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M.Sc. Thesis in Food Engineering

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

**DEVELOPMENT AND CHARACTERIZATION OF SOME
COMPOSITE (WPI, SPI AND CMC BASED)
EDIBLE FILMS**

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

**BY
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(WPI, SPI and CMC Based)
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**M.Sc. Thesis
in
Food Engineering
University of Gaziantep**

**Supervisor
Prof. Dr. Sevim KAYA**

**By
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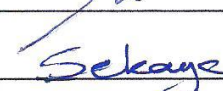
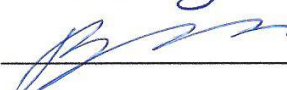

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Burcu GÖKKAYA

ABSTRACT

DEVELOPMENT AND CHARACTERIZATION OF SOME COMPOSITE (WPI, SPI AND CMC BASED) EDIBLE FILMS

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Edible films based on different proteins, polysaccharides and/or lipid were developed with their optimal proportions. Whey protein isolate (WPI), soy protein isolate (SPI) and carboxymethyl cellulose (CMC) were used as protein and polysaccharide resources, respectively. Besides, sunflower oil (SO) was added into the films. Moisture absorption, mechanical, barrier, physical and thermal properties of the composite films were investigated. Moisture absorption of the films containing single component was tested at three different relative humidity (30, 50 and 80%) conditions at room temperature using dynamic vapor sorption instrument. Water vapor permeability (WVP) and oxygen permeability (OP) of the films were determined using analytical and instrumental techniques, respectively.

Significant reduction in WVP and OP of the WPI films was obtained upon addition of SO. Mechanical properties also affected by addition of SO that percent elongation (%E) was increased by four times. It was determined that glass transition temperature was increased proportionally with SO amount for WPI films but SPI-SO blend film had less value than that of SPI. CMC addition improved the WVP but it caused negative effect on OP. WS1 (0.05% SO added WPI) and SPI-CMC were assumed to be the best two combinations among all studied films.

Key words: Composite edible film, whey protein isolate, soy protein isolate, carboxymethyl cellulose, sunflower oil

ÖZET

BAZI KOMPOZİT (WPI, SPI VE CMC BAZLI) YENİLEBİLİR FİLMLERİN GELİŞTİRİLMESİ VE KARAKTERİZASYONU

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İdeal karışım oranlarında protein, polisakkarit ve/veya lipit orijinli kompozit yenilebilir filmler üretildi. Peyniraltı suyu protein izolesi (WPI), soya protein izolesi (SPI) ve karboksimetil selüloz (CMC) sırasıyla protein ve polisakkarit kaynakları olarak kullanıldı. Ayrıca ayçiçek yağı (SO) ise lipit kaynağı olarak kullanıldı. Üretilen kompozit filmlerin nem soğurma, mekanik, bariyer, fiziksel ve termal özellikleri incelendi. Tek bileşen içeren (WPI, SPI veya CMC) filmlerin nem soğurma özellikleri üç farklı bağıl nemde (%30, 50 ve 80) ve oda sıcaklığında DVS cihazı kullanılarak test edildi. Su buharı geçirgenliği (WVP) ve oksijen geçirgenliği (OP) sırasıyla analitik ve aletli yöntemle belirlendi.

SO eklenmesi ile WPI bazlı filmlerin WVP ve OP değerlerinde belirgin bir azalma gözlemlendiği tespit edildi. Aynı zamanda SO'nun bahsedilen filmlerin mekanik özelliğinde de iyileşme gösterdiği, yüzde uzama miktarlarının ise dört kata kadar arttığı saptandı. Camsı geçiş sıcaklığının SO miktarı ile orantılı olarak arttığı belirlendi, fakat SPI-SO kompozit filminin SPI filminden daha düşük bir değere sahip olduğu görüldü. CMC eklenmesi ile elde edilen kompozit filmin WVP değerinin azaldığı, fakat OP'un arttığı belirlendi. Çalışılan film kompozisyonları arasında, %0.05 SO içeren WPI ve CMC içeren SPI filmleri elde edilen en iyi filmler olarak kabul edildi.

Anahtar kelimeler: Kompozit yenilebilir film, peynir altı suyu protein izolatu, soya protein izolatu, karboksimetil selüloz, ayçiçeği yağı

Dedicated to my father Sami GÖKKAYA...

