDAN YENİLEBİLİR FİLM ÜRETİMİ

Esra KOCA

Gıda Mühendisliği Anabilim Dalı

Danışman: Dr. Öğr. Üyesi Levent Yurdaer AYDEMİR

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Yenilebilir film üretiminde daha çok proteinler, polisakkaritler, lipitler gibi "GRAS" sınıfında bulunan gıda polimerleri ve bu polimerlerin oluşturduğu filmlerin esnekliğini artırmak için çeşitli plastikleştiriciler kullanılır. Polisakkaritler arasında, ekonomik olması nedeniyle nişasta öne çıkmaktadır. Ancak nişasta tabanlı filmler genellikle higroskopiktir ve bu nedenle zayıf bariyer ve mekanik özelliklere sahiptir. Bu özelliklerini geliştirmek için nişastanın çapraz bağlanması veya nişastanın diğer doğal polimerlerle kompleks oluşturması gibi uygulamalar gerçekleştirilebilir. Bu çalışmada, karabuğday nişastası ve farklı yağ asitleri kullanılarak olluşturulmuş amiloz-lipid komplekslerinden film üretim parametreler optimize edilmiştir. Amiloz-lipid kompleks oluşumunda yağ asidi olarak kaprik asit ve miristik asit, film çözeltisinde plastikleştirici olarak sorbitol kullanılmıştır. Optimizasyondan sonra en iyi mekanik dayanıma sahip filmler fiziksel, bariyer, optik, özellikler açısından karakterize edilmiştir. Karabuğdaay nişastası ve amiloz-lipid kompleksi filmlerinin üretiminde önem taşıyan üç farklı parametre (sorbitol konsantrasyonu, pH ve film hazırlama çözeltisinin sıcaklığı) optimize edilerek en iyi mekanik dayanıma sahip filmlerin elde edileceği koşullar belirlenmiştir. Karabuğday nişastası filmlerine kıyasla, amiloz-lipid kompleksi (ALC) filmlerinin daha elastik olduğu ancak daha düşük gerilme mukavemetine sahip olduğu bulunmuştur. Optimize edilmiş filmlerin fiziksel, mekanik, optik ve bariyer özellikleri araştırıldı. Doğal karbuğday nişasta, kaprik asitle modifiye edilmiş nişasta ve miristik asit ile modifiye edilmiş nişasta filmlerinin gerilme dirençleri sırasıyla 0.339, 0.139, 0.08 MPa, uzama değerleri 45.62, 99.44, 127.94 % ve elastic modülleri 0.062, 0.051, 0.0015 MPa olarak bulunmuştur. Filmler su buharı geçirgenliği, suda çözünürlük ve nem içeriği açısından değerlendirildiğinde, BS ve ALC filmlerinin suda çözünürlükleri ve nem içerikleri

birbirlerinden önemli ölçüde farklı değilken, filmlerin su buharı geçirgenlikleri sırasıyla 0.312, 0.147, 0.196 g.mm/ m^2.h.kPa olarak belirlenmiştir. Sonuç olarak, modifiye filmlerin gerilme direnci, elastik modül, su buharı geçirgenlikleri, doğal karabuğday nişasta filmlerinden daha düşük, uzama değeri ise daha yüksek bulunmuştur.

Anahtar Kelimeler: Yenilebilir Film, Karbuğday Nişastası, Yağ Asitleri, Amiloz-Lipit Kompleks, Mekanik Dayanım

To my family

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

EF	: Edible film
EC	: Edible coating
ALC	: Amylose-lipid complex
BS	: Buckwheat starch
CA	: Capric acid
MA	: Myristic acid
BS-CA	: Buckwheat starch - capric acid complex
BS-MA	: Buckwheat starch - myristic acid complex
TS	: Tensile strength
EM	: Elastic modulus
EAB	: Elongation at break
MC	: Moisture content
WVP	: Water vapour permeability
N	: Newton
s	: Second
mm	: Millimeter
FTIR	: Fourier-transform infrared spectroscopy
XRD	: X-ray diffraction
AFM	: Atomic force microscopy
SEM	: Scanning electron microscopes

1. INTRODUCTION

Although most foods can be consumed directly after harvest, it takes time to deliver food product to the consumer due to transportation, distribution, and storage requirements. During food delivery process to the market, some precautions must be taken to protect food from drying out, deterioration, and the formation quality defects contributing negatively to appearance, flavour and nutritional value. Therefore, the use of packaging materials are mostly required to keep food fresh and in high quality during the period between harvest and consumption (Skurtys et al., 2010). Synthetic polymers which are light in weight, highly formabale and cost efficient and have higher strength, are used to manufacture food packaging materials. The most commonly used packaging materials are petroleum-based materials such as polyethene and polypropylene. However, these materials are becoming less available and more expensive due to rapidly consumption of crude oil resources or increasing oil prices. Moreover, synthetic polymers are becoming less preferable because they cause severe environmental problems such as being non biodegradable and existing in the nature for hundreds or thousands of years. These properties lead the production and use of plastic polymers to be unsustainable. Besides plastic packaging materials, synthetic food additives such as antioxidants and antimicrobials also have been extensively used in food industry to increase food shelf life by reducing food deterioration caused by oxidation reactions or microbial infections which adversely affect human health. Therefore, the environmental and health concerns have led to find solutions to decrease plastic polymer dependence and synthetic food preservative use. From this perspective the use of biodegradable packaging materials instead of plastic-based ones is getting popular and many researches have been conducting studies to find alternative packaging materials based on natural sources as carbohydrates, proteins and lipids. Natural food-based polymers which are classified as edible films and coatings have the potential to be an alternative to synthetic polymeric packaging materials. The studies showed that these edible films and coatings could meet the customer expectations providing sufficient shelf life and maintaining fresh food quality without negative impact on the environment. (Chen et al., 2021; Hassan et al., 2018; Mohamed et al., 2020).

As the food application of edible films and coating seems to be new, it is reported that this practice is very ancient. Wax coating first were used to prevent in China. In the 15th century, an edible coating known as Yuba, obtained from boiled soy milk traditionally used in Asian countries, was used to preserve food quality and improve its appearance. In the 16th century, meat products were coated with oil to prevent shrinkage, and sucrose was first used as an edible protective coating to prevent oxidation and spoilage during the storage of walnuts, almonds, and hazelnuts. The commercial use of edible films and coatings was limited to covering the fruit surface with a layer of wax in the 1960s. But the tendency for such applications increased, with about 600 companies has started to use films and coatings (Debeaufort et al., 1998; Dursun & Erkan, 2009; Embuscado, M. E., & Huber, 2009)

Today, there are various companies that produce commercialedible films and coatings such as BluWrap (USA), Devro Plc. (UK), JRF Technology LLC (USA), MonoSol LLC (USA), Safetraces, Inc. (USA), Skipping Rocks Lab. (UK), Tate & Lyle Plc (UK), Watson, Inc. (US), WikiCell Designs Inc (US).

Edible films and coatings are thin layers coated on food and used to create a barrier between food and the environment. These films and coatings have many advantages, but the most important ones are their biodegradability and edibility.To provide these two features, the selected ingredients must be environmentally safe, odourless, tasteless, colourless, transparent, clear, and compatible with the foodstuff as much as possible not to cause any negative effects during consumption (Talens et al., 2010). These films exhibit many functions, such as separating food from the surrounding environment, acting as a barrier against moisture and gas transmission, prolonging shelf life by preventing food spoilage, and protecting food against physical impacts. These films also prevents aroma loss by protecting the volatile organic compounds in food and avoids the light-induced chemical reactions until consumption (Fig.1.1) (Mohamed et al., 2020; Otoni et al., 2017).

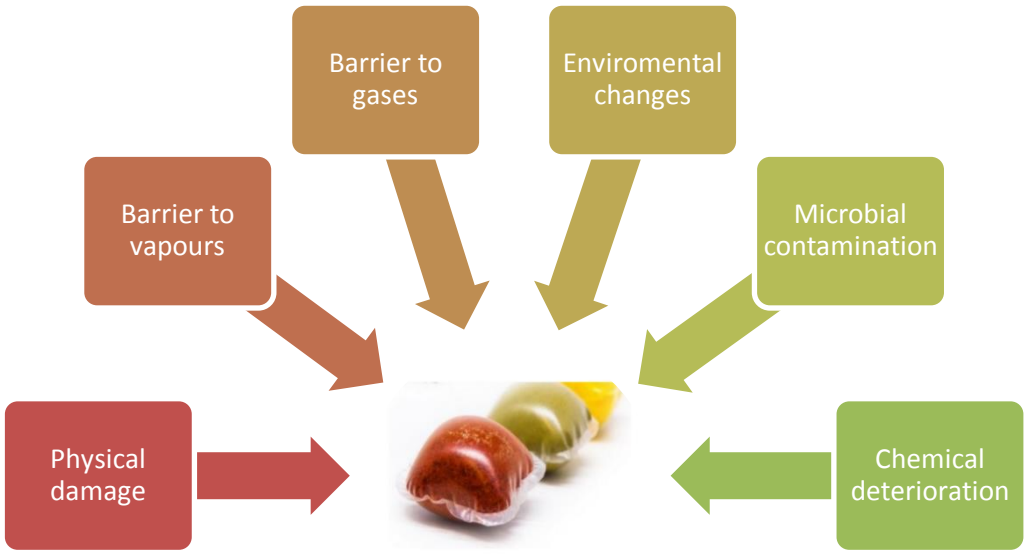


Figure 1.1. Functions of edible films and coatings

Although edible films and coatings are thought to be the same because they have similar properties, they are actually different concepts. As can be seen in Figure 1.2., the main difference between them is that their application methods are different. While the coating solution is applied as a liquid on food surfaces by methods such as dipping, spraying, painting, etc., the films are obtained by pouring technique and are applied by wrapping the food surface as a solid. (Mohamed et al., 2020; Suhag et al., 2020).

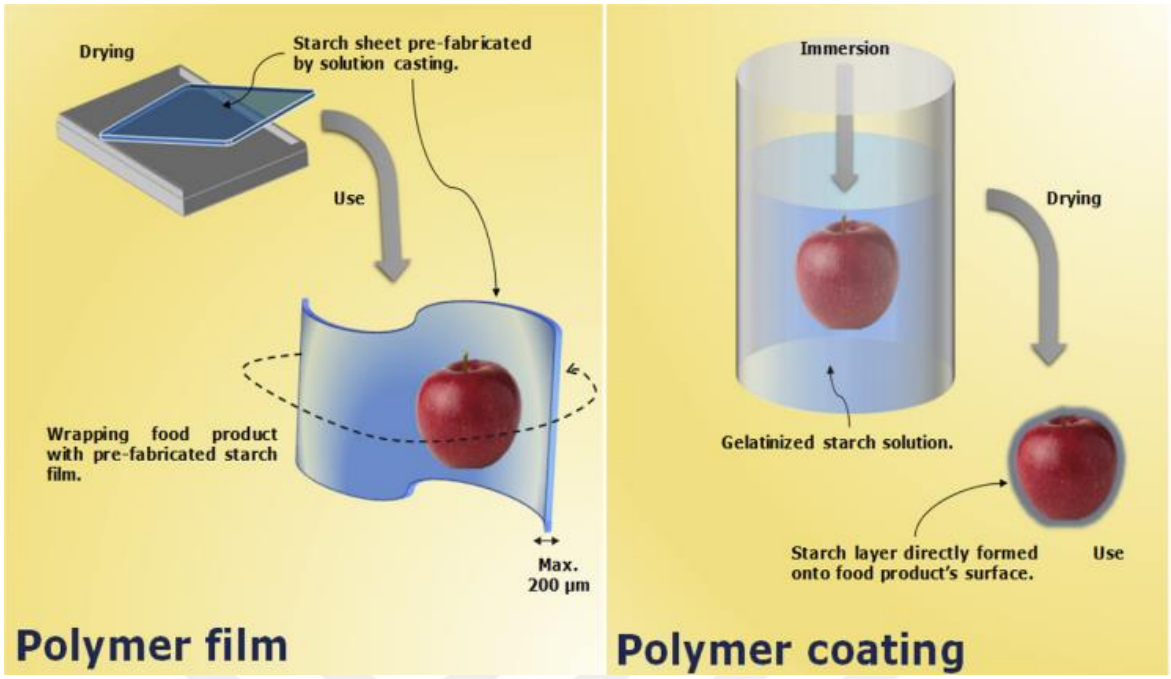


Figure 1.2. Differences between edible film and coating (Pelissari et al., 2018)

While methods like dipping and spraying are utilized to create edible coatings, two different methods—wet and dry—are employed to produce edible films. The wet method, often referred to as solution casting involves dissolving or dispersing polymers and other components in a proper solvent and then pouring the solution over a smooth surface to form the film structure, followed by drying. This method is straight and simple, so it is extensively used in laboratory conditions. The main advantage of this method is that the films are produced at a low cost without using special equipment. In addition, in this method, the dissolution of the polymer in the solvent leads to better particle-particle interaction, resulting in a more homogeneous film. Another advantage is that by producing films at low temperatures, irreversible structural changes that occur at very high temperatures are prevented. However, scaling up of film production process from laboratory scale to industrial scale is difficult since the several quality differences might emerge due to the difficulties in controlling process variables such as agitation speed, process temperature, and heating. This situation hinders edible film and coating development for commercial scales. In the dry method, films can be produced by extrusion and heat pressing without using solvents such as alcohol and water. The biopolymer is combined with plasticizers in the first phase and extruded in the next step; a film is prepared from the paste or pellets produced by injection moulding or thermal pressing (Chen et al., 2021; Hassan et al., 2018; Suhag et al., 2020).

At least one component with adequate adhesion and the ability to form a structural film matrix should be used in the formulation of films and coatings. The major components used in the edible film preparation are biopolymers such as polysaccharides, lipids and proteins (Figure 1.3). These polymers are included in the film formulation independently or as composites according to the properties of the food products to which the edible films or coatings applied and the advantages/disadvantages which they provide

Protein-based films are produced by using both animal and plant-based proteins. Animal-based proteins mostly include casein, keratin, collagen, and whey protein, while plant-based proteins mostly include soy protein, wheat gluten, zein, wheat germ protein, and peanut protein. Since proteins may create stronger intermolecular covalent bonds, protein-based films have superior mechanical characteristics and they can also act as a robust barrier to oxygen. However, they have a high water vapour permability because of their hydrophilic nature(Hassan et al., 2018). Lipids as oils, waxes, fatty acids can be used in the production of edible films and coatings for foods with high moisture contents, such as meats, seafood, fruits and vegetables. They can both prevent moisture loss and improve the product's appearance because these compounds have a hydrophobic character. Additionally, lipid films can also prolong shelf life by controlling oxygen transfer in the product they are used in. Furthermore, lipid-based films have poor mechanical properties they are brittle, and stick poorly to foods with hydrophilic surfaces. Therefore, they are rarely used to produce independent films and are commonly used by combining with hydrocolloid compounds (Chen et al., 2021; Hassan et al., 2018). Polysaccharides are frequently used to produce edible films or coatings owing to their low cost, simple availability, and effective film-forming capabilities. Edible films produced from polysaccharides generally act as a strong barrier against oxygen, flavour, and oil transfer due to their hydrophilic nature, yet they have poor mechanical characteristics and water vapour barrier properties. Alginate, pectin, dextrin, carrageenan, starch, chitosan, cellulose derivatives, and pullulans are polysaccharides that are generally used for film-forming (Chen et al., 2021). Consequently, in this field, lipids are used to minimize water transport, polysaccharides are used to regulate the movement of oxygen and other gases, and proteins are used to impart mechanical strength to edible films (Tural et al., 2017). In addition to these polymers, various plasticizers are added into film forming solutions to provide the films with higher flexibility and various additives can be added or polymer modifications can be performed to improve physical properties such as gas barrier and moisture barrier of the films (Chen et al., 2021).