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CONTENTS

ABSTRACT.....	v
ÖZET.....	vi
ACKNOWLEDGEMENTS	viii
CONTENTS.....	ix
LIST OF FIGURES.....	xii
LIST OF TABLES	xiii
LIST OF APPENDICES.....	xiv
LIST OF ABBREVIATIONS.....	xv
CHAPTER 1	1
INTRODUCTION	1
1.1 General Objective.....	1
CHAPTER 2	3
LITERATURE SURVEY	3
2.1 Food Packaging	3
2.1.1 The Functions and Importance of the Food Packaging	3
2.2 Edible Films and Coatings.....	4
2.2.1 Manufacturing of Edible Films and Coatings.....	7
2.2.2 Biopolymers Used For Manufacturing of Edible Films and Coatings	7
2.2.2.1 Protein Based Edible Films.....	7
2.2.2.1.1 Whey Protein	8
2.2.2.1.2 Soy Protein	9
2.2.2.2 Polysaccharide Based Edible Films	10
2.2.2.2.1 Carboxymethyl Cellulose	11
2.2.2.3 Lipid Based Edible Films.....	11
2.2.2.3.1 Sunflower Oil	12
2.2.2.4 Composite Films	12
2.3 Methods For Determining the Properties of the Films.....	14
2.3.1 Mechanical Properties.....	14

2.3.2 Barrier Properties	17
2.3.2.1 Water Vapor Permeability of Edible Films.....	17
2.3.2.2 Oxygen Permeability of Edible Films	17
2.4 Differential Scanning Calorimetry (DSC).....	18
2.5 Fourier Transform Infrared Spectroscopy (FTIR).....	19
2.6 Moisture Absorption Isotherms	20
CHAPTER 3	21
MATERIAL AND METHOD	21
3.1 Materials	21
3.2 Experimental Studies.....	21
3.2.1 Preparation of the Films.....	21
3.2.1.1 WPI Based Edible Film.....	22
3.2.1.2 SPI Based Edible Film	22
3.2.1.3 WPI-SO Blend Edible Films	23
3.2.1.4 SPI-SO Blend Edible Film	23
3.2.1.5 SPI-CMC Blend Edible Film	23
3.2.1.6 CMC Based Edible Film	23
3.2.2 Characterization of Edible Films	24
3.2.2.1 Thickness	24
3.2.2.2 Mechanical Properties	24
3.2.2.3 Barrier Properties	25
3.2.2.3.1 Water Vapor Permeability.....	25
3.2.2.3.2 Oxygen Permeability	26
3.2.2.4 Physical Properties	26
3.2.2.4.1 Gloss and Haze	26
3.2.2.4.2 Color	27
3.2.3 Thermal Properties.....	27
3.2.4 FTIR.....	28
3.2.5 Moisture Absorption	28
3.2.6 Statistical Analysis.....	28
CHAPTER 4	29
RESULT AND DISCUSSION	29
4.1 Mechanical Properties	29

4.2 Barrier Properties	32
4.2.1 Water Vapor Permeability	32
4.2.2 Oxygen Permeability	34
4.3 Physical Properties	36
4.3.1 Gloss and Haze	36
4.3.2 Color	37
4.4 Thermal Properties	38
4.5 FTIR	39
4.6 Moisture absorption.....	41
CONCLUSION	43
REFERENCES.....	44
APPENDIX.....	50

LIST OF FIGURES

	page
Figure 2.1 Schematic presentations of components of the edible films.....	6
Figure 2.2 Principle of tensile strength.....	14
Figure 2.3 The graph of the percent elongation.....	15
Figure 2.4 The graph of the tensile strength.....	15
Figure 2.5 Stress-Strain curve.....	15
Figure 2.6 The diagram of the heat flux into DSC.....	18
Figure 2.7 A representative of thermogram of DSC.....	19
Figure 3.1 Representation of placing films on texture analyzer in a tensile test.....	24
Figure 3.2 Schematic presentation of the experiment of water vapor permeability.....	25
Figure 4.1 Tensile strength of WPI and WPI-SO blend films.....	30
Figure 4.2 Elongation of WPI and WPI-SO blend films.....	30
Figure 4.3 Comparison of elongation values of SPI and CMC films.....	31
Figure 4.4 Effect of SO concentration on the WVP of WPI films.....	33
Figure 4.5 Effect of CMC and SO incorporation into SPI films on WVP.....	34
Figure 4.6 Gloss and haze results for studied films.....	37
Figure 4.7 Spectra of FTIR spectroscopy of SPI (green line), SPI-SO (blue line), SPI-CMC (red line), CMC films (black line).....	40
Figure 4.8 Spectra of FTIR spectroscopy of WPI (black line), WS1 (red line), WS2 (blue line), and WS3 (green line).....	40
Figure 4.9 Moisture absorption of the pure films at 30%RH.....	41
Figure 4.10 Moisture absorption of the pure films at 50%RH.....	42
Figure 4.11 Moisture absorption of the pure films at 80%RH.....	44

LIST OF TABLES

	page
Table 2.1 WVP values for some of the films.....	16
Table 3.1 The combinations of the compounds used in film formation in preliminary study.....	21
Table 4.1 Mechanical properties of edible films.....	29
Table 4.2 Water vapor permeability of edible films.....	32
Table 4.3 Oxygen permeability of edible films.....	35
Table 4.4 Gloss and haze of edible films.....	36
Table 4.5 Color parameters of the studied edible films.....	37
Table 4.6 Glass transition temperatures and heat of fusions for films as determined by DSC.....	38

LIST OF APPENDICES

		page
Table A1	Effect of sunflower oil concentration on tensile strength to WPI films.....	50
Table A2	Effect of sunflower oil concentration on elongation to WPI films...	51
Table A3	Effect of sunflower oil and carboxymethyl cellulose including on tensile strength to SPI films.....	52
Table A4	Effect of sunflower oil and carboxymethyl cellulose including on elongation to SPI films.....	53
Table A5	Effect of sunflower oil concentration on water vapor permeability to WPI films.....	54
Table A6	Effect of sunflower and carboxymethyl cellulose including on water vapor permeability to SPI films.....	55
Table A7	Effect of sunflower oil concentration on oxygen permeability to WPI films.....	56
Table A8	Effect of sunflower oil and carboxymethyl cellulose addition on oxygen permeability to SPI films.....	57
Table A9	Effect of sunflower oil addition on glass transition to WPI films....	58
Table A10	Effect of sunflower oil addition on heat of fusion to WPI films.....	59
Table A11	Effect of sunflower oil and carboxymethyl cellulose addition on glass transition to SPI films.....	60
Table A12	Effect of sunflower oil and carboxymethyl cellulose addition on heat of fusion on SPI films.....	61
Table A13	Effect of sunflower oil addition on total color to WPI films.....	62
Table A14	Effect of sunflower oil and carboxymethyl cellulose addition on total color on SPI films.....	63

LIST OF ABBREVIATIONS

BW	Beeswax
CMC	Carboxymethyl cellulose
CarW	Carnauba wax
CS	Corn starch
DSC	Differential scanning calorimetry
DVS	Dynamic vapor sorption
EPA	Environmental protection agency
FTIR	Fourier transform infrared spectroscopy
Gly	Glycerol
HPMC	Hydroxypropyl methylcellulose
MG	Mesquite gum
OP	Oxygen permeability
OTR	Oxygen transmission rate
%E	Percent elongation
RH	Relative humidity
SO	Sunflower oil
SPC	Soy protein concantrate
SPI	Soy protein isolate
SPI-CMC	Soy protein isolate-carboxymethyl cellulose
SPI-SO	Soy protein isolate-0.06% sunflower oil
TS	Tensile strength
WPC	Whey protein concentrate
WPI	Whey protein isolate
WS	Wheat starch
WS1	Whey protein isolate-0.05% sunflower oil
WS2	Whey protein isolate-0.10% sunflower oil
WS3	Whey protein isolate-0.15% sunflower oil
WVP	Water vapor permeability

CHAPTER 1

INTRODUCTION

1.1 General Objective

There are increasing trend to formulate edible films and coatings which are consumable with the product or if not consumed with food, caused no environmental problem because they are biodegradable, and safe to use as food packaging materials. Edible coating is a thin material created as a coating on the food product directly, whereas edible film is firstly produced and later placed on or between food materials (Bravin et al., 2006). In general, natural edible coatings and films are composed of polysaccharides, proteins, lipids or various composites of these (Galus et al., 2012). The main functions of the edible film are to protect the food product from possible physical, chemical and microbiological deteriorations. Also their suitability for use as food, compatibility of the environment, being non-toxicity and nonpolluting are the other important causes for preference of them. Edible films can function as secondary packaging materials to provide food quality and safety by working as water and oxygen barriers after the primary packaging is opened. Additionally, their protective and mechanical function may also be improved with the addition of some supplement such as antimicrobials, antioxidants, flavors, nutrients, aroma etc. (Salgado et al., 2009). Interactions within food products and their packaging materials cause some doubts in consumers' mind about safety of food products. Usage of edible films may provide a layer between the product and package and helpful to increase safety of food with preventing contact of them to each other, no matter edible films consumed with food or not. Also unemployed cultivation materials are evaluated with using the production of the edible films without environmental pollution.

There are different kind of sources to form edible films, hydrocolloids (proteins and polysaccharides) and lipids (Martinez et al., 2011). Hydrocolloids have been commonly studied to form of edible films. However, their applications are restricted.

Since hydrocolloid origin films have high water vapor permeability (WVP) than others owing to their hydrophilic nature (Valenzuela et al., 2013) and also because of using plasticizing agents to obtain flexible films (Gounga et al., 2007; McHugh et al., 1994). Having high WVP is an important problem because the efficient control of moisture transfer is a wanted property for a large amount of foods. To develop the water barrier properties of protein or polysaccharide based films, lipid compounds are commonly included into mentioned structures. But also lipid materials may decrease mechanical stability of films and cause poor mechanical strength (Martinez et al., 2011).