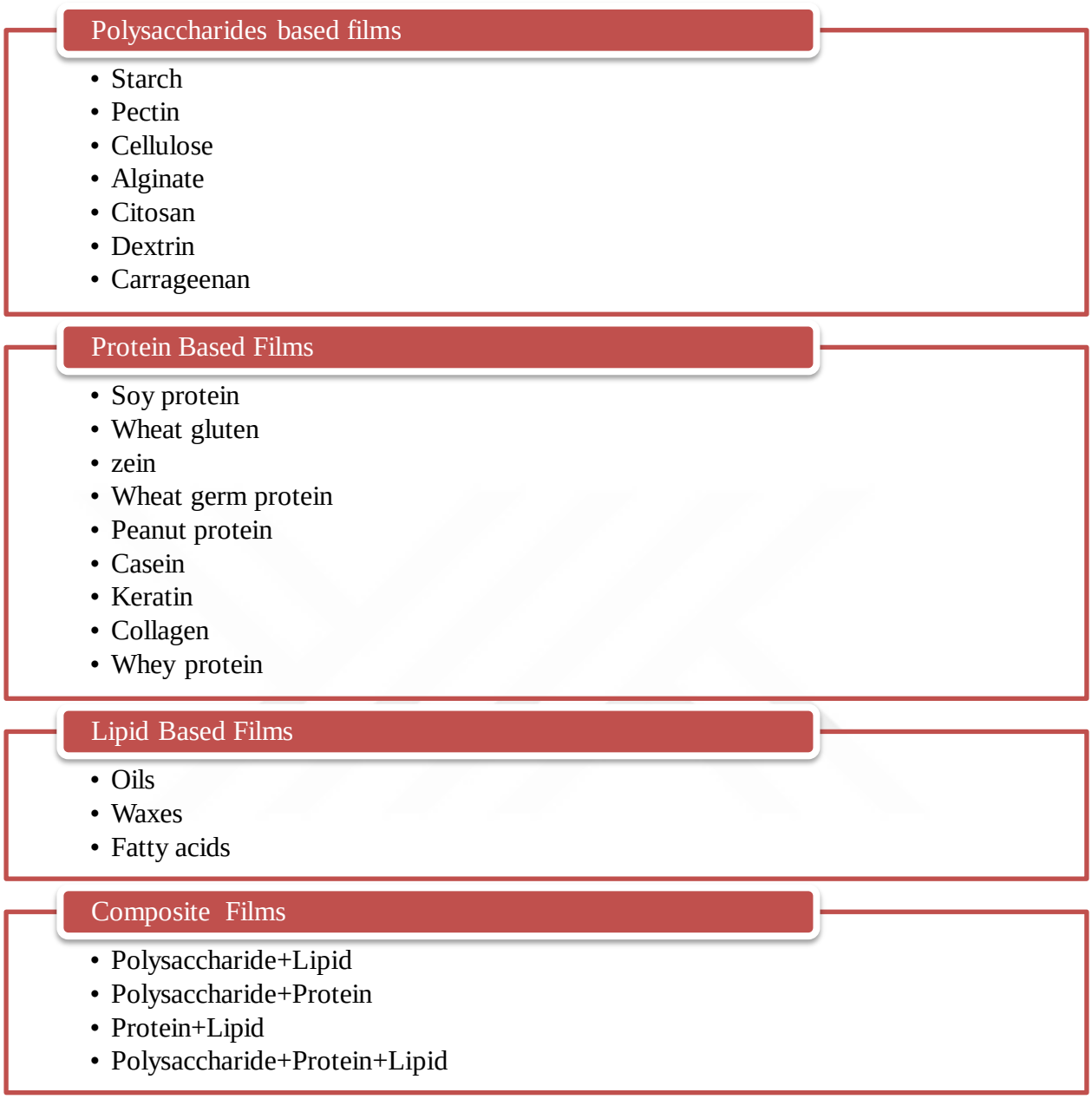


Figure 1.3. Categorization of edible films and coatings

In the edible films based on polysaccharides and proteins, the rigid film structures are formed due to the polymer-polymer interactions. The problems of unfavourable brittleness can be brought on by cohesive forces in polymer films. Cohesion and adhesion forces are two forms of interactions which are necessary for film formation. To prevent film brittleness, plasticizers which are low molecular weight compounds can be added to the polymeric film forming components. Plasticizers enhance intermolecular space, thus, increase film flexibility by decreasing intermolecular hydrogen bonding between polysaccharide or protein polymers.

Additionally, they increase the film's elongation, strength, processability, and reduce glass transition temperature (Tg) (Embuscado, M. E., & Huber, 2009; Sothornvit & Krochta, 2005). However, water molecule is considered as a plasticizer in film forming solutions, its plasticizing effect is very limited due to forming H-bonds between the components. Plasticizers that commonly used in edible film processing are glycerol, glycol, xylitol, sorbitol, ethanolamine, urea, formamide, and acetamide. Among them, polyols (glycerol, glycol, xylitol, sorbitol, etc.) are stated to be more effective in plasticizing hydrophilic polymers (Pelissari et al., 2018; Suhag et al., 2020). One of the polyols frequently utilized in starch-based films is sorbitol. It is a suitable plasticizer for starch-based films because it is more likely to interact with polymeric chains since its molecular structure is comparable to glucose units in starch chains. The schematic representation of the hydrogen bonds and intermolecular space that causes the increase in sorbitol-plastified flexibility is given in Figure 1.4. Compared to other plasticizers, the addition of sorbitol improves the mechanical properties of the film and increases the permeability less. Its major drawback is a propensity to crystallize in time, which reduces the flexibility of the film and eventually result in the loss of continuity (Embuscado, M. E., & Huber, 2009; Thirathumthavorn & Charoenrein, 2007).

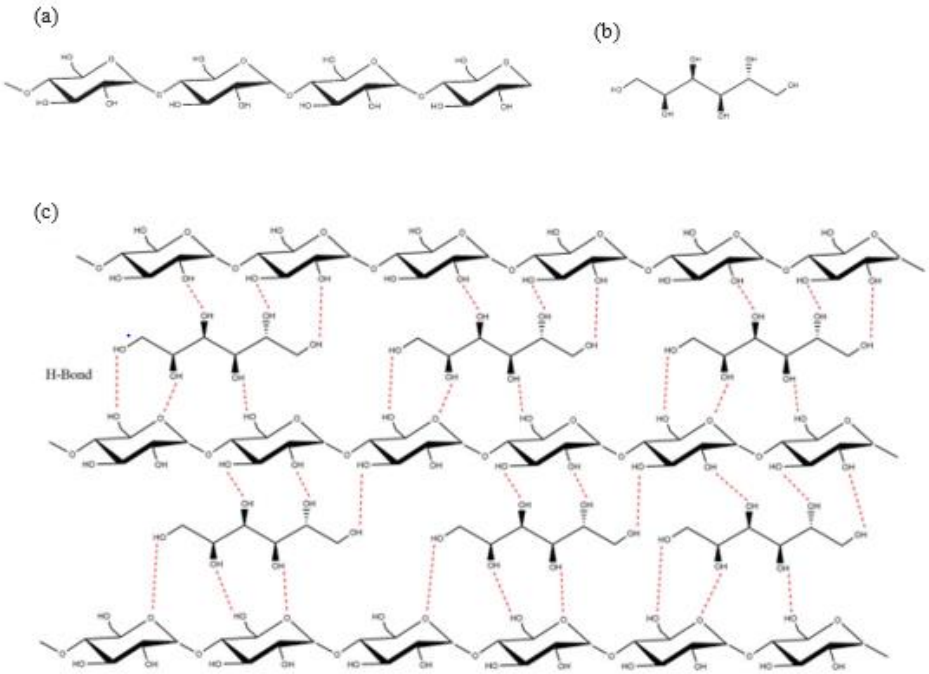


Figure 1.4. (a) Chemical structure of starch, (b) chemical structure of sorbitol and (c) the hydrogen bond formations between starch and sorbitol

Starch is one of the polysaccharides which is frequently used in the production of the films and coatings. since it is inexpensive, naturally occurring, simple to use, and ecologically acceptable (Vellaisamy Singaram et al., 2021). Starch films are also superior in terms of transparency and tasteless and they are strong CO_2 and O_2 barriers(Hassan et al., 2018).

Starch is a D-glucose polymer found in granular form in its sourcesand is chemically composed of amylose and amylopectin. A linear chain polymer called amylose is made up of 500-6000 glucose units connected by α-(1-4) D-glycosidic linkages. Its molecular weight varies between $1x10^5$-$1x10^6$ Dalton. Amylopectin, on the other hand, has a branched structure and is a polymer formed by connecting 25-30 glucose units to each other with α-(1-4) and α-(1-6) glycosidic bonds. Its molecular weight varies between $1x10^7$-$1x10^9$ Dalton (Figure 1.5).

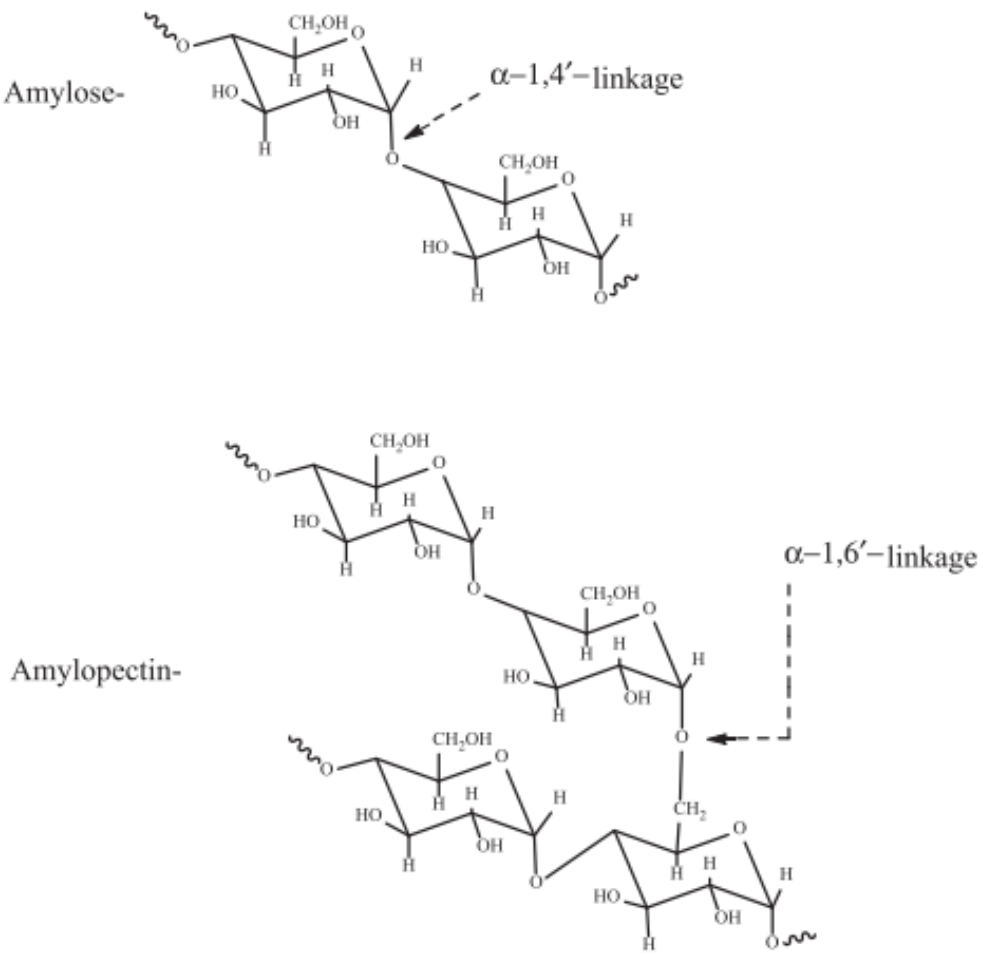


Figure 1.5. Structure of amylose and amylopectin (Agarwal, 2021)

In comparison to amylopectin, amylose has a greater tendency to scatter, to form gels, and to recrystallize in the aqueous media. Moreover, because of its primary linear structure the film formation and coating ability of amylose is better than amylopectin. Although the amylose: amylopectin ratio differs according to the source of starch, the amylose content of most starches is around 20-30%. Any kind of amylose-containing starch can be used to prepare a film or coating (Cazón et al., 2017; Embuscado, M. E., & Huber, 2009; Ratnayake & Jackson, 2008; Skurtys et al., 2010).

Besides its amylose content, starch produces stronger and better films due to its strong gelling capabilities and helical linear polymer structure (Pelissari et al., 2018). The preparation of the starch film starts with the preparation of a viscous solution by heating starch granules in excess water. Thus, starch gelatinization plays a crucial role in transforming starch into biodegradable films by destroying the semi-crystalline structure and obtaining a homogenous film solution. Figure 1.6 shows a schematic representation of the gelatinization of starch. Since the hydrogen bonds hold the starch chains together, the starch granules do not dissolve in cold water, but when heated, water enters the structure of starch granules, and it becomes swollen. As a result of the interactions between water molecules and the hydroxyl groups of amylose and amylopectin, the granules' crystal structure deteriorates. Thus, the reversible partial dissolution occurs. As the starch solution continues to be heated, water enters the amorphous area of the starch and amylose leaks out, disrupting the double helix structures of amylopectin. This results in the loss of birefringence in the starch granule. As a result of this process, the solution's viscosity rises and it progressively loses its crystalline structure, turning into a gel. The temperature at which 98 % of the birefringence of starch granules is lost is known as the gelatinization temperature. The temperature at which gelatinization occurs, which depends on how long the branch chain of amylopectin is, ranges from 65 to 110 °C. While smaller amylopectin chains easily form hydrogen bonds with water molecules, longer chains interact with one another instead of water molecules, resulting in a higher gelatinization temperature. (Agarwal, 2021; Cazón et al., 2017; Pelissari et al., 2018).

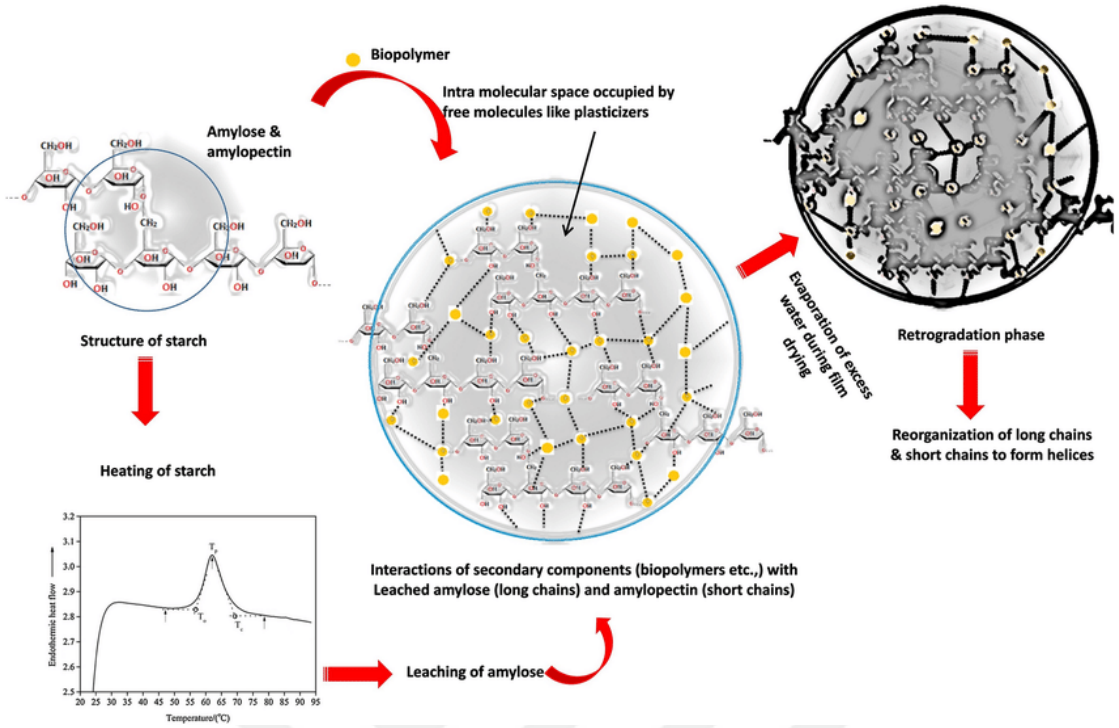


Figure 1.6. Schematic diagram of starch gelatinization (Thakur et al., 2019)

Starch is found in various plants, including wheat, corn, rice, beans, buckwheat, potatoes, etc. Its granules differ in form, size, structure, and chemical structure depending on the botanical source. Among these plants, buckwheat is a member of the Polygonaceae family, and there are two types grown around the world depending on the region of production. These varieties include common (*Fagopyrum esculentum Moench*) and tartary buckwheat (*Fagopyrum tataricum*). Buckwheat's main ingredient, starch, makes about 70% of the grain and has a considerable impact on the flavor, consistency, and quality of food products. There are 25% amylose and 75% amylopectins in buckwheat starch. It is a member of the A-type crystallinity group, and the amylose polymerization degree ranges from 1020 to 1380. Additionally, BS has a lower retrogradation rate than maize and wheat starch due to its lower molecular weight and degree of polymerization (Oskaybaş-Emlek et al., 2022; Zhu, 2016). Despite these benefits, BS has not been well investigated for edible film and coating applications according to our knowledge.

Since the films obtained from natural starch are not sufficient in terms of mechanical properties, natural starch is not used alone in the production of films and coatings. However, the properties of these films can be improved by including other polymers and plasticizers in the film

formulation or by modifying the natural starch. The natural starch can be modified as chemically, physically and enzymatically. Acid hydrolysis, oxidation, the formation of amylose-lipid complexes, and cross-linking are examples of chemical modification. Among these methods, chemical modification except the amylose-lipid complex formation, causes an environmental problem due to using a chemical substance. Lipids are extensively employed in starch modifications because they are food and environmental freidnlyl compounds (Oskaybaş-Emlek et al., 2022; Shah et al., 2016). Lipids are non-polar substances and hydrophobic in nature. They act as an excellent barrier against moisture migration, and have poor mechanical properties. Therefore, incorporating lipid in protein or polysaccharide-based films can cause the reduction in strength, increase in flexibility and reduce water vapour permeability. The majority of the lipids are made up of fatty acids which are compounds containing aliphatic chain with a carboxylic acid group. Most fatty acids in nature containing carbon atoms in number varying between 14 and 24, but some fats, such as tropical oils and dairy fats, include fatty acids which have 14 or fewer carbon. Common fatty acids used are shown in Figure 1.7. (Gregory, 2007; Sothornvit & Krochta, 2005).

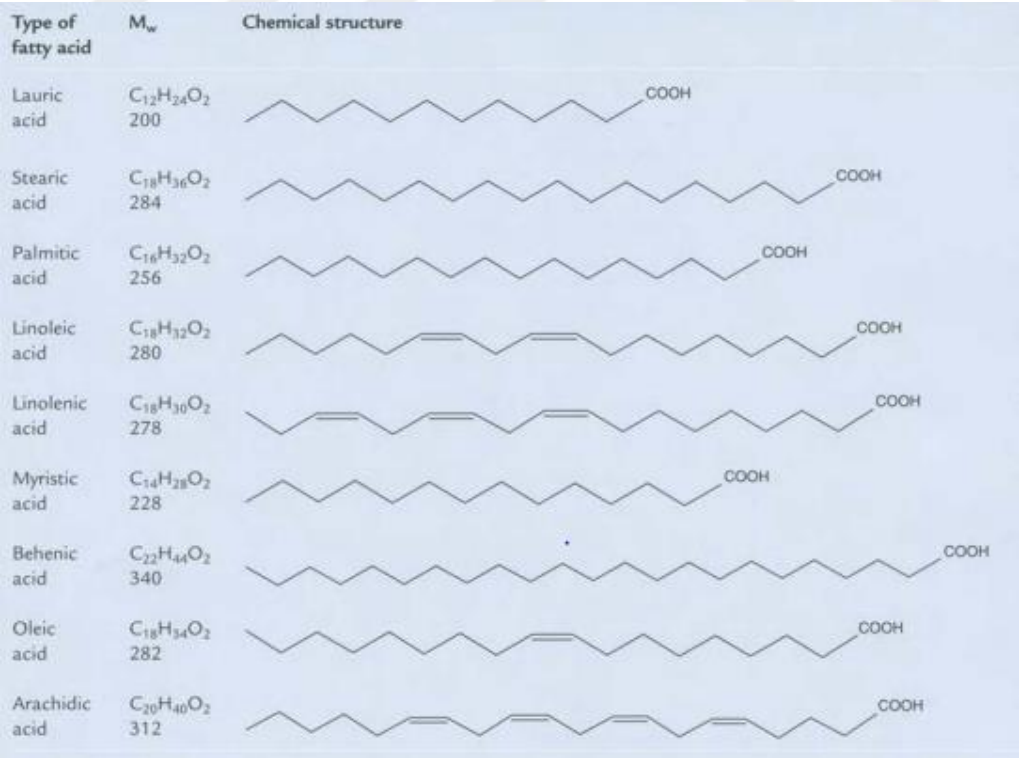


Figure 1.7. Fatty acids used in edible films and coatings **(**Sothornvit & Krochta, 2005)

Amylose-Lipid Complexes (ALCs) are amorphous or highly crystalline complexes formed by amylose and lipid, and are classified as resistant starch types III or V. ALCs are naturally found in starch at low levels, and their production might be developed by adding different fatty acids such as myristic acid, stearic acid, linoleic acid and etc. which increase ALC formation during thermal processing (Panyoo & Emmambux, 2017). Amylose forms single helical complexes (-helix) with a variety of substances, including iodine, monoglycerides, phospholipids, and fatty acids, depending on its long, linear structure in the starch. The hydroxyl groups of the glucose units in this helical form are thought to be on the outside of the helix, whereas the hydrophobic regions are located in the inner part of the helix. Because of this, the hydrophilic groups stretch toward the outside of the helix while the hydrophobic components awhich will make up the complex are found within the helical structure. The aliphatic hydrocarbon chain of the fatty acids interacts with the hydrophobic region of the inside of the helix to fill the space within the helix in amylose-lipid complexes generated by amylose and fatty acids (Hasjim et al., 2013) (Figure 1.8).

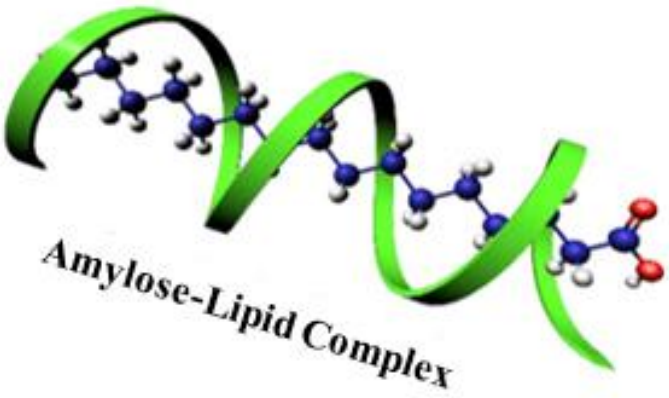
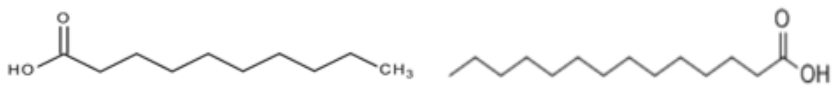


Figure 1.8 Amylose-lipid complex

The aim of this study was to optimize the film preparation conditions contributing to the mechanical properties of the edible films produced from buckwheat starch coplexed with different fatty acids and to characterize the films prepared at optimum conditions in terms of water solubility, moisture content and water vapor permeability. Capric and myristic acids were used to form amylose-lipid complexes. The structures of capric and myristic acids are given in Figure 1.9. Capric acid is a saturated fatty acid which has 10 carbon medium-chain and is also known as decanoic acid. Myristic acid is a 14-carbon straight-chain saturated fatty acid, that is

commonly present in the lipids of plants and animals. These fatty acids can be used as a plasticizer in the film production and provide the films with flexibility (Sothornvit & Krochta, 2005)



Capric acid Myristic acid

Figure 1.9. Structure of capric and myristic acids

2. LITERATURE REVIEW

In the literature, the starch films prepared by using the starch samples obtained from different plant sources have been extensively studied. Because of the various amylose:amylopectin ratios, the films prepared from these starches have different mechanical and barrier characteristics. Cano et al. (2014) investigated the mechanical characteristics of the different starch-based films and they discovered that films with a high amylose content had a lower oxygen permeability and a higher water-binding capacity. Additionally, it was noted that the film prepared from pea starch, which had the highest amylose-amylopectin ratio, exhibited the highest strength, but the film prepared from cassava starch, which had the lowest amylose-amylopectin ratio, had the greatest flexibility (Cano et al., 2014).

It has been noted in many studies that the structure of starch films is fragile and rigid. Therefore, plasticizer addition or modification of starch with different methods was needed to improve the film structure. Commonly, sorbitol and glycerol are employed as plasticisers in several studies (Ballesteros-Mártinez et al., 2020; Miller et al., 2021; Talja et al., 2007). Nordin et al. (2020) investigated the effect of different plasticizers and their combinations on the physical, optical, mechanical and thermal properties of the films. They reported that while the film thickness raised, the moisture content, solubility, and transparency of the films formed in the presence of both glycerol and thymol decreased. Although thymol and glycerol work together synergistically, glycerol has a more pronounced impact on the tensile strength of corn starch films than thymol (Nordin et al., 2020). Additionally, Martinez et al., (2020) studied the effects of the plasticizer concentration and type (glycerol and sorbitol) on the physical such as textural, optical, and barrier of sweet potato starch based edible films. For both plasticizers, they reported that the tensile strength was decreased whereas percentage elongation water solubility and water permeability increased as the plasticizer concentration increased. (Ballesteros-Mártinez et al., 2020). In another study, Miller et al., (2021) investigated the mechanical, barrier and optical properties of potato skin-based films produced by the solutions having glycerol and sorbitol in different concentrations. They reported that films' permeability to oxygen and water vapour increased when plasticizer content raised, and glycerol-containing films had greater water vapour and oxygen permeabilities than sorbitol-containing films. Also, they found that a high sorbitol content reduced the mechanical strength (TS, EM, and EAB) of the film, whereas high

glycerol concentration reduced TS and EM values but had no discernible effect on the elongation value (Miller et al., 2021).

In the literature, in addition to the use of plasticizers, there are also many studies on the starch modification to improve the film structure. In these studies, different chemical and physical starch modification methods were employed. For instance, Gomez et al. (2021) determined the effects of yam starch modification and glycerol concentration on the mechanical, barrier and optical properties of the films. They reported that the water vapour permeability of starch modified by oxidation decreased compared to natural starch and the mechanical properties of the films produced from oxidized starch increased as the glycerol concentration increased. Singaram et al. (2021) investigated the effects of the chemical modification of mango kernel starch on the film properties. They observed that the modified starch films' tensile strength was more than twice of the unmodified starch film. They also found that the L* value increased upon chemical modification indicating that the films prepared from modified starch were lighter in color than the film produced form unmodified starch.

Due to the use of additives which might leave residues in chemically synthesized film component and constitute a risk to the environment, consumers do less prefer chemical modification. Instead, fatty acids which have a "clean label" can be used for the modification of edible polymers. Fatty acids can be used by adding directly to the film solution or by forming a complex with amylose (Mapengo et al., 2019; Oskaybaş-Emlek et al., 2022). Wang et al. (2021) investigated the morphological, optical, mechanical and barrier properties of corn starch-based films containing varying concentrations of *Zanthoxylum bungeanum* essential oil (ZYO). When films containing ZYO were investigated, although the tensile strength, moisture content, water solubility and water vapour permeability rate significantly decreased in the films as the ZYO concentration increased, whereas the elongation at break significantly increased ($p<0.05$). In addition to mechanical properties, corn starch-based films containing different concentrations of ZYO had good antibacterial activity against both Gram-negative and Gram-positive bacteria (Wang et al., 2021).

The number of studies using amylose-lipid complex starches in the production of edible films is quite limited, and any research on amylose–lipid complex by buckwheat starch has not been reached in the literature. There is only one study about film formation obtained from native buckwheat starch. In that study, the effects of the addition of zinc oxide nanoparticles on the mechanical, optical and barrier properties of the buckwheat starch films were investigated (Kim

et al.,2018). Antimicrobial activity of the films against *Listeria monocytogenes* during the storage of fresh-cut mushrooms were also evaluated It was reported that there was a decrease in the film thickness and tensile strength and an increase in percentage elongation and the water vapour permeability with the increasing Zn oxide nanoparticle concentration. Furthermore, it was noted that the films showed an antimicrobial activity against *Listeria monocytogenes.*

Jimenez et al. (2013) aimed to minimize the films' hygroscopicity and enhance their water vapour permeability by incorporating fatty acids (stearic, palmitic, and oleic acid) into corn starch films containing glycerol as a plasticizer. They reported that the addition of fatty acids caused a decrease in the mechanical resistance, water vapor permeability, gloss and transparency. However, Wang et al., (2019) developed the production of potato starch-lauric acid complexes using various methods and their impact on the mechanical and barrier properties of the film. According to the researchers, the PS-LA complex produced films with stronger tensile strength, lower elongation at break, and lower moisture permeability than the natural starch-based film. Liu et al. (2018) employed sweet potato starch and lauric acid to form the amylose-lipid complex by ultrasonic treatment and investigated the effecs of sweet potato starch-lauric acid complex on the structural and physicochemical characteristics of the resultant films. The ultrasonically-treated films had stronger light transmission, lower elongation at break, higher tensile strength, and superior moisture barrier properties than the untreated starch films. It can be attributed to the more inclusion complexes that are formed by ultrasonic treatment, which have provided the film matrix more strength throughout the film-making process (Liu et al., 2018).

Liu et al. (2016) studied the effects of saturated (stearic acid) and unsaturated fatty acids (oleic and linoleic acids) on the mechanical properties and water permeability of the sweet potato starch-based films. They reported that sweet potato starch (SPS) -saturated fatty acid composite films exhibited higher tensile strength, lower elongation at break, and lower water vapour permeability than SPS-unsaturated fatty acid composite films (Liu, Sun, Hou, et al., 2016). In another study (Liu, Sun, Lu, et al., 2016), the effects of adding stearic acid (SA) before heating (Method I) and after heating (Method II) to the SPS on the properties of SPS-based films were investigated. It was reported that the films prepared by Method I had higher tensile strength and elongation values than the films prepared by Method II, and their moisture barrier properties were found to be better.

3. MATERIALS AND METHODS

3.1. Materials

Native buckwheat starch and amylose-lipid complexes were produced in Niğde Ömer Halisdemir University using the buckwheat (Mert Organik, İstanbul) procured from Ekotime company. Sorbitol was purchased from Sigma-Aldrich (Germany) while sodium hydroxide (NaOH) and hydrochloric acid were obtained from Tekkim (Turkey)

3.2. Methods

3.2.1. Preparation of Film

The effects of plasticizer concentration, pH and temperature of film forming solutionare important factors effecting the film properties. In order to determine the effects of these parameters on the mechanical properties of the films and to optimize the film preparation conditions central composite design was used. The lower and upper levels of these parameters used in the trial design are given in Table 3.1. The film forming solutions containing 3 (w/v) % native buckwheat starch or amylose-lipid complexes and varying concentrations of sorbitol at three different pH values (6-8-10) were prepared according to experimental design. Then, the film solution was homogenized for 4 minutes at 12000 rpm (Isolab, Germany), and heated by stirring for 30 minutes in a magnetic stirrer (IKA, CMAG HS7, Germany) at 70, 80 and 90˚C. The solutions were directly poured into polystyrene petri dishes (120 mm x 120 mm) then cooled down to room temperature and dried at 50 % relative humidity and 25 °C.

Table 3.1. Upper and lower levels of factors in the trial design to be used for starch-based edible film production

	Factor levels		**Responses**
Factors	**Lower Level**	**High Level**	
Sorbitol /Starch (g/100 g)	30	70	**Mechanical Properties**
pH of film solution	6	10	
Temperature of film forming solution	70	90	

3.2.2. Experimental Design

The effects of film-forming conditions on the mechanical properties of films were assessed using the response surface method (RSM). The factors evaluated were the sorbitol concentration, pH, and temperature of film forming solution, and with levels ranging from 30 to 70 g / 100 g starch, 6 to 10, and 70 to 90˚C, respectively. The trial design was formed using the face-centred composite design. Four center points were added to the selected design and a total of 18 trial points were determined (Table 3.2). Films in each run were produced and their mechanical properties were examined

Table 3.2. Face Centered Composite Design

Run	Sorbitol/Starch (g/100g)	pH	Temperature of film forming solution (˚C)
1	30	6	70
2	70	6	70
3	50	8	70
4	30	10	70
5	70	10	70
6	50	6	80
7	30	8	80
8	50	8	80
9	70	8	80
10	50	10	80
11	30	6	90
12	70	6	90
13	50	8	90
14	30	10	90
15	70	10	90
16	50	8	80
17	50	8	80
18	50	8	80

3.2.3. Mechanical properties

The ASTM D882 (ASTM, 2001) standard method was used for the determination of the mechanical properties of the films by using a 5 kg load cell with a Universal Tester (TA.XT plus, Stable Micro Systems, England). The films were conditioned for at least 40 hours in a test cabinet (Nüve TK 120, Türkiye) at 50 % RH and 25˚C before mechanical testing. Then, the films were cutted into strips with 7 mm wide and 60 mm long, were placed in the device and the analysis were performed at a speed of 30 mm/min. Tensile strengths (MPa), elongations at break (%) and elastic modulus (MPa) of the films were determined from the stress-strain curves using equations (1) and (2). A representative stress-strain curve is given in Figure 3.1.

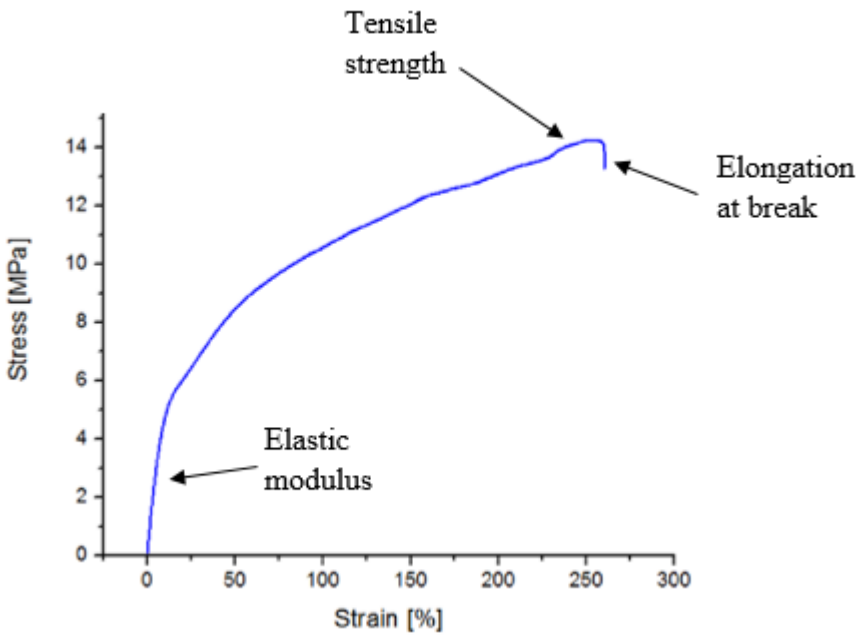


Figure 3.1. Stress-strain curve for mechanical properties

$$TS = \left(\frac{F}{A}\right) \quad (1)$$

where F is the maximum force and A is the cross-sectional area of the film.

$$EAB\ (\%) = \frac{(Lf - Li)}{Li} \times 100 \quad (2)$$

where L_f is the final length at a break and L_i is the initial length of the film

3.2.4. Moisture Content and Water Solubility

The moisture content (MC) of films was determined by gravimetric method. The films cut into 3 cm x 4 cm rectangles were weighted and the masses were denoted as m_0. They were dried at 105 °C until constant weight and final masses were recorded (m_1). The MC of the films was determined according to the following equation (3);

$$Moisture\ Content = \left(\frac{m0 - m1}{m0}\right) * 100 \quad (3)$$

The water solubility tests were carried out using the modified method reported by Kim et al (2018). For this purpose, the films were cut into square pieces (5x5 cm^2) and dried in an oven at 105 ˚C until they reached a constant weight, and the dry weights were recorded m_0. Dried films were then immersed in 30 mL distilled water and incubated in a shaking incubator for 24 hours at 25 °C and 200 rpm (IKA KS3000 IC, IKA®-WerkeGmbH&Co. KG, Staufen, Germany). At the end of 24 hours-incubation in water, films were removed and dried in an oven at 105 °C until constant weight. The masses of the films were recorded and denoted as m_1. The water solubilities of the films in percentages were calculated according to the following equation (4);

$$Solubility\ of\ Film\ (\%) = \left(\frac{m0 - m1}{m0}\right) * 100 \quad (4)$$

3.2.5. Film Thickness

The thicknesses of the films with an accuracy of 0.001 mm. The thickness was calculated by taking the average of thicknesses measured at five randomly selected distinct sites on each film.

3.2.6. Optical Properties

Colour of the films were measured using a Konica Minolta CR5 (Japan). According to the CIE colour system, L* is the indicator of brightness and ranges between 0 (black) and 100 (white). While a* represents red-green component of a color, b* indicates yellow-blue component. The calibration of the device was performed by using the standard white plate (L*=96.80, a*=-0.25-0,09, b*=-0.11).