In the current study, it was aimed to form composite edible films from different kind and concentrations of hydrocolloids and lipid to develop mechanical, physical and barrier properties of pure whey protein isolate (WPI), carboxymethyl cellulose (CMC) and soy protein isolate (SPI) based films.

The steps of the study can be defined as:

1. Developing composite edible films from WPI, SPI, CMC and Sunflower oil (SO) with different combinations and concentrations.
2. Determining the mechanical, barrier, thermal and physical properties of the formed films.
3. Evaluating moisture absorption capacity of the main film forming materials: WPI, SPI and CMC based films at three different relative humidity conditions (30, 50, and 80%) at 25°C.

CHAPTER 2

LITERATURE SURVEY

2.1 Food Packaging

Packaging of the foods helps in protection of all types of foods and also their raw materials, from chemical, microbiological and physical deterioration during distribution and marketing of foodstuff. Without proper packaging, it is hard to get effective food distribution. The food product is surrounded and protected by packaging, from processing to the final state, until it reaches the consumer.

2.1.1 The Functions and Importance of the Food Packaging

The packaging has several functions. They can be summarized as below:

Protection: It is generally accepted as the most important function of the package. A package should defend the food product from:

- (a) Biological agents; e.g. rats, insects, and microbes;
- (b) Mechanical damage: e.g. product abrasion, compressive forces, vibration and crushing;
- (c) Chemical degradation e.g. oxidation;
- (d) Moisture transfer from the food or to food through packaging material; e.g. drying, caking or shrinkage.

Packaging is one of the most important and critical stage of the preservation process that the food product can be endangered if the unity of the package is damaged.

Suitability: Modernization and industrialization have occurred new life styles and the industry of packaging has had to adjust these changes. Last few years more people prefer to live alone and a greater percentage of women are in the workforce than ever before. Therefore, products are designed around principles of desirable “consumer”

size packaging and also easy-preparation and cooking. Therefore packaging has an important role in supplying these kinds of needs of the consumers.

Communication: Actually it can be said that the package works as a “silent salesman”. The package should give information about the product such as expiration date, nutritional information and ingredients generally with label text and graphics and it enables markets to function on a self-service basis.

Environmental issues: It is important to provide reusing or recycling of the packaging material or reduce the size of the packaging to protect the environment.

Package safety: Before using a certain type of package for food product it is very important to ensure that the material of package is safe to use for the food being considered and there are no adverse interactions such as metal contamination issues from a can or any plastic contamination from a bottle between the package and the food.

2.2 Edible Films and Coatings

Since environmental pollution caused by plastic films, research has been focused on developing edible packaging technology from renewable biopolymers (Ou et al., 2005). Traditional plastic packaging materials, for instance polypropylene, cannot recycle and they are non-biodegradable also they raise amount of the waste. Environmental Protection Agency (EPA) explained that approximately more than 12 million tons of plastic packaging wastes were produced in the United States in 2010, plastic materials such as plastic bags and wraps are cause of nearly 35% of it and these amounts have almost twofold raised since 1990.

Last a few years edible films and coatings attract more attention of scientists worldwide because of their biodegradability and reutilization compared to conventional petroleum based synthetic polymers. They need rather short period of time for completely biodegradation than synthetic materials. They are accepted as biodegradable and their usage in industry descend the environmental dirties. Biodegradability and edibility are some of the most important characteristics of edible films and coatings not offered by plastic packaging materials. In addition to

above properties, they also have to be non-toxic and inexpensive, have good sensorial qualities, high barrier and mechanical properties (Debeaufort, et al. 1995; Kokoszka et al., 2010). They also decrease the possibility of food quality spoilage and prolonging the shelf life of foods (Lee et al., 2008).

Actually, the method of using edible film or coating to protect the fresh food products from environmental hazards and to extend their shelf life is not a novel approach. The idea comes from the natural protective coating on some foods that skin of the fruits and vegetables. For example paraffin waxes have been used for years for citrus fruits as a coating and it retards the moisture loss. Also sausages have been used for meat product as a casing agent commercially for long years.

Wax the first edible coating has been used on fruits to retard water loss, resulting in shelf-life extension in the early twelfth century in China (Tharantan, 2003). However, waxes have been the only commercial protective coatings for apples and pears since 1930s. Paraffin waxes have been commercially available for coating citrus fruits in the United States since 1930s, and carnauba wax, beeswax and their emulsions have been used for coating fresh fruits and vegetables since late 20th century. To enhance protection of meat and cheese products against shrinkage, lipid coatings have been used since ages.

Yuba, the first free-standing edible film produced from boiled skin of soy milk, was conventionally used in Asian countries to enhance color and protection of some food products since the 15th century. Currently, edible films and coatings are used in different food applications, mostly fruits, vegetables, candies, and some nuts (Debeaufort, et al. 1998).