Opacity of the films were measured according to ASTM D523. Rectangular films were placed in spectrophotometer cuvettes and opacity values were measured at 550 nm by using a spectrophotometer (ONDA spectrophotometers Vis 325-1000, V-10 plus)

3.2.7. Water Vapour Permeability

The water vapour permeabilities of BS and ALC films were analyzed according to ASTM E96/E96M standard method. The films were conditioned at 50 % RH in a dessicator. Glass test cups with a 5 cm internal diameter and a 3 cm depth were employed. RH of the measurement systems were adjusted using water (RH=100%) and silica gel (RH=0%). Films were sealed firmly over the cup containing silica gel, and the cups were weighted every hour periodically to evaluate the weight gain within a day. The WVP values were calculated using the equation (5);

$$WVP = \frac{\Delta m}{\Delta t \times \Delta P \times A} * d \qquad (5)$$

WVP is the water vapor permeability (g.mm / m^2.h. kPa), $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/h), A is the film area exposed to the moisture transfer (m^2), ΔP is the water vapor pressure difference (kPa), and d is the film thickness (mm)

3.2.8. Statistical Analysis

The results were assessed by one-way ANOVA and single sample T-test using SPSS programme (IBM SPSS Statistics for Windows, Version 15.0.). The Duncan multiple range test was used to identify statistical differences between means at a significance level of 5%.

4. RESULTS AND DISCUSSIONS

4.1.Optimization and validation

The film preparation parameters contributing to the mechanical properties of the films were optimized using “Response Surface Methodology”. The parameters used in the optimization were sorbitol concentration (30-70 g/100 g starch), pH (6-10) and temperature of film forming solution (70-90˚C). The experimental design used in the film production was constructed by the "Face Centered Composite Design". In line with the experimental design, 18 films were prepared by the selected parameters. The mechanical properties of the films were determined using the tensile strength, elongation at break and elastic modulus of the films. Edible films should exhibit sufficient mechanical strength and high flexibility to endure the possible external forces exerted during processing, transportation and storage. For this reason, mechanical strengths of the films prepared by using natural buckwheat starch or modified starch with two different fatty acids (capric and myristic acids) were selected as a response in the optimization of the film prepration parameters. Tensile strengths (TS-MPa), elastic modulus (EM-MPa) and percent elongations at break (EAB-%) were computed from the stress-strain curves obtained by texture analyzer. Tensile strength is expressed as the ratio of the maximum strength the film can withstand before breaking during the tensile test to the film area. Elongation is the maximum change in length of the sample before breaking and is expressed as the per cent change in the original length of the material. The stress-strain ratio in the first linear region of the stress-strain curve is known as the modulus of elasticity, commonly known as Young's modulus (Skurtys et al., 2010).

4.1.1. Mechanical properties of films obtained from BS

The TS, EAB and EM of the films prepared from the BS under different conditions varied between 0.2 and 0.489 MPa, 21.2% to 102.2%, and 0.059 to 0.548 MPa, respectively (Table 4.1). At constant temperature of film forming solution and pH, increase in the concentration of sorbitol in the film forming solution led to a decrease in the TS and EM yet increase in the EAB of the BS films. Because the sorbitol interferes with the polymer chain and this interaction improves the film flexibility and reduces the rigidity. Similar to these results, Martinez et al. (2020) reported a decrease in TS and an increase in EAB for increasing sorbitol concentration (Ballesteros-Mártinez et al., 2020).

Table 4.1. Mechanical properties of BS films

Run	Sorbitol/Starch (g/100g)	pH	Temperature of film forming solution (°C)	Mechanical Properties		
				TS (MPa)	EAB (%)	EM (MPa)
1	30	6	70	0.200	81.775	0.059
2	70	6	70	0.305	67.990	0.085
3	50	8	70	0.409	74.916	0.204
4	30	10	70	0.322	61.444	0.170
5	70	10	70	0.259	75.823	0.075
6	50	6	80	0.325	92.904	0.115
7	30	8	80	0.406	21.189	0.409
8	50	8	80	0.330	95.404	0.127
9	70	8	80	0.256	75.740	0.075
10	50	10	80	0.339	96.569	0.143
11	30	6	90	0.455	42.016	0.548
12	70	6	90	0.270	90.627	0.072
13	50	8	90	0.309	79.811	0.187
14	30	10	90	0.489	68.928	0.514
15	70	10	90	0.305	82.655	0.124
16	50	8	80	0.260	102.2	0.074
17	50	8	80	0.305	87.165	0.120
18	50	8	80	0.317	81.399	0.120

Table 4.2. ANOVA table for mechanical properties of BS films

		TS (MPa)		EAB (%)		EM (MPa)	
	sd	***F***	***p***	***F***	***p***	***F***	***p***
A (Concentration)	1	6.26	0.0369*	6.12	0.0384*	31.83	0.0005*
B (pH)	1	0.70	0.4267	0.045	0.8367	0.43	0.5308
C (Temperature)	1	3.05	0.1191	1.936E-003	0.9660	14.36	0.0053*
AB	1	0.96	0.3551	0.025	0.8782	0.032	0.8633
AC	1	5.89	0.0413*	2.11	0.1840	15.71	0.0042*
BC	1	2.16E-003	0.9641	0.55	0.4802	0.17	0.6889
A^2	1	0.031	0.8647	10.93	0.0108*	3.28	0.1075
B^2	1	0.024	0.8816	3.12	0.1151	0.64	0.4478
C^2	1	0.35	0.5701	0.019	0.8940	0.55	0.4793
Model	9	1.96	0.1866	2.42	0.1138	7.62	0.0044*
R^2		0.6829		0.7314		0.8956	

*Statistically significant ($p<0.05$)

The effects of the film preparation parameters on the mechanical properties of the resultant films were evaluated by central composite design The parameters with a $p<0.05$ significance level in the obtained quadratic model were considered as the parameters having significant effects on the results.

In the fully quadratic model, the TS and EAB models were found to be insignificant, but the sorbitol concentration (A) and the concentration-temperature (AC) interaction had a significant effect on the TS. In addition, the sorbitol concentration (A) and interaction of A^2 had also a significant effect on the elongation of the films. For the elastic modulus, the model was found to be significant ($p<0.05$) and it was observed that the concentration (A), temperature (C) and interaction of concentration-temperature (AC) had an effect on the elastic modulus of the films (Table 4.2).

Table 4.3. Statistical values in reduced quadratic model for films produced from BS according to face centered composite design

	TS (MPa)	EAB (%)	EM (MPa)
Lack of fit	0.1404	0.2042	0.1448
R^2	0.4817	0.5255	0.8154
Adjusted R^2	0.4126	0.4622	0.7758
Predicted R^2	0.1168	0.2826	0.7026
Adeq Precision	8.656	5.669	13.508
PRESS	0.080	4814.86	0.46

Various statistical parameters such as lack of fit, R^2, adjusted and predicted R^2, adeq precision, and predicted residual error sum of square (PRESS) were obtained in the reduced quadratic model, which was created by selecting the factors that had a significant effect in the full quadratic model (Table 4.3). Model adequacy criteria were accepted as lack of fit >0.05, R^2 > 0.90, adj-R^2-pre-R^2 < 0.2, pre-R2 > 0.7 (Salum & Erbay, 2019). There was a decrease in the R^2 value for TS, EAB, and EM in the reduced model compared to the full model. The pre- R^2 values of TS and EAB are 0.1168 and 0.2826, respectively, indicating that the predictive ability of this model is moderate. In the EM, the pre-R^2 value is 0.7026, which indicates that the predictive ability is good. The minimum adeq precision values of the models developed in this study are 5.669, and an adeq precision value greater than 4 means that the model precision is statistically acceptable.

The equations created according to the factors coded with the reduced quadratic model are as follows:

$$TS=+0.33-0.048\times A-0.052\times AC$$

$$EAB =+88.80+11.75 \times A-21.98 \times A^2$$

$$EM =+0.36-0.25\times A+0.17\times C-0.20\times AC$$

According to the obtained equations, sorbitol concentration and concentration-temperature (AC) interaction had a negative effect on the TS and EM of the films as sorbitol concentration contributed positively to the EAB. Additionally, film forming solution's temperature also contributed positively to the EAB of the films.

In the films obtained from BS, there was no significant change in TS as the concentration increased at low temperature (70˚C) whereas a decrease was observed at high temperature (90˚C). On the other hand, the TS increased with increasing temperature at low sorbitol concentration and decreased at high sorbitol concentration. It was determined that the TS reached its maximum in combination of high temperature and low sorbitol concentration (Figure 4.1).

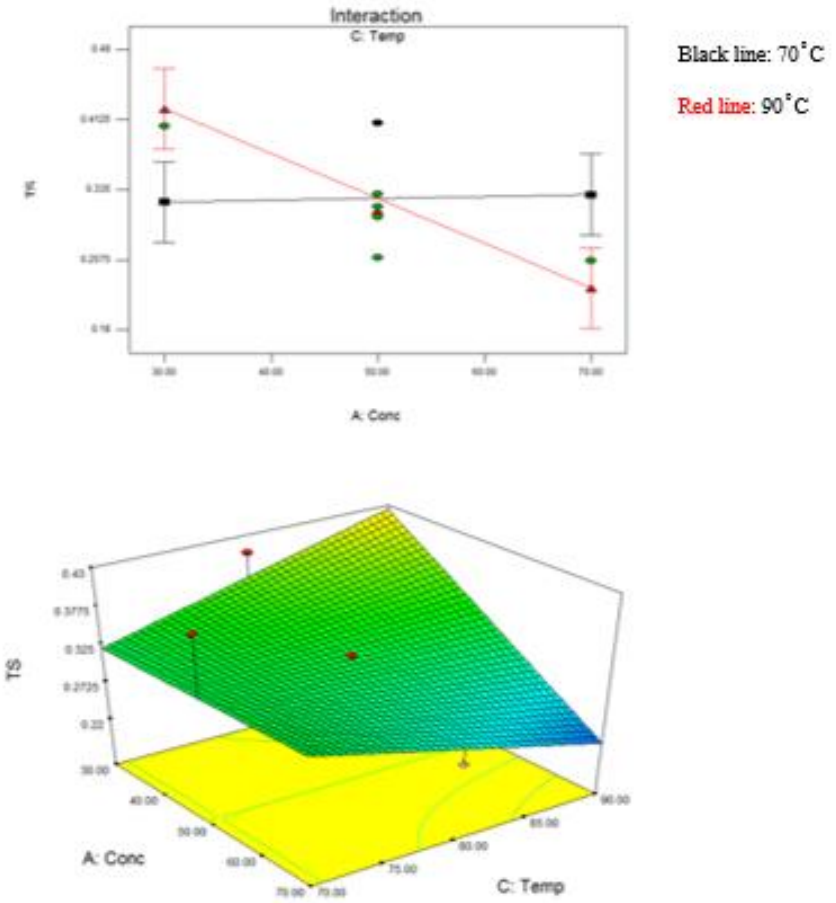


Figure 4.1. The effect of sorbitol concentration-temperature interaction on TS of films obtained from BS

As the sorbitol concentration was increased, the EAB of the BS films was increased, and the TS reached its maximum value when the sorbitol concentration was approximately 50 g/100 g starch. (Figure 4.2)

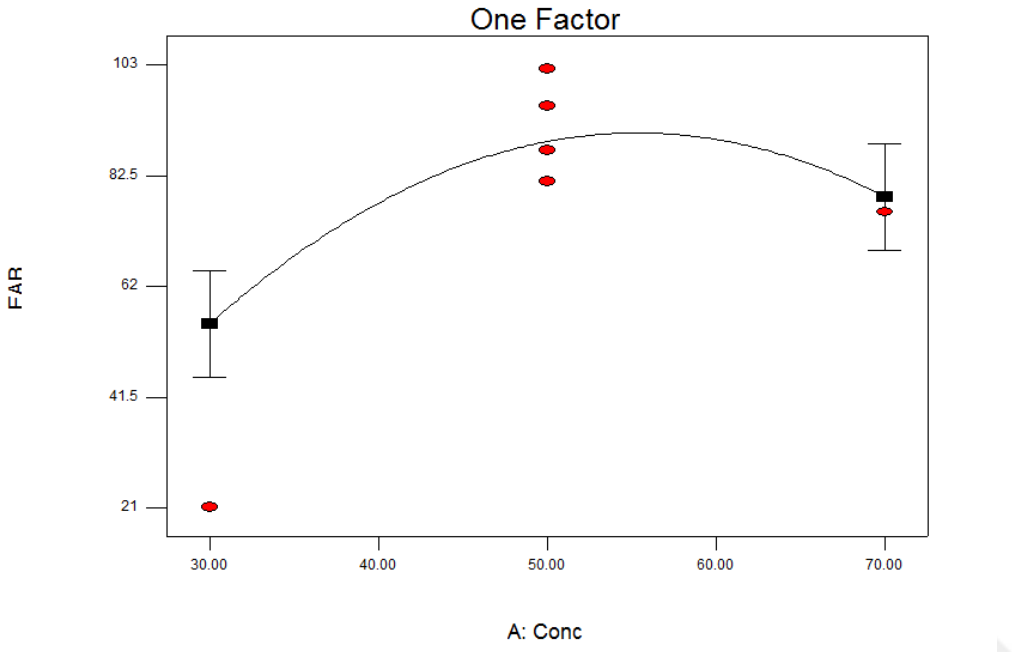


Figure 4.2. The effect of sorbitol concentration on EAB of films obtained from BS

In the BS films, no significant change was observed in the EM values as the concentration increased at low temperature (70˚C), while a decrease in EM was found at high temperature (90˚C). At low sorbitol concentration, while the EM of the films increased with the increasing temperature. but no significant change was found at high sorbitol concentration. It was determined that the EM of the films reached a maximum at the combination of high temperature (90˚C) and low sorbitol concentration, and reached a minimum at low temperature (70˚C) - low sorbitol concentration combination (Figure 4.3).

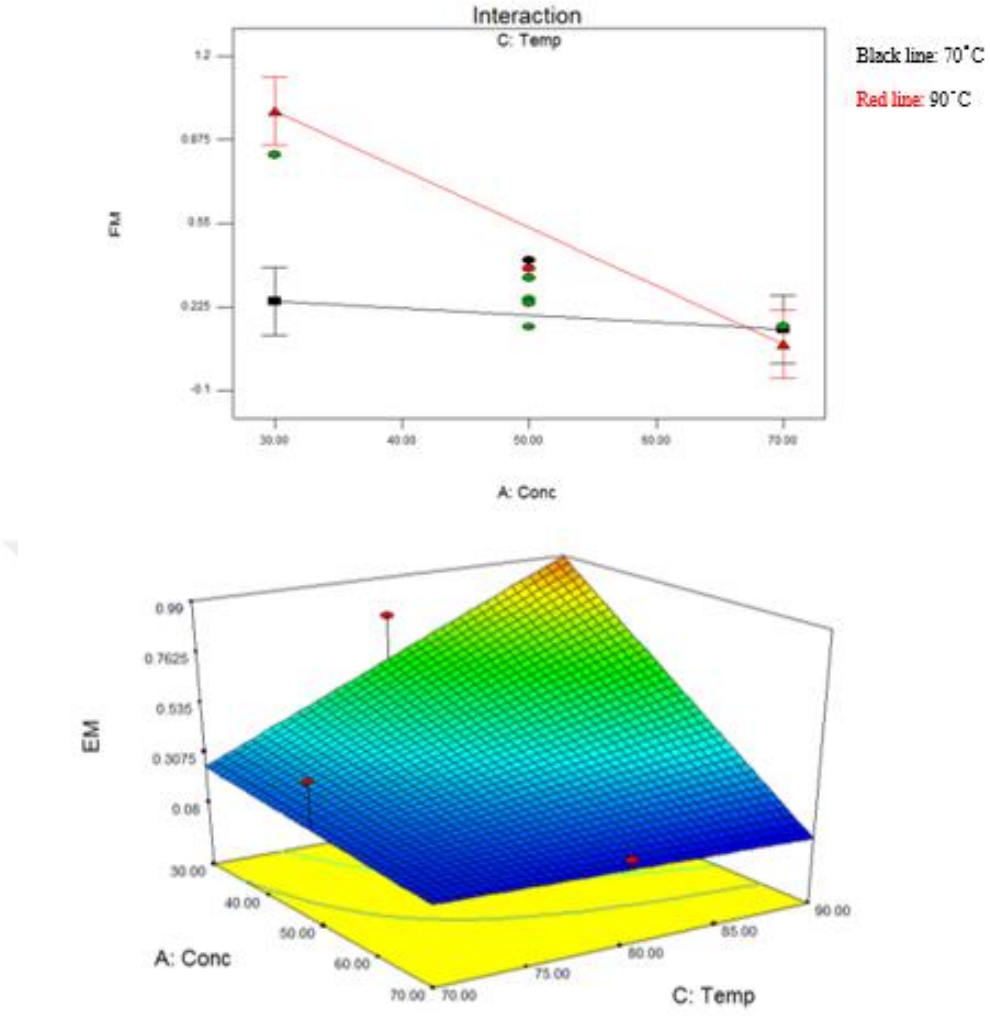


Figure 4.3. The effect of sorbitol concentration-temperature interaction on EM of films obtained from BS

Based on the models obtained from the ANOVA analysis, the criteria aimed in the optimization were chosen as the maximum value for TS and EAB, and the minimum value for EM. As a result of the surface response studies. significant results were obtained for the EM among the mechanical test results of the BS films ($P<0.05$). For the validation test, the film production condition was optimized so that the values of EM become minimum. The optimum trial condition obtained as a result was determined at a 100% desirability rate. As a result of the desirability function; optimum film production conditions were calculated as 58.57 (g/100g starch) sorbitol concentration. pH 6.62, and temperature of film forming solution 70.62 °C. Whether there is a statistically significant difference between the mean result of the experimental data obtained for the EM of the films and the value estimated from the model was

determined by applying the single sample t-test. It was found that there was no significant difference ($p>0.05$) between the experimental values and the estimated values from the model (Table 4.4)

Table 4.4. Validation of optimum conditions with experimental data for BS films

	EM (MPa)
Estimated Value	0.056
Actual Value	0.062±0.02
p-value	0.148

4.1.2. Mechanical Properties of Films Produced from BS-CA

The TS of the films obtained from BS-CA starch varied between 0.039 and 0.230 MPa, while the EAB and the EM ranged between 78.723 and 127.283%, and between 0.0015 and 0.131 MPa, respectively (Table 4.5). In the films obtained from BS-CA, with the increase in solution sorbitol concentration at constant temperature and pH, a decrease in both TS and EM and an increase in the EAB were observed. Since fatty acids are hydrophobic molecules, they can contribute to the improvement of the mechanical properties of films by exhibiting a plasticizing effect during the film formation. (Thakur et al. 2016).