The advantages of edible films over traditional synthetic polymeric packaging materials can be listed as below:

- The films can be consumed with the packaged product and in this manner, no residual left to be disposed of. If the consumer does not want to consume the films, still the pollution of the environment is prevented because they are likely to degrade

more easily than synthetic polymeric materials and conversely formed from edible ingredients.

- Edible films can carry food additives (e.g. antioxidants and antimicrobials) to add different components such as flavorings, colorings and sweeteners.
- Especially protein originated edible films can be contributed to the nutritional value of the packaged product.
- Edible films can be successfully applied for individual packaging of small portions of the food such as strawberry and nuts.
- The films can be used inside heterogeneous foods at the interfaces between different layers of the components. The film prevents the product from deteriorative water and migration of solute in foods such as pizzas.
- The films can be used with other food packaging materials together with non-edible or synthetic polymeric materials by this way the edible films would be the inner layers in directly contacted with food product. In this manner food materials can be more efficiently protected from the deteriorative external factors and also amount of use of the synthetic polymeric materials can be reduced.

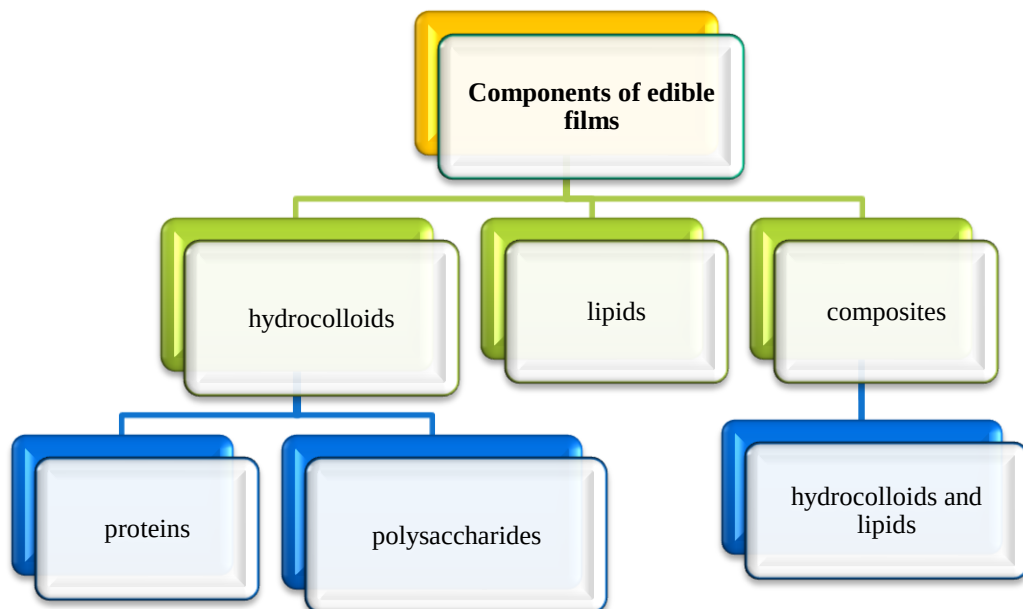


Figure 2.1 Schematic presentations of components of the edible films

2.2.1 Manufacturing of Edible Films and Coatings

Films and coatings vary in their mode of development and application to foods. Coatings are produced directly on the food products. Coatings may be applied by dipping, spraying, foam application and brushing. For instance in dip method coating, food is immersed into the composite coating formulations, then take off from the film solution and allowed to dry. Another coating method is foam application method that is used for coating emulsions (Tharanathan, 2003). On the other hand for production of edible films, the film structure is formed separately and later applied to food products.

2.2.2 Biopolymers Used For Manufacturing of Edible Films and Coatings

Materials for biopolymer edible packaging obtained from renewable and biodegradable resources such as hydrocolloids, lipids and composites were given in Figure 1.

2.2.2.1 Protein Based Edible Films

Protein based films, the most commonly researched biopolymers in the field of edible films, are the efficient alternatives for traditional food packaging which ensure barriers to water and oxygen, improve mechanical property of the food product, and are completely biodegradable, reducing thus environmental pollution (Yoshida et al., 2004; Kokoszka et al., 2010). Interest in protein-based films has centered on generally WPI (Osés et al., 2009), SPI (Cho et al., 2006), and casein (Ponce et al., 2008).

Proteins from plant (such as zein, soy, corn) and animal (milk and whey proteins) origin films have good mechanical properties and provide good barrier properties to oxygen owing to the skill of proteins to form stronger intermolecular covalent bonds (Perez-Mateos et al., 2009) but because of their hydrophilic character protein based edible films have high WVP (Mauri and Anon, 2008).