It was determined that the model used for TS in the created full quadratic model was significant and sorbitol concentration (A), temperature (C), the interaction of concentration-pH (AB), concentration-temperature (AC) and A^2 had an effect on the TS of the films. The model used for the EAB value was found to be insignificant, but it was determined that the sorbitol concentration (A) had an effect on the EAB value of the films. For EM, the model and the effects of concentration (A), pH (B), temperature (C), interaction of concentration-pH (AB) concentration-temperature (AC) and A^2 on the EM of the films were found to be significant.

Table 4.5. Mechanical properties of films obtained from BS-CA

Run	Sorbitol/Starch (g/100g)	pH	Temperature of film forming solution (˚C)	Mechanical Properties		
				TS (MPa)	EAB (%)	EM (MPa)
1	30	6	70	0.109	99.856	0.046
2	70	6	70	0.063	111.91	0.003
3	50	8	70	0.062	112.97	0.007
4	30	10	70	0.208	96.963	0.130
5	70	10	70	0.075	127.28	0.003
6	50	6	80	0.075	85.020	0.019
7	30	8	80	0.203	85.087	0.132
8	50	8	80	0.117	108.85	0.010
9	70	8	80	0.063	112.96	0.002
10	50	10	80	0.098	102.79	0.021
11	30	6	90	0.191	99.220	0.128
12	70	6	90	0.056	94.517	0.004
13	50	8	90	0.094	123.03	0.026
14	30	10	90	0.230	78.723	0.198
15	70	10	90	0.039	94.818	0.004
16	50	8	80	0.084	96.075	0.012
17	50	8	80	0.079	113.22	0.015
18	50	8	80	0.051	87.194	0.005

Table 4.6. ANOVA table for mechanical properties of films produced from BS-CA according to face centered composite design

		TS (MPa)		EAB (%)		EM (MPa)	
	sd	*F*	*P*	*F*	*p*	*F*	*p*
A (Concentration)	1	130.43	0.0001*	5.41	0.0484*	344.78	<0.0001*
B (pH)	1	2.70	0.1390	2.80	0.1330	26.29	0.0009*
C (Temperature)	1	7.53	0.0253*	0.082	0.7817	21.79	0.0016*
AB	1	8.66	0.0186*	0.97	0.3525	24.89	0.0011*
AC	1	7.84	0.0232*	1.55	0.2485	26.81	0.0008*
BC	1	3.10	0.1164	1.08	0.3283	0.20	0.6690
A^2	1	18.20	0.0027*	0.62	0.4543	59.59	0.0001*
B^2	1	0.71	0.4250	4.11	0.0770	0.015	0.9057
C^2	1	5.736E-004	0.9815	2.39	0.1608	0.18	0.6789
Model	9	20.52	<0.0001*	1.92	0.1864	60.78	<0.0001*
R^2		0.9585		0.6830		0.9856	

*Statistically significant ($p<0.05$)

A reduced model was created by selecting the parameters that have a significant effect in the obtained full quadratic model, and the various statistical paramaeters were obtained from this model. No significant changes were found for the R^2 values of the TS and the EM in this model. Furthermore, adj and pre- R^2 values were found to be close to 0.90 and a model with high predictive power was obtained. The pre-R^2 value of the EAB is 0.0149 indicating that the predictive power is very low (Table 4.7).

Table 4.7. Statistical values in reduced quadratic model for films obtained from BS-CA according to face centered composite design

	TS (MPa)	EAB (%)	EM (MPa)
Lack of fit	0.8806	0.5391	0.0869
R^2	0.9241	0.2145	0.9849
Adj- R^2	0.8925	0.1654	0.9767
Pre- R^2	0.8035	0.0149	0.9561
Adeq Precision	17.704	3.966	34.86
PRESS	0.012	3061.27	0.011

The equations created according to the factors coded with the reduced quadratic model are given below:

$$TS = +0.083 - 0.064 \times A + 0.015 \times C - 0.019 \times AB - 0.018 \times AC + 0.041 \times A^2$$

$$EAB = +101.69 + 8.16 \times A$$

$$EM = +0.028 - 0.12\ A + 0.034 \times B + 0.031 \times C - 0.037 \times AB - 0.039 \times AC + 0.10 \times A^2$$

According to the obtained equations, sorbitol concentration negatively affected the TS and EM of the films, while it positively affected the EAB value. While temperature of film forming solution (C) and A^2 interaction contributed positively to the TS and EM of the films, the concentration-pH (AB) and concentration-temperature (AC) interactions of the film contributed negatively. On the other hand, pH (B) contributed positively to the EM of the films.

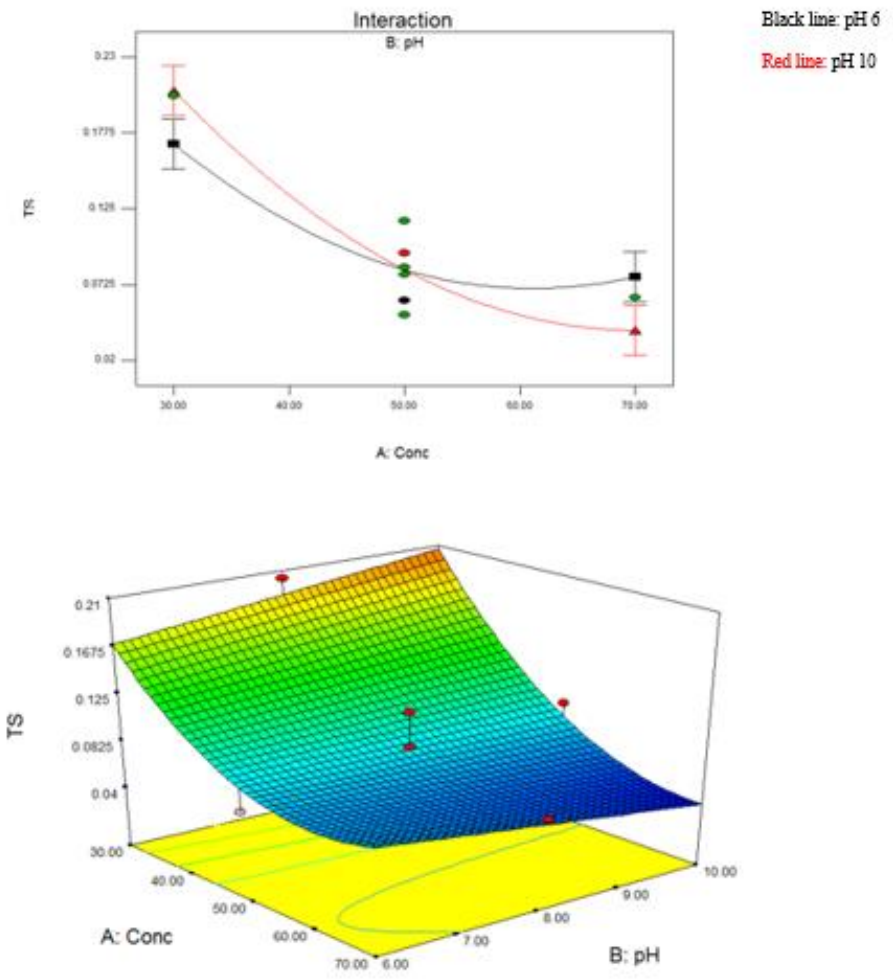


Figure 4.4. The effect of sorbitol concentration-pH interaction on TS of films obtained from BS-CA

The TS of the films obtained from capric acid-amylose complex starch decreased at 3 different pH values (6-8-10) with the increase in sorbitol concentration, and the highest TS was found for the combination of low sorbitol concentration and high pH value.

In the concentration-temperature interaction, a significant decrease was found for the 3 temperature values (70-80-90˚C) as the sorbitol concentration increased. While there was no significant change in TS with the increase in temperature at high sorbitol concentration, an increase with an increase in temperature at low sorbitol concentration.

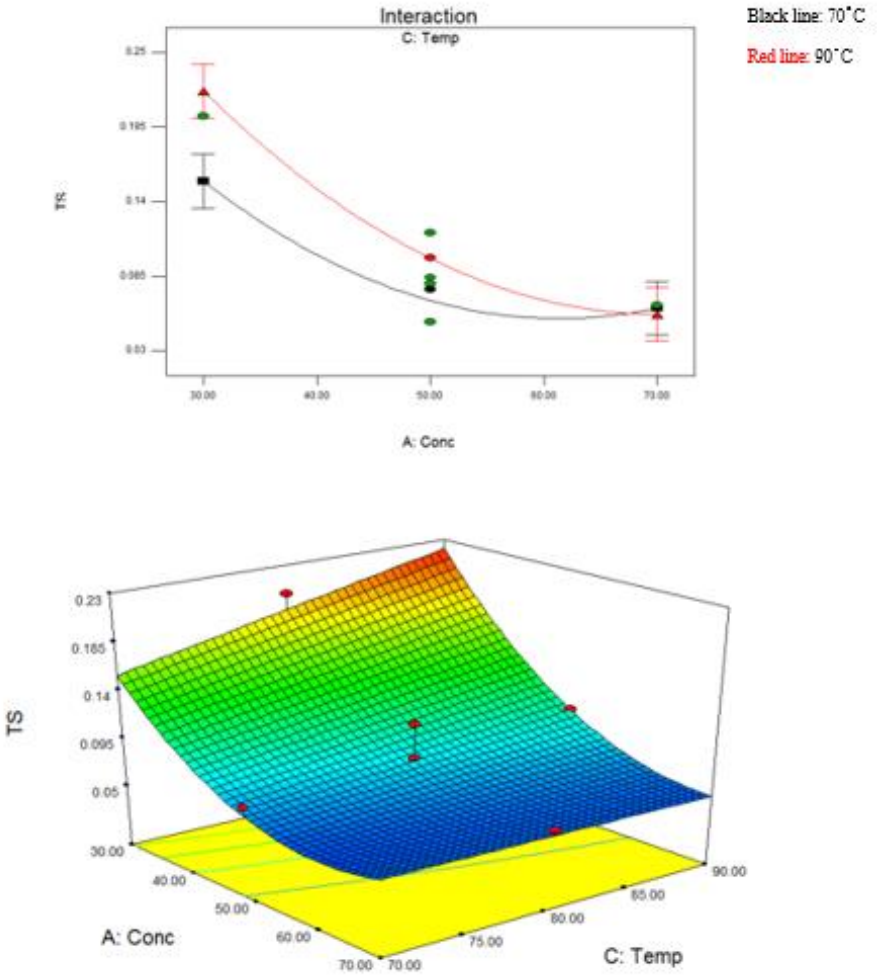


Figure 4.5. The effect of sorbitol concentration-temperature interaction on TS of films obtained from BS-CA

With the increase in concentration, a decrease was found in the EM of the films at 3 different pH values. As the pH increased at low concentration, the EM of the films increased, but no change was observed at high sorbitol concentration (Figure 4.6).

As the sorbitol concentration increased, the EM of the films decreased for the also 3 different temperature values. While the modulus of elasticity of the films increased with the increase in temperature at low sorbitol concentration, no change was observed at high sorbitol concentration (Figure 4.7).

As a result of the surface response studies, significant results were obtained for TS, and EM among the mechanical strength results of capric acid-amylose complex starch films ($p<0.05$). The film production condition was optimized so that the maximum values of TS and EAB the minimum value of EM. The optimum trial condition obtained as a result were determined at a 62.3 % desirability rate. As a result of the desirability function; optimum film production

conditions were calculated as 37.49 (g/100g starch) sorbitol concentration. pH 6.0, and 90 °C. Whether there is a statistically significant difference between the mean result of the experimental data obtained for the TS and EM of the films and the value estimated from the model was determined by applying the single sample t-test. It was found that there was no significant difference ($p>0.05$) between the experimental data obtained and the values estimated from the model. (Table 4.8)

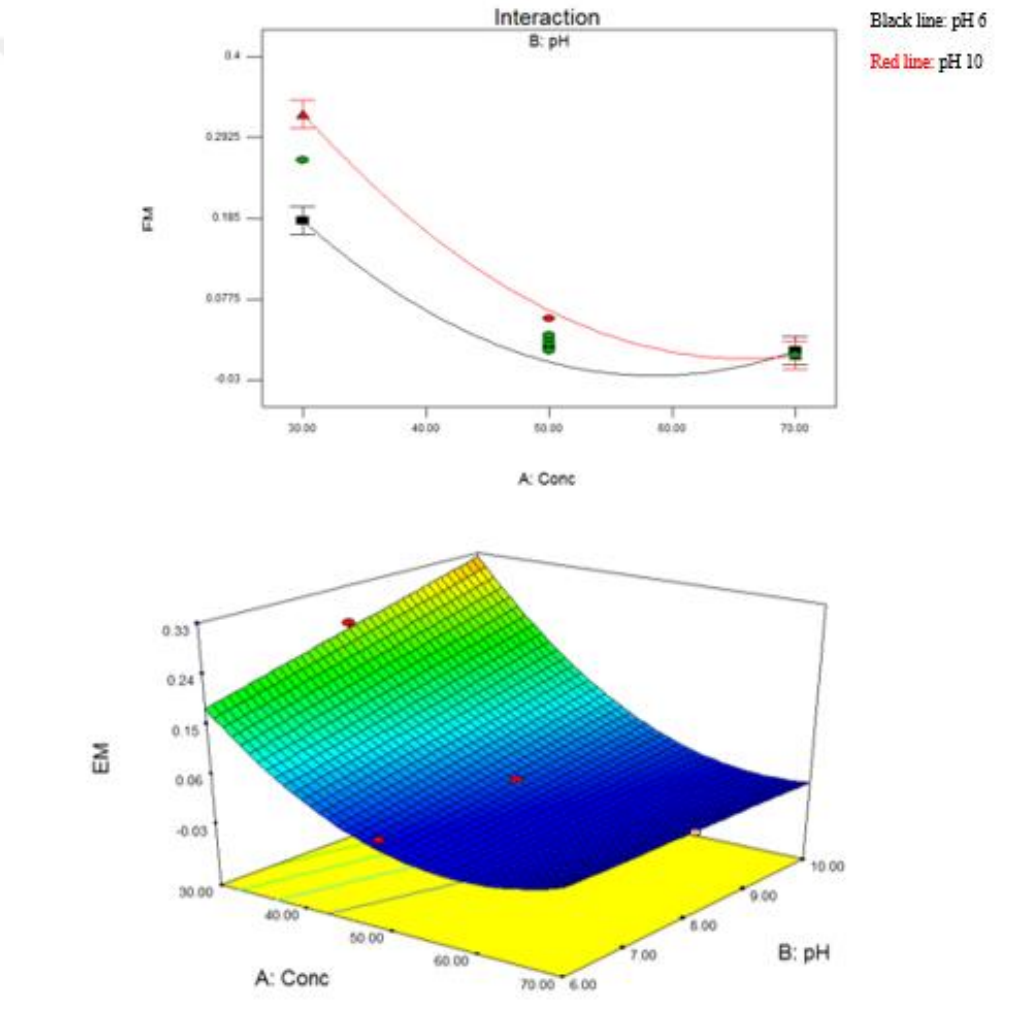


Figure 4.6. The effect of sorbitol concentration-pH interaction on EM of films obtained from BS-CA

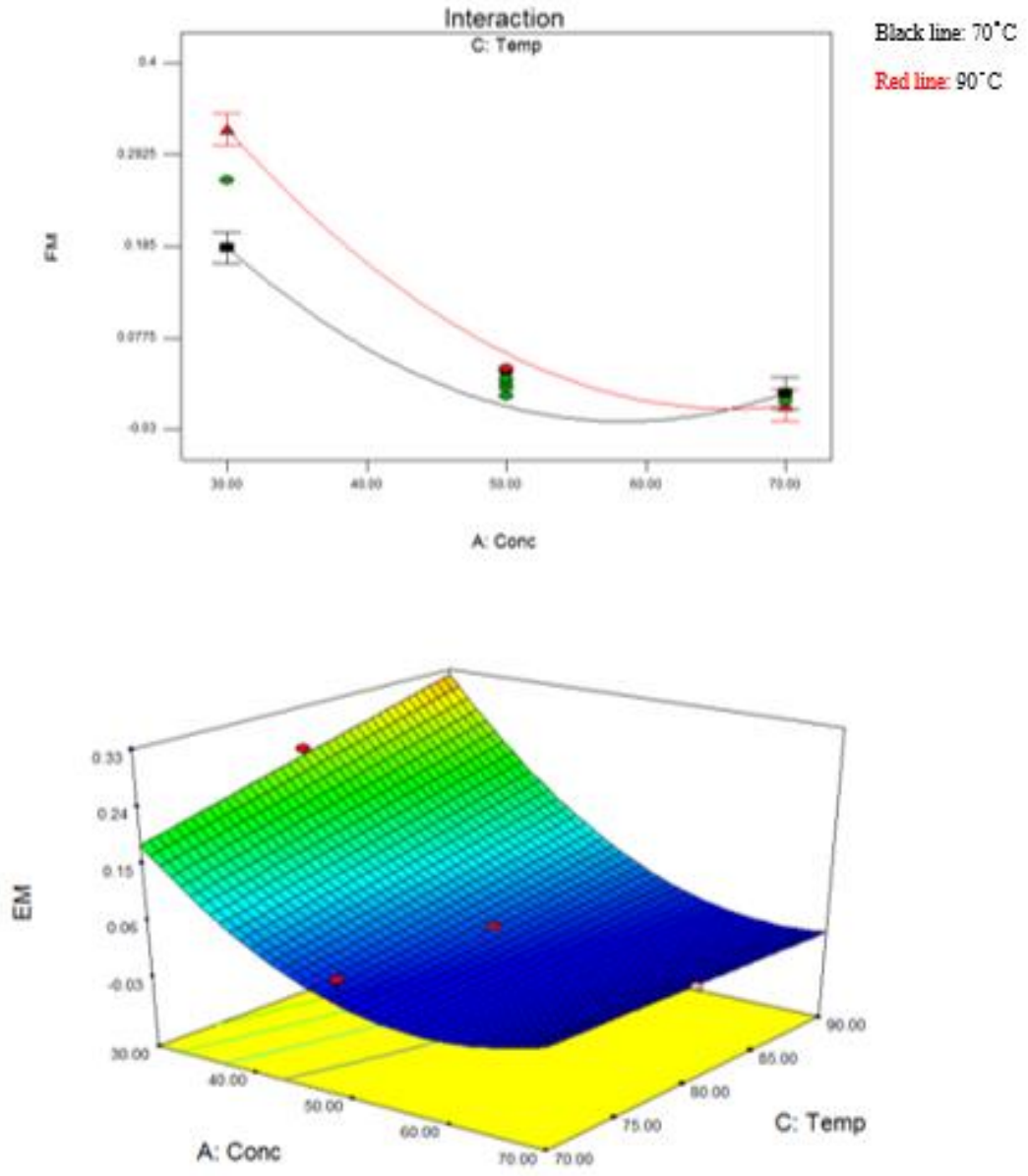


Figure 4.7. The effect of sorbitol concentration-temperature interaction on EM of films obtained from BS-CA

Table 4.8. Validation of optimum conditions with experimental data for films obtained from BS-CA

	TS (MPa)	EM (MPa)
Estimated Value	0.154238	0.071
Actual Value	0.1388±0.02	0.05±0.05
p-value	0.134	0.099

4.1.3. Mechanical properties of films obtained from BS-MA

The TS, EAB, and EM of the films obtained from the BS-MA complex under different conditions ranged from 0.048 to 0.241 MPa, 56.589% to 138.3%, and 0.009 to 0.134 MPa, respectively (Table 4.9). In the films produced using complex starch, as the concentration increased at constant temperature a decrease in TS at pH 6 and 8 and an increase at pH 10 were observed. As the concentration increased at the same temperature, the EAB value of the films decreased at pH 6 and increased at pH 8 and 10. The TS of the complex starch films was lower than those of the BS film, the EAB was higher. Hydrophobic molecules such as fatty acids can contribute to the improvement of the mechanical properties of the films by exhibiting a plasticizing effect during the film preparation (Thakur et al. 2016). As the concentration increased, a decrease was found in the EM of the films at constant temperature and pH, but an increase was found at pH 10 and 90 °C.

It was determined that the created full quadratic model was significant only for EAB and the sorbitol concentration (A), pH (B), concentration-pH (AB) and A^2 interaction were significant. The model was found to be insignificant for the TS and EM of the films, but the effects of concentration (A), pH (B) on the EM, and the effects of C^2 interaction on the TS were found to be significant (Table 4.10).

There was a decrease in the R^2 value for TS, EAB and EM in the reduced model compared to the full model. The minimum adeq. precision values of the models developed in this study are 5.257 for TS, and an Adeq Precision value greater than 4 implies that the model precision is statistically acceptable (Table 4.11).

Table 4.9. Mechanical properties of films obtained from BS-MA

Run	Sorbitol/Starch (g/100g)	pH	Temperature of film forming solution (˚C)	Mechanical Properties		
				TS (MPa)	EAB (%)	EM (MPa)
1	30	6	70	0.115	115.002	0.021
2	70	6	70	0.048	94.301	0.009
3	50	8	70	0.112	117.129	0.036
4	30	10	70	0.095	65.296	0.085
5	70	10	70	0.112	88.881	0.022
6	50	6	80	0.147	138.300	0.011
7	30	8	80	0.241	56.589	0.134
8	50	8	80	0.194	107.957	0.068
9	70	8	80	0.147	117.875	0.028
10	50	10	80	0.184	112.053	0.058
11	30	6	90	0.157	116.265	0.040
12	70	6	90	0.083	108.172	0.005
13	50	8	90	0.131	122.802	0.031
14	30	10	90	0.079	59.069	0.014
15	70	10	90	0.112	114.006	0.017
16	50	8	80	0.156	111.339	0.031
17	50	8	80	0.093	94.240	0.017
18	50	8	80	0.185	107.221	0.014

Table 4.10.ANOVA table for mechanical properties of films produced from BS-MA according to central composite design

		TS (MPa)		EAB (%)		EM (MPa)	
	sd	*F*	*p*	*F*	*p*	*F*	*p*
A (Concentration)	1	2.54	0.1498	6.82	0.0311*	8.67	0.0186*
B (pH)	1	0.074	0.7928	9.74	0.0142*	6.11	0.0386*
C (Temperature)	1	0.49	0.5020	0.87	0.3777	1.64	0.2360
AB	1	3.36	0.1043	7.96	0.0224*	3.57	0.0957
AC	1	7.536E-003	0.9330	1.34	0.2811	0.092	0.7700
BC	1	0.80	0.3965	9.803E-003	0.9236	0.49	0.5020
A^2	1	0.28	0.6109	11.91	0.0087*	0.13	0.7308
B^2	1	0.58	0.4666	1.42	0.2668	2.42	0.1584
C^2	1	7.56	0.0251*	0.31	0.5936	0.28	0.6096
Model	9	2.39	0.1168	4.38	0.0246*	2.82	0.0797
R^2		0.7291		0.8313		0.7602	

* Statistically significant ($p<0.05$)

Table 4.11. Statistical parameters in reduced quadratic model for films obtained from BS-MA according to face centered composite design

	TS (MPa)	**EAB (%)**	**EM (MPa)**
Lack of fit	0.8076	0.1507	0.4492
R^2	0.4604	0.7314	0.4429
Adj- R^2	0.4266	0.6487	0.3686
Pre- R^2	0.3097	0.4844	0.1141
Adeq Precision	5.257	10.177	7.537
PRESS	0.027	4421.21	66.53

The equations created according to the factors coded with the reduced quadratic model are as follows:

$$TS=+0.17-0.064 \times C^2$$

$$EAB =+113.88+11.10 \times A-13.27 \times B +13.41 \times AB-20.33 \times A^2$$

$$1.0/Sqrt\ (EM) =+4.86 + 1.40 \times A – 1.17 \times B$$

According to the obtained equations, C^2 interaction contributed negatively to the TS of the films. Sorbitol concentration (A) and the concentration-pH (AB) interaction affected positively the EAB of the films, while pH and A^2 interactions negatively.

In the films obtained from myristic acid-amylose complex starch, the TS of the films increased as the temperature increased, but the TS reached its maximum when the temperature was 80 °C. The TS values of the films prepared from the film forming solutions subjected to 70 and 90 °C were found be similar to each other (Figure 4.8).

In the concentration-pH interaction, a significant increase was found for pH of 10 value as the sorbitol concentration increased, while there was no significant change in TS with the increase in sorbitol concentration for pH 6. At low sorbitol concentration, TS was found to be higher in the case of pH 6 than pH 10, while at high sorbitol concentration it was found to be similar for both pH values.

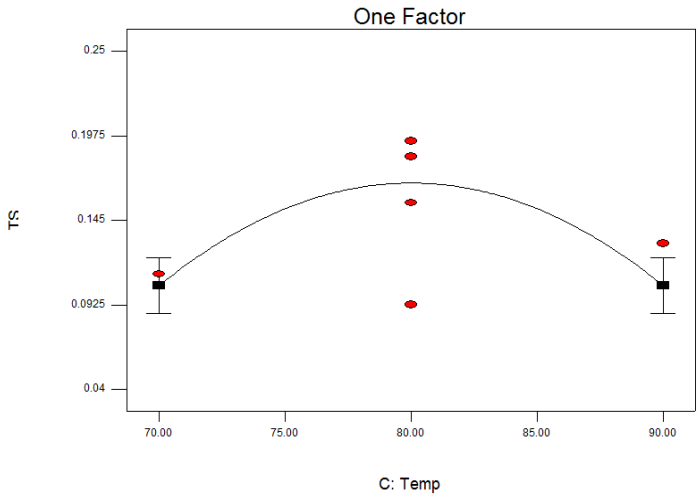


Figure 4.8. The effect of temperature on TS of films obtained from BS-MA

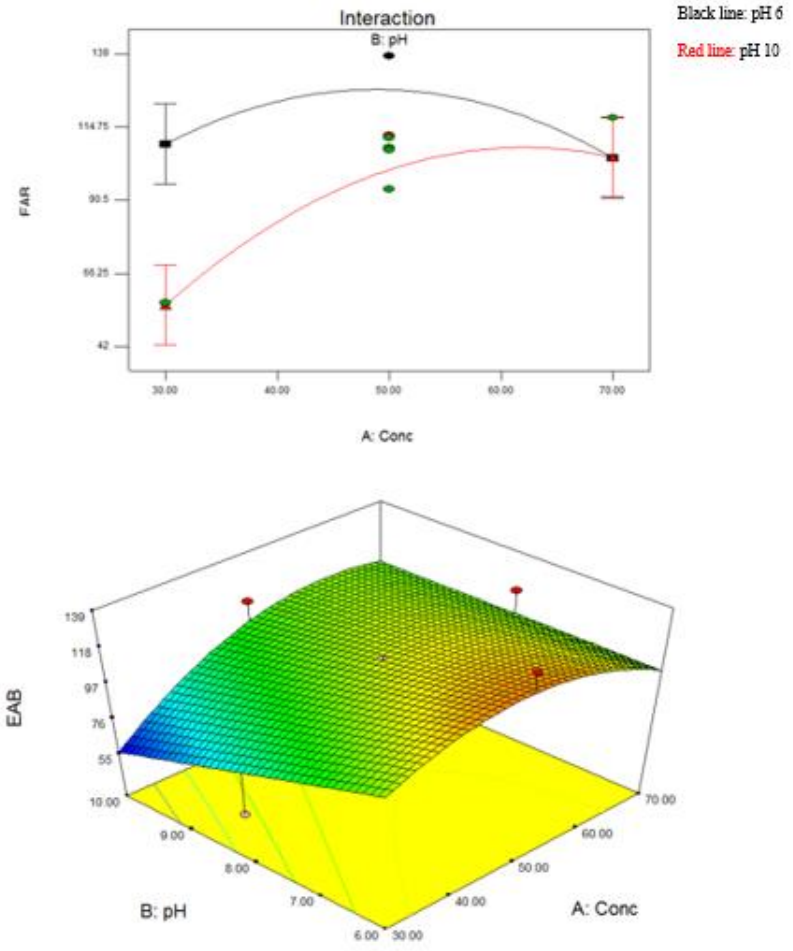


Figure 4.9. The effect of sorbitol concentration-pH interaction on EAB of films obtained from BS-MA

As a result of the surface response studies, significant result was obtained for EAB among the mechanical properties of myristic acid-amylose complex films ($p<0.05$). The film production condition was optimized so that the EAB become maximum, and determined at an 86.4 % desirability rate. In this case, the optimum trial condition obtained is as follows: 48.85 (g/100g starch) sorbitol concentration. pH 6, and 80.86 °C. The EAB of the film predicted from the model and obtained by experimental data were found to be 127.22 and 128.22, respectively. It was seen that there was no significant difference ($p>0.05$) between the experimental data obtained and the values estimated from the model. (Table 4.12)

Table 4.12. Validation of optimum conditions with experimental data for films obtained from BS-MA

	EAB (%)
Estimated Value	127.22
Actual Value	127.98±5.49
p-value	0.706

4.2. Characterization of Films

4.2.1. Mechanical Properties

The mechanical characteristics of films play a key role in determining whether they may be used as packing materials. The mechanical properties of the films were determined by assesing the tensile strength, elongation at break, and elastic modulus from the stress-strain curve. Table 4.13 shows the mechanical properties of films produced from BS (Buckwheat starch) and ALCs (Amylose-Lipid Complexes) according to optimized conditons. The highest TS was found to be 0.3 MPa in the BS film, but it was found to be 0.139 MPa and 0.080 MPa in the films made from ALCs produced with capric acid (BS-CA) and myristic acid (BS-MA), respectively. The EAB of the films varied between 45.62% and 128.22%, and the highest elongation was determined in the BS-MA film. Compared to the BS film, ALCs films were found to have a higher elongation. When the EAB values of the films prepared by using ALCs were compared, BS-MA film had a higher elongation than BS-CA film. While the TS of ALCs films decreased as the chain length of fatty acids increased, the EAB also increased. Free fatty acids in the film matrix can cause weakness in the film and also increase the elongation value by showing a plasticizing effect. Therefore, the change in mechanical strength can be attributed to the free

fatty acids present in the film matrix (Liu. Sun. Hou. et al.. 2016). EM is a mechanical property which indicates the resistance of the films to elastic deformation. It is about film's rigiditiy or stiffnes. The EM of the BS, BS-CA, and BS-MA films were found to be 0.062, 0.051, and 0.015 MPa, respectively. The EM of ALCs films was lower than BS film. Kim et al (2018) determined the TS and EAB values of BS film as 15 MPa and 25 %, respectively. When the finding of this study was compared to the findings of Kim et al. (2018), the TS found for the BS film prepared in this studywas lower than that reported by Kim et al. (2018). The EAB of the BS film determined in this study was however higher than that found by Kim et al. (2018).

Table 4.13. Mechanical Properties of the BS and ALCs films

	TS (MPa)	EAB (%)	EM (MPa)	Thickness (mm)
BS	0.339 ± 0.03^{a}	45.62 ± 2.76^{c}	0.062 ± 0.02^{a}	0.077 ± 0.01^{a}
BS-CA	0.139 ± 0.02^{b}	99.44 ± 3.08^{b}	0.051 ± 0.05^{a}	0.052 ± 0.01^{b}
BS-MA	0.08 ± 0.02^{c}	127.98 ± 5.49^{a}	0.0015 ± 0.00^{b}	0.069 ± 0.01^{a}

Data are given as means±SD

Significant differences are indicated by different letters in the same column ($p<0.05$) by Duncan test

4.2.2. Film Thickness

The film properties such as opacity, the TS and the VWP as well as the shelf life of the food, to which an edible film is applied, are directly affected by the film thickness. The film thickness is greatly influenced by a number of factors, including the structure of the ingredients added to the film formulations together with their distribution in the final film matrix and the drying conditions (Skurtys et al.. 2010). The thickness of the films ranged from 0.077 mm to 0.052 mm, and the highest thickness was found in the BS film. Thicknesses of ALCs films were slightly higher than that of the BS film. While there was no significant difference in the thicknesses of the BS and BS-MA films. The thickness of the BS-CA film was found to be significantly lower than those of the BS and BS-MA films. Similar results were reported in the scientific literature (Kim et al,, 2018 and Sindhu et al., 2007).

4.2.3. Moisture Content and Water Solubility

The amount of water present in the films is represented by the film moisture content. The moisture content is one of the significant factors determining the film properties, for example

overdry films may exhibit brittle characteristics (Embuscado, M. E., & Huber, 2009). The MCs of the films produced from BS and ALCs are given in Table 4.14. The MCs of the BS, BS-CA and BS-MA films were found to be 9.78, 10.38 and 10.54 %, respectively. No significant changes in the MCs of the BS and ALCs films were observed. ALCs films have a higher MC than BS however BS-MA is the highest among the ALCs. Similarly, Kim et al. (2018) determined the MC of sorbitol plasticized buckwheat starch films to be 7.07%.

The WS of films varied between 47.60 % and 49.78 % for BS and BS-MA, respectively. There was no noticeable variation in the WS of the films formed by BS and ALCs. While the BS-MA has higher solubility, BS has a lower. Due to the interaction of the fatty acids in the structure with amylose, the interaction of amyloses among themselves is restricted, so a strong intermolecular bond could not be established, which can be attributed to increased solubility. It is also thought that free fatty acids in the structure may cause an increase in solubility (Sindhu & Singh Khatkar, 2007; Thakur et al., 2016).

Table 4.14. Barrier properties of the BS and ALCs films

	MC (%)	**WS (%)**	**WVP (g.mm /m^2.h.kPa)**
BS	9.78±0.5^a	47.60±5,71^a	0.312±0.09^a
BS-CA	10.38±1.25^a	48.43±6.05^a	0.147±0.024^b
BS-MA	10.54±0.85^a	49.78±2.02^a	0.196±0.016^b

Data are given as means±SD

Significant differences are indicated by different letters in the same column ($p<0.05$) by Duncan test

4.2.4. Water Vapour Permeability

Table 4.14 shows the water vapour permeability of the BS and ALCs films. As it can be inferred from Table 4.14, the highest WVP (0.312 g.mm/m^2.h.kPA was found for the BS film. The films produced from ALCs had lower permeability values compared to the BS films. When the water vapor permeability values of ALC films were compared, the BS-MA film had a WVP higher than the BS-CA film. The lower WVP of ALC films compared to BS may be due to the hydrophobicity of fatty acids. These is because of the strong networks caused by covalent and non-covalent bonds. These networks cause BS films to have higher permeability as a result of the formation of extra intermolecular spaces and due to the high diffusion rate (Thakur et al.,

2017). Similarly, Liu et al (2016) and Schmidt et al. (2013) reported the decrease in the WVP of films as a result of the improved barrier properties of films against the water vapour (Schmidt et al.. 2013). This decrease in the WVP can be explained by the hydrophobicity and low water affinity of the carbon chains found in the polymer matrix (Liu, Sun, Hou, et al. 2016).