Shellhammer and Krochta(1997), used milk proteins for edible films and coatings and they reported that milk protein based films and coatings may slow down moisture loss and they are excellent oxygen barrier and have high tensile strength. The films made by milk proteins are also flexible and with having no flavor and

taste, they have good organoleptic properties. Mauer et al. (2000) investigated that β -casein films had better WVP than caseinate-based films and composite caseinate-lipid films described in the literature. β -casein films had higher mechanical properties than other caseinate-based films. However, because of temperature sensitiveness of β -casein, marketable manufacture of β -casein films could be narrowed.

2.2.2.1.1 Whey Protein

WPC and WPI are widely used as food ingredients in confectionery, bakery and ice cream products, health foods, sport bars and infant formula because of their high nutritional quality and also functional properties. The most widespread basis of proteins used to get films and coatings are casein and whey proteins.

The protein content of industrially produced WPC is lower (25-80%) than WPI (>80%) that means WPC has far more impurities (for example lactose). These impurities may cause higher permeability and worse mechanical properties due to discontinuities in the protein matrix (Khwaldia et al., 2004).

Whey, yellow-green liquid, is separated from the curd during production of cheese. The major components of WPI are β -lactoglobulin and α -lactalbumin (Fairley et al., 1996) also it contains negligible amounts of fat, sodium, and lactose and is an excellent source of essential amino acids.

WPI is a known material has a good film forming ability and the film-forming properties of whey proteins have been ensured to produce transparent, stretchy and odor-free films (Gounga et al., 2007). WPI-based films were found that they are brilliant oxygen barrier at generally low or intermediate RH (Anker et al., 2002) but have poor water barrier properties because of their hydrophilic nature (Oses et al., 2009).

Ramos et al. (2013) made a research about relationship of features of WPI and WPC with variation of glycerol content. According to their results, the whey protein origin films showed fine mechanical and very good oxygen barrier properties than other protein based films such as zein, wheat gluten and SPI or polysaccharide based films.

Both whey protein films, and especially WPI with 40 or 50% (w/w) glycerol (Gly), showed good mechanical properties. Therefore it was suggested that WPI films can minimize the deterioration rate of minimal processing of fresh fruits and vegetables. Besides, it has been reported that whey protein coating markedly retarded color change in fresh cut apples and Russet type potatoes.

2.2.2.1.2 Soy Protein

Soybeans have been cultivated for more than 3000 years and have been an important protein source for especially Asian countries. The yuba from soybean milk has traditionally been used in the Orient to wrap ground meats and vegetables. However, the process of yuba was unwieldy and not adaptable mechanization (Wu Y., 1999). During a few years soy films could be prepared from soy protein isolate (SPI) and soy protein concentrate (SPC). SPI is one of the most important commercially available soy protein products. Production of the films from soy proteins can be explained as a method which includes the denaturation of proteins and drying of them on a surface. Denatured proteins are generated the film structure through disulfide cross-linking and hydrophobic bonds (Cho et al., 2007). SPI is more commonly utilized than SPC, that non-protein compounds of SPC affects adversely the film forming ability over time, when soy protein based edible film is obtained (Cho et al., 2007).

SPI is a complex combination of proteins with commonly different molecular properties. According to their sedimentation coefficients soy proteins can be fractionated as 2S, 7S, 11S and 15S. The fractions of 7S and 11S have the ability of polymerization and generate around 37% and 31% of the total extractable protein respectively (Kokoszka et al., 2010; Cho et al., 2007).

The abilities of film forming and oxygen barrier properties of soy protein based edible films are good but because of the hydrophilic nature its water vapor permeability is high (Krochta, 1994).

SPI is a raw material that has high nutritional value, easily accessible, low-cost and biodegradable (Galus et al., 2012). Besides, soy protein generates more stretchy, smooth and clear films than films obtained from other plant protein sources (Cho et al., 2007).

WVP of SPI based films were tested by Gennadios et al. (1993) and they found that WVP was affected by altering pH of the SPI film solution. It was reported that when pH was increased more than the isoelectric point of SPI, WVP decreased. Kokoszka et al., (2010) studied to identify the influence of both soy protein and glycerol contents on physical and chemical features of SPI films. According to study it was found that WVP of SPI films had affected from amount of SPI and glycerol content. At the end of this study the film which had the lowest SPI (%6)-glycerol (%40) content were found to have the best barrier properties. Probably protein network is less organized at higher concentration. Varying amounts of plasticizer (glycerol) on WVP of SPI films were tested by Stuchell and Krochta (1994) and observed that WVP increased as the concentration of plasticizer increased. Actually glycerol is a considerably hygroscopic compound which increases the wetting of the surface and moisture absorption.