4.2.5. Colour and Opacity

Colour parameters of the BS and ALCs are shown in Table 4.15. The L* values of the films ranged from 96.95 to 98.22, and the L* of the BS film was significantly higher than those of the ALCs films ($p<0.05$). The L^* values of the BS and ALCs are too close to the values of the white plate (L=96.80), so these films tend to be transparent. The a* value specifies the red (-a) –green (+a) scale and the b* value specifies the yellow (-b) -blue (+b) scale. The a* and b* values varied between 0.05 to 0.18 and 0.29 to 0.39, respectively.

The a* value of the BS film was the highest among the a* values measured for all films indicating that the colour of the BS film was much closer to green than the colours of the ALCs films. While the b* values of the BS and BS-MA films were similar, b* value of the BS-CA film was significantly lower than those of the BS and BS-MA films. If the film is to be utilized as a surface food covering, opacity is a crucial parameter. Film opacity values were found to be between 4.26 and 6.24, and the BS film was opaquer than ALCs films. The opacity of the BS-CA film was measured as 4.26 whereasthe opacity of the BS-MA films was determined as 5.29, and the opacity was found to increase with the increase in the fatty acid chain length.

Table 4.15. Optical properties of the BS and ALCs films

	L*	**a***	**b***	**Opacity**
BS	98.22 ± 0.35^{a}	0.18 ± 0.04^{a}	0.39 ± 0.04^{a}	6.24 ± 0.53^{a}
BS-CA	96.95 ± 0.04^{b}	0.05 ± 0.00^{c}	0.29 ± 0.02^{b}	4.26 ± 0.16^{c}
BS-MA	97.35 ± 0.13^{b}	0.12 ± 0.03^{b}	0.39 ± 0.04^{a}	5.29 ± 0.12^{b}

Data are given as means±SD

Significant differences are indicated by different letters in the same column ($p<0.05$) by Duncan test

5. CONCLUSIONS

The carbohydrate based edible films were prepared by using native and modified buckwheat starches which were obtained by the complexation of buckwheat starch molecules with two different fatty acids, a 10-carbon containing fatty acid (capric acid) and a 14 carbon-containing fatty acid (myristic acids) in this study. The purpose of the use of modified starches in this study was to obtain edible films with, improved mechanical properties. The sorbitol was employed as a plasticizer and also contributed to the flexibility in some extent by the formation of voids between starch molecules. The films of buckwheat starch and lipid modified buckwheat starch were prepared. The effects of significant parameters such as sorbitol concentration, pH and the temperature of the film forming solutions on the mechanical properties, tensile strength, elongation at break and elastic modulus, of the films were studied. Optimum film preparation conditions were determined by central composite design. Water solubilities and water vapor permeabilities as well as the optical properties of the films prepared at optimum conditions were finally determined. The conclusions drawn from experimental finding of this study are as follows:

- The EAB values of the films increased as the sorbitol concentration in the film forming solution was increased. The higher sorbitol addition to the film solution might provide more distances between starch molecules by forming H-bonds between sorbitol and glucose molecules producing more elastic films.
- The TS values of the films decreased with the increase in the sorbitol concentration of the film forming solution. In consistent with the previous result, the higher sorbitol concentration reduced the rigidity of the films hence increased the flexibility of the films.
- The modified films were found to be more flexible than native BS films due to the increased hydrophobicity.
- On the other hand, the modified films had lower TS values than native BS films. This could be attributed integrity of starch molecules due to structure disruption during fatty acid complexation.

- The modified films were thinner than the films prepared from the native BS. Water vapor permeability of modified films were lower than native BS films due to increased film hydrophobicity.
- The water solubilities and moisture contents of all films were not significantly different from each other ($P<0.05$).
- The modified films were more transparent than native starch film.
- BS-MA film was more elastic but had lower TS than BS-CA film which attributed to the carbon chaing length.
- The BS-CA film was more transparent and exhibited better water vapour permeability than the BS-MA film.

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APPENDICES

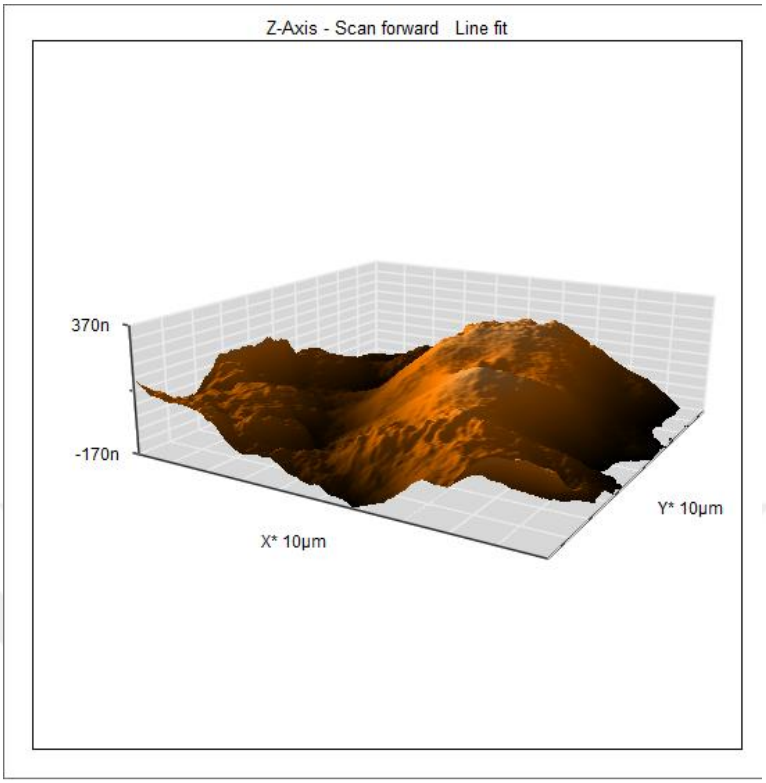

Figure A1. AFM image of natural buckwheat starch film

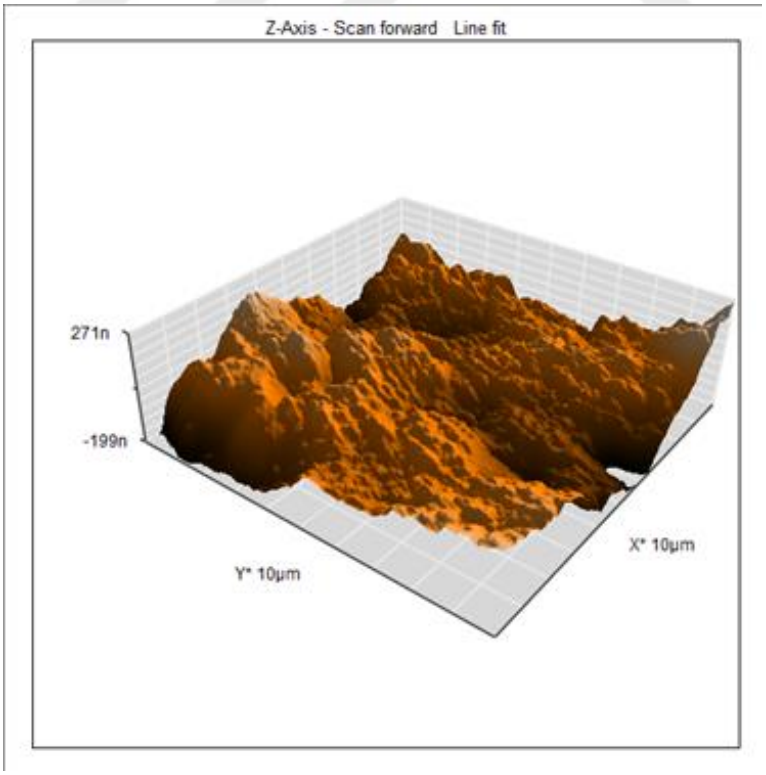

Figure A2. AFM image of film of modified buckwheat starch with capric acid

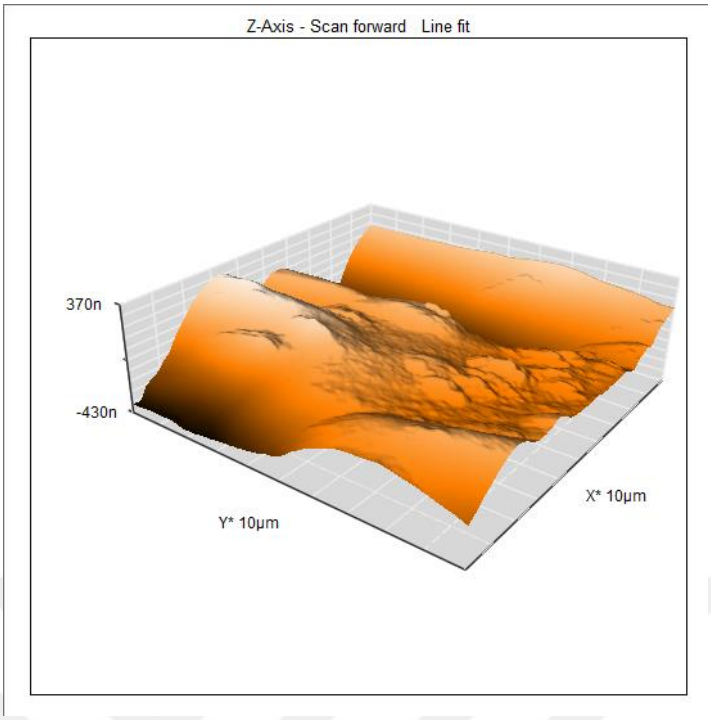

Figure A3. AFM image of film of modified buckwheat starch with myristic acid

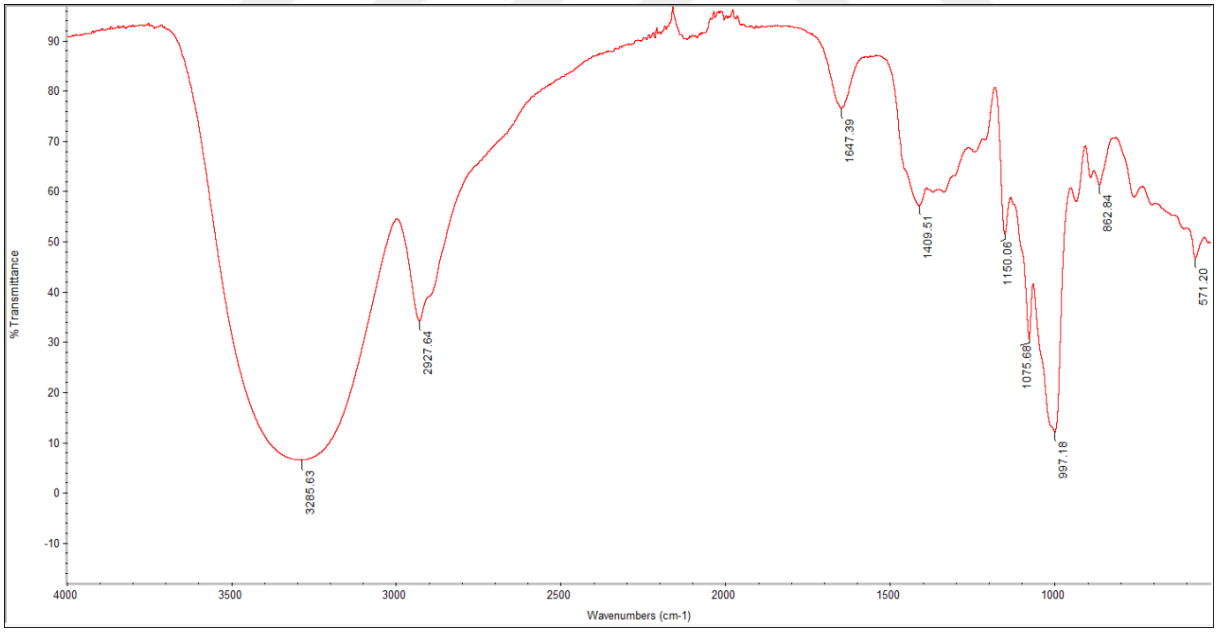

Figure A4. FTIR spectrum of natural buckwheat starch film

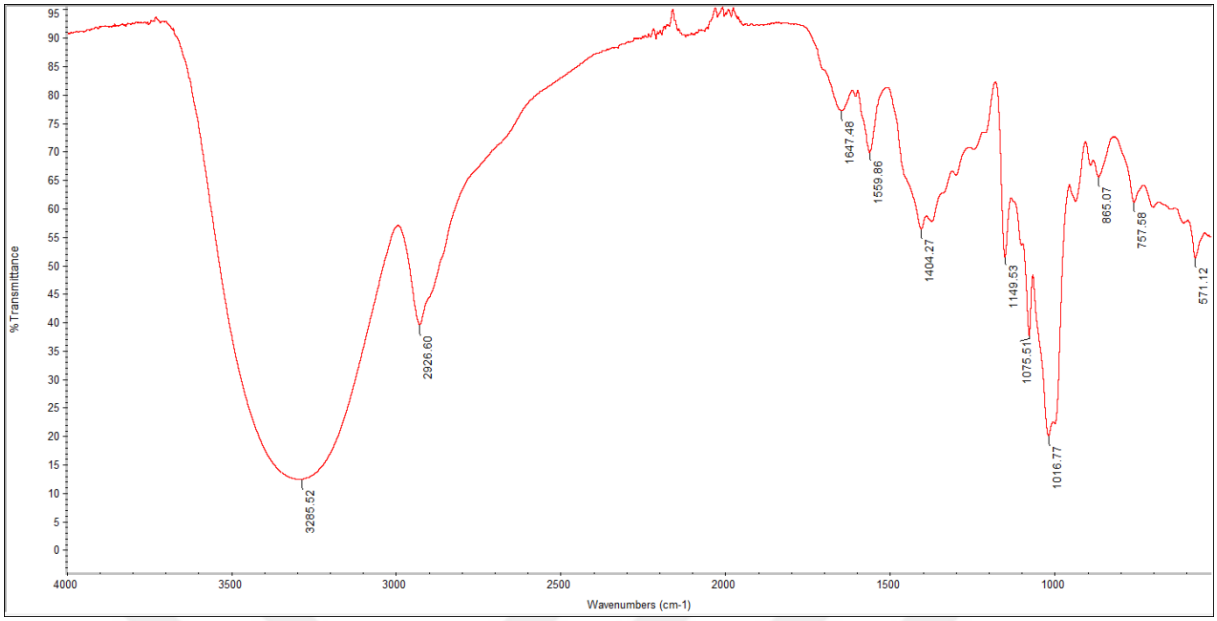

Figure A5. FTIR spectrum of film of modified starch by capric acid

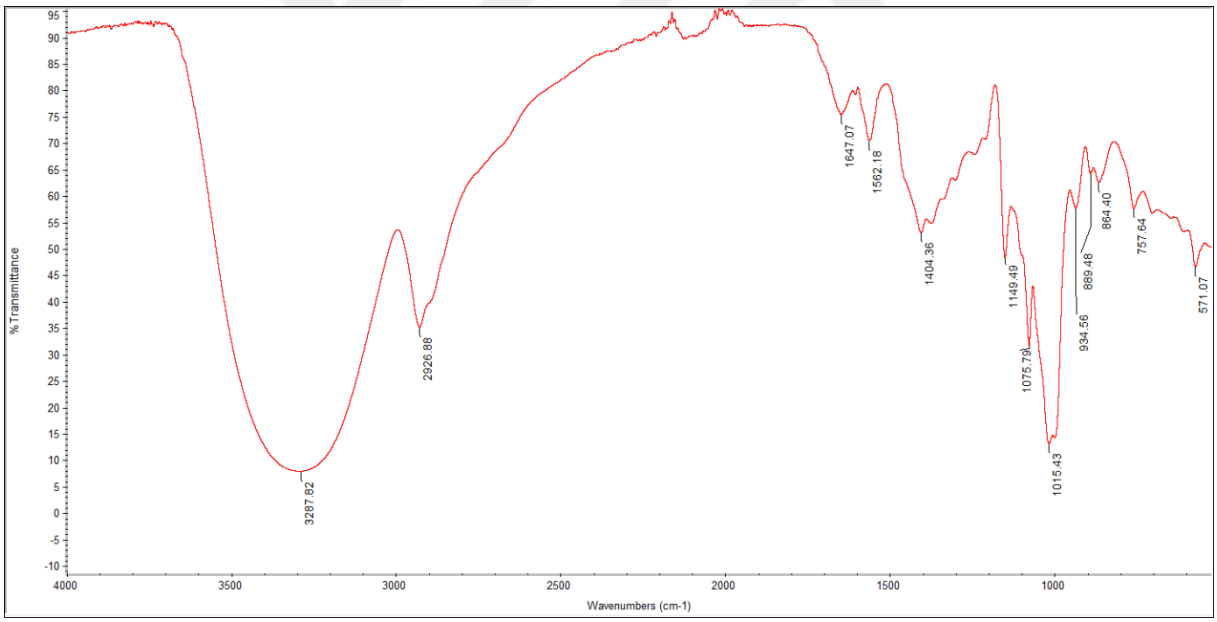

Figure A6. FTIR spectrum of film of modified starch by myristic acid

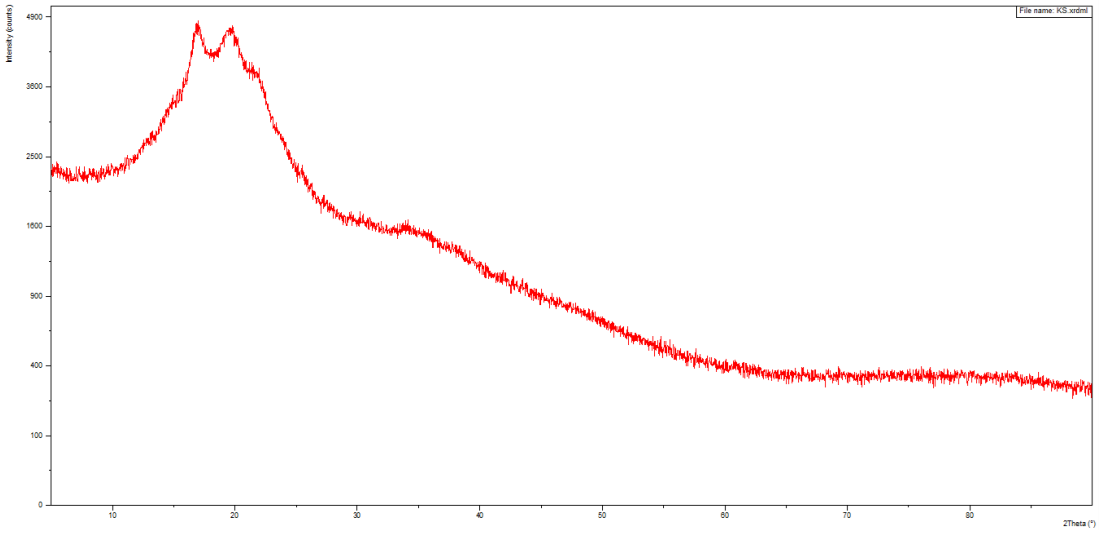

Figure A7. XRD spectra of native buckwheat starch.