2.2.2.2 Polysaccharide Based Edible Films

Polysaccharide based edible films have good mechanical resistance, low oxygen permeability and good film forming ability; therefore they can help to preserve certain foods. On the other hand, polysaccharide films show poor barrier against to moisture because of their hydrophilic nature. To have better moisture barrier properties, hydrophobic compounds can be added to hydrocolloid films but it can decrease the mechanical strength (Guilbert et al., 1996). Polysaccharides used in the edible film include alginate, pectin, starch and cellulose derivatives. Polysaccharides have an important role in food industry as they are cheap, abundant in nature, nontoxic and can be produced from various resources.

Polysaccharide-based coatings have color-free appearance, low caloric content and can be used to increase the shelf life of foods such as fruits and vegetables with significantly reducing dehydration, weight loss and also darkening of the surface (Carneiro-daCunha, 2009).

Zhang et al. (2006) investigated the influence of plasticizers on mechanical properties of pea starch films. They used different monosaccharides (mannose, galactose and fructose) and polyols (glycerol and sorbitol) as plasticizer to form starch films. According to research monosaccharide-plasticized films have better

plasticizing effect in terms of physical, mechanical (higher tensile strength and elongation), and WVP than polyol-plasticized films. Further, Maran et al. (2013) suggested using glycerol as a plasticizer raised the WVP and OP of tapioca starch, agar and span films due to its hydrophilic nature.

2.2.2.2.1 Carboxymethyl Cellulose

Cellulose is the structural component of the plant and the most abundant source of complex carbohydrate. They are water-soluble cellulose derivatives and also non-toxic materials. CMC is an efficient additive to get better product and it could be used for processing in different fields of such as foodstuffs, cosmetics and textile industries (Su et al., 2010). Because of the its polymeric structure and high molecular weight chains CMC produces excellent films (Ghanbarzadeh, 2010). It is an anionic linear polysaccharide obtained from cellulose and it has high viscosity. It is a low-cost material, probably because of its insolubility in water that hydrophilic nature of cellulose. This problem can be solved by derivatization of cellulose with etherification and esterification reactions (Alyanak, 2004). Esters (e.g., cellulose acetates, propionates, and mixed derivatives) are soluble in organic solvents, while ethers (e.g., methyl, hydroxyalkyl, and carboxymethyl cellulose) dissolve in water.

Cellulose derivative based edible films are transparent, flexible, flavorless, colorless, and tasteless. Besides they are water soluble and good barriers to oxygen. Nevertheless, their barrier properties for water vapor are not good due to the hydrophilic nature of polysaccharides and they own poor mechanical properties. Ghanbarzadehet al. (2010) investigated that incorporation of CMC to starch-glycerol blend films enhanced the moisture resistance of the created starch based composites. The percentage of 1.5 (w/w) CMC, composite films had the lowest WVP values at 2.34×10^{-7} g/(m)(h)(Pa). Besides, it was determined that CMC had an significant effect on the mechanical and physical properties of the resulted blend films that it increased the TS of starch based blend films by almost 59%.

2.2.2.3 Lipid Based Edible Films

Lipid based edible films and coatings can be produced from a wide variety of lipid substances involving oils, waxes, fatty acids and surfactants (Alyanak, 2004). They are used because they are generally shows good barrier properties against to moisture

(Martinez et al., 2011). To have less WVP make the lipid films appropriate for coating of meat products. Moreover, lipid films have been used to make the appearance of fruits and vegetables glossy. It is also known that they are efficient on protecting fruits from mold growth. Lipid films are used also as coating agents for adding gloss to confectionary products too. Their use in a pure form as free-standing film is restricted, because not to have enough structural integrity and durability (Krochta et al., 1994).

Waxes are widely used for coating fruits and vegetables to decrease moisture loss. Although fatty acids and fatty alcohols are effective barriers to water vapor, their fragility requires that they can be used in conjunction with a supporting matrix. Lipid materials provide better moisture barriers than hydrocolloids because of their hydrophobic character (Oms-Oliu et al., 2008). However, they have poor mechanical strength, show little resistance to gas transfer and have poor mechanical stability (Martinez et al., 2011).

2.2.2.3.1 Sunflower Oil

Sunflower oil is non-volatile oil obtained from sunflower (*Helianthus annuus*) seeds. It contains mainly linoleic acid. It has high Vitamin E content. It is considerably abundant and inexpensive material.

2.2.2.4 Composite Films

Generally one kind of film forming hydrocolloid (protein or polysaccharide), or lipid used to form film with some plasticizing additives. Each kind of these materials has some disadvantages and advantages, hence to enhance advantages and eliminate disadvantages, there was a need to form composite films. In this way, edible films with developed and modified properties could be get according to the specifications of each application (Debeaufort et al., 1998). There are different techniques to form them such as bi-layer films, lamination and blending them to form films.