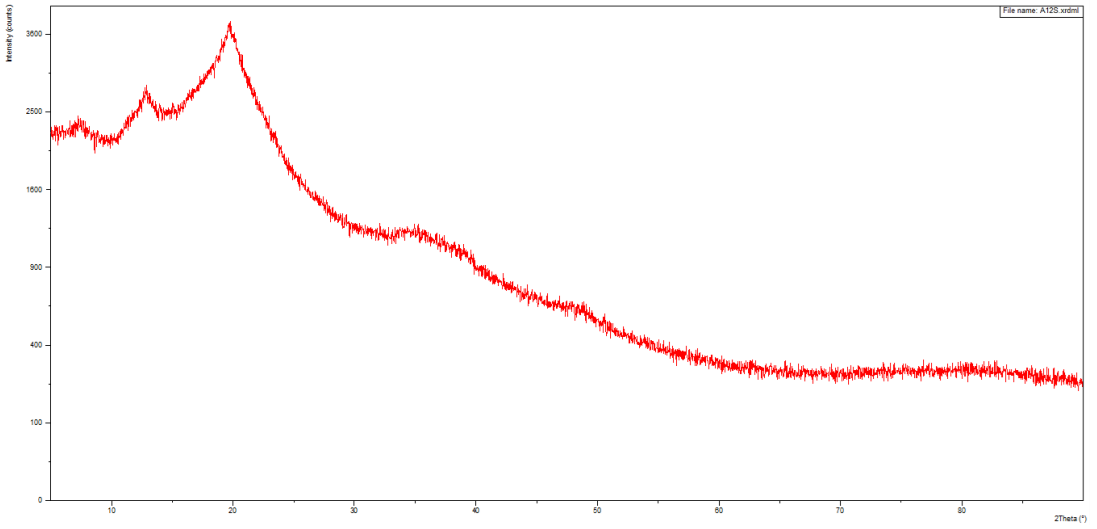

Figure A8. XRD spectra of film of modified starch by capric acid

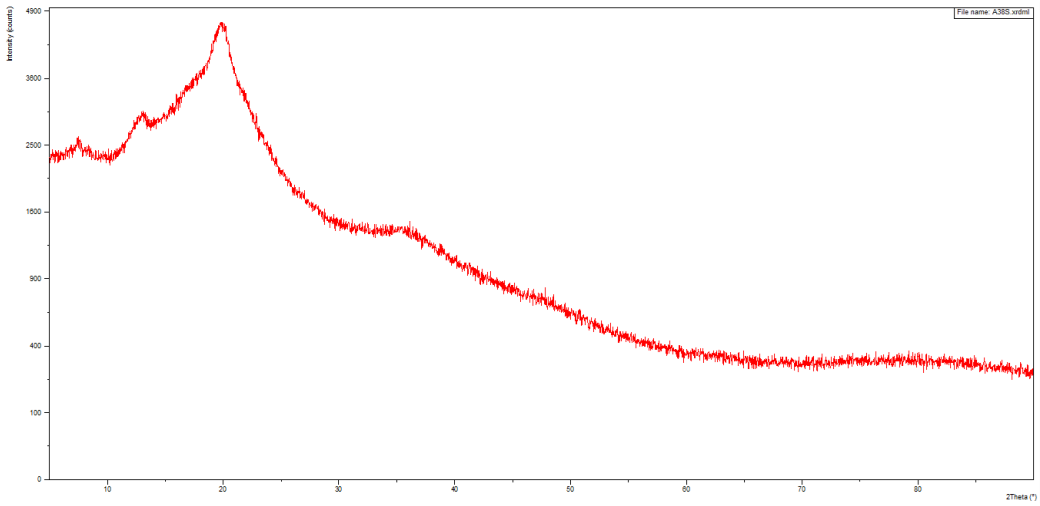

Figure A9. XRD spectra of film of modified starch by myristic acid

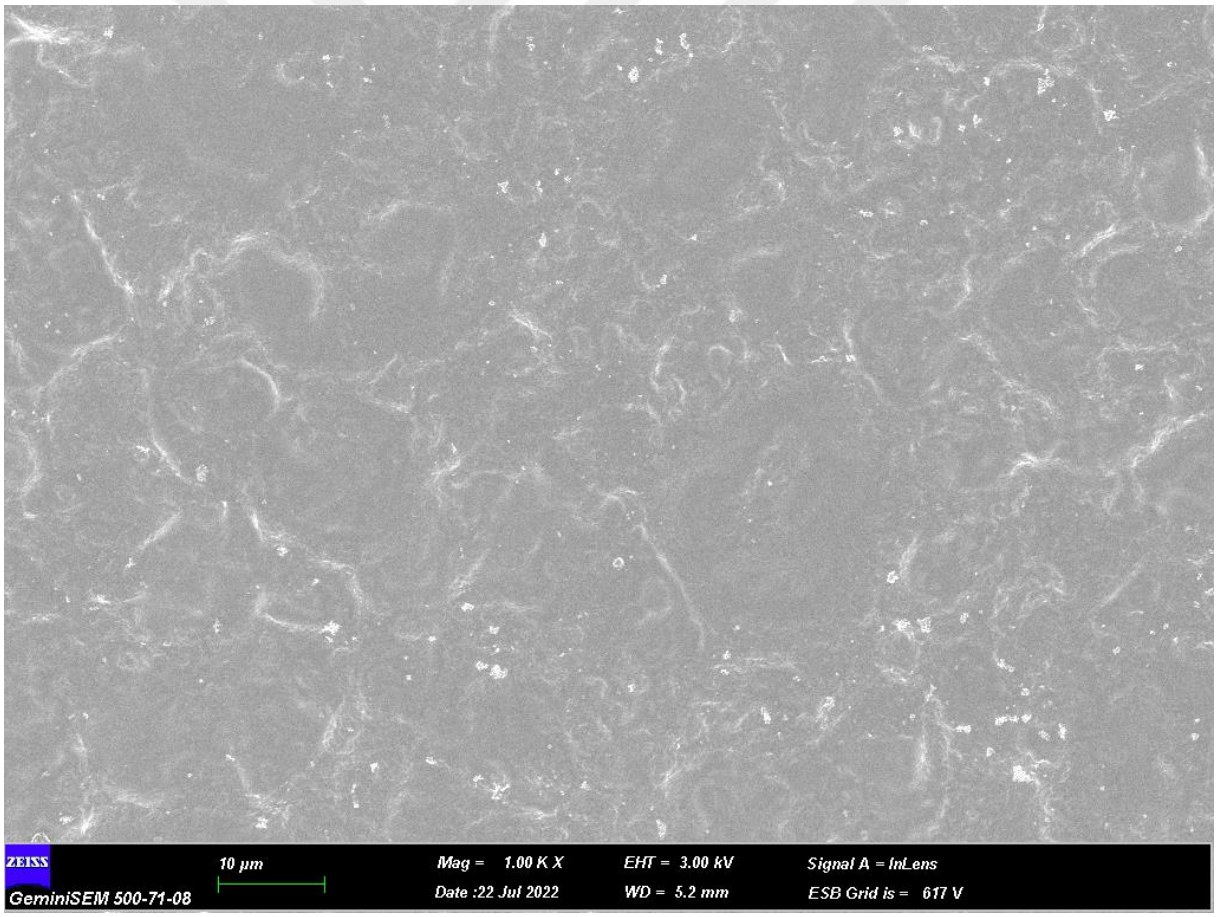

Figure A10. Surface SEM image of natural buckwheat starch film

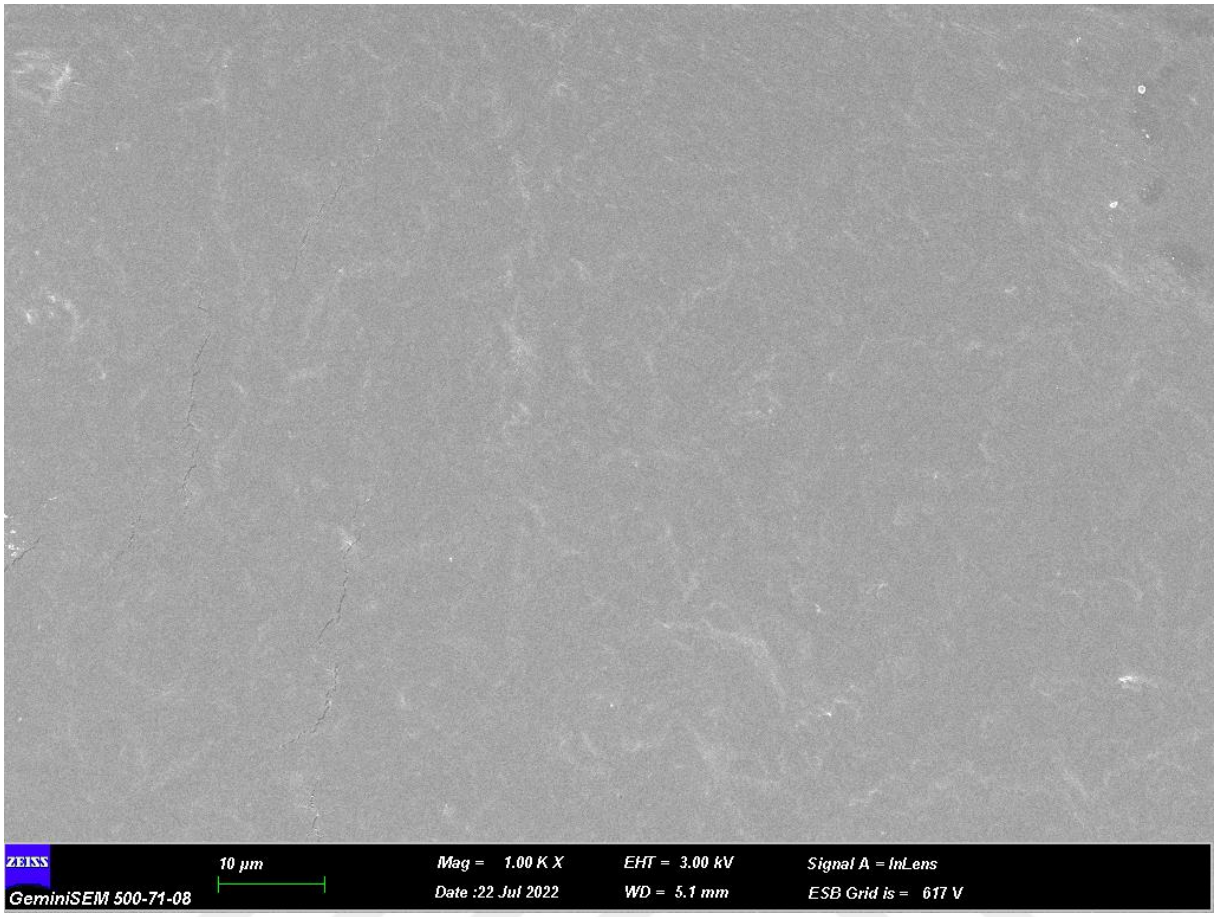

Figure A11. Surface SEM image of film of modified starch by capric acid

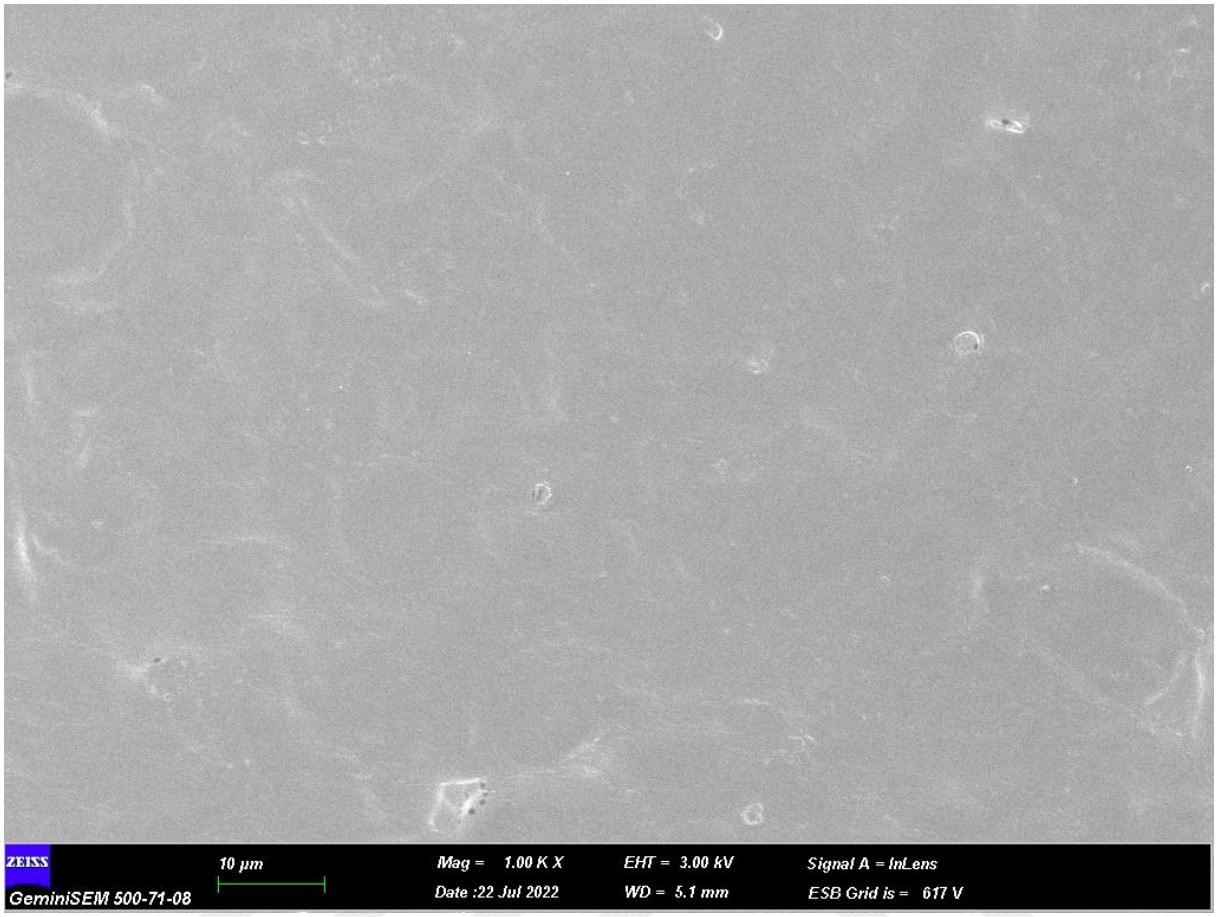

Figure A12. Surface SEM image of film of modified starch by myristic acid

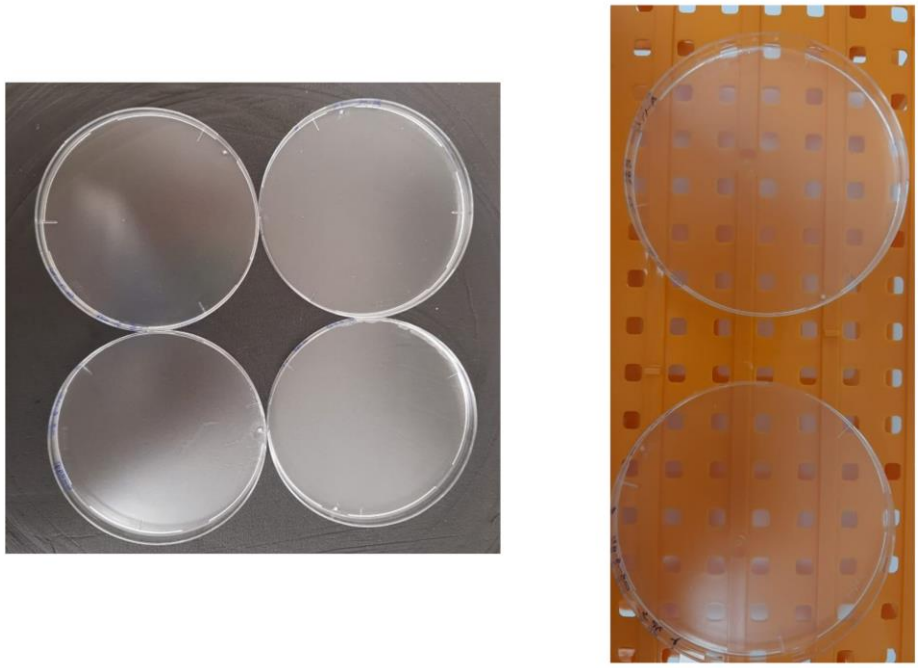

Figure A13. The native BS films