Film forming materials interacts physically or chemically with each other. Knowledge about them gives facilitate to design of the films with specific structural characteristics and barrier properties. Shellhammer and Krochta (1997) investigated that rising the amount of milkfat; carnauba wax, candelilla wax, or beeswax more than 40% in WPI films considerably improved their WVP. Perez-Gago et al., (2005)

made research on the effect of usage of composite coatings formed with protein (WPI or WPC) or cellulose (HPMC) and beeswax (BW) or carnauba wax (CarW) on browning of fresh-cut apples. They reported that whey protein based coatings reduced enzymatic browning more than cellulose coatings probably because of upper oxygen barrier property that the protein performs.

Addition of olive oil to chitosan film was studied and it was suggested that because of the developed interaction between lipid and carbohydrate phases, the tensile properties such as strength and elongation increased with olive oil concentration (Pereda et al., 2012).

Oses et al. (2009) studied the physical properties and microstructure of films made of WPI and mesquite gum (MG). They indicated that incorporation of MG on the WPI solution improved mechanical properties, specifically increased flexibility without increasing plasticizer content. Then they supported that the use of MG will be an option of using more amount of plasticizers.

A different approach has been tried using same kind of film forming materials with different origin instead of combining different kinds. For example, Arnon et al., (2015) had seen that, the layer-by-layer coatings, ground on to use with two polysaccharides, CMC and chitosan ensured mandarins with the better firmness, lower weight loss and good gloss, while not influence natural taste. Another example was reported by Ghanbarzadeh et al. (2006) starch-CMC films had better mechanical properties and moisture resistance than films formed with starch.

Valenzuela et al., (2013) determined mechanical, barrier and structural properties of quinoa protein-chitosan-sunflower oil blend film. They found that water vapor permeation of the composite film decreased compared with quinoa protein-chitosan and chitosan films as a result of the hydrophobic and the non-polar inherent of the lipids. However, addition of sunflower oil to the film reduced the mechanical properties and raised oxygen permeability owing to the creation of micropores. Plasticizing characteristics of the quinoa protein and lipid increased elongation without addition of any plasticizer.

2.3 Methods For Determining the Properties of the Films

2.3.1 Mechanical Properties

Mechanical properties of edible films are important to ensure that the film has enough mechanical strength and integrity during transportation, processing and storage of foods coated with edible films. The film must be durable to breakage and abrasion to protect the food from environmental effects and also film must be flexible to adapt to possible deformation of the filling without breaking (Guilbert et al. 1996). The most important mechanical properties are tensile strength (TS) and percent elongation (%E) to investigate the quality of the edible films and coatings.

Tensile strength is the stress needed to break a sample and it is an indicator how strong the film is. The tensile stress on a material is defined as the force per unit area as the material is stretched (Figure 2.2).

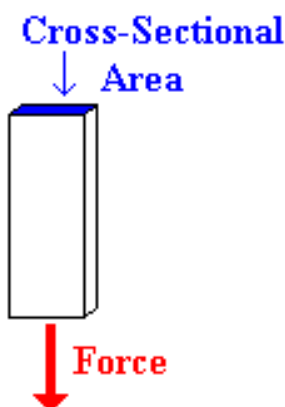


Figure 2.2 Principle of tensile strength

Pascal, a pressure unit, is used as a unit for the stress. TS increases with an increase in molecular mass and increase in intermolecular forces, but also decreases with the presence of plasticizers.

The strain is (Figures 2.3-2.5) a measure of the change in length of the sample. The strain generally is expressed as elongation. %E is a quantitative representation of a film's ability to stretch and it is the length of the displacement per original length ($\Delta L/L_0$).

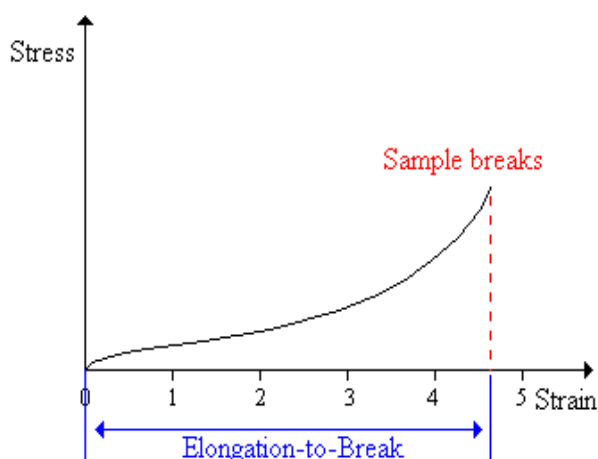


Figure 2.3 The graph of percent elongation

The strain is a unitless number. A tensile stress-strain curve is a plot of stress on the y-axis versus strain on the x-axis (Figure 2.5). In the plot given below, strain is expressed as elongation. Stress-strain curve is measured with an instrument specially designed for tensile testing such as Texture Analyzer.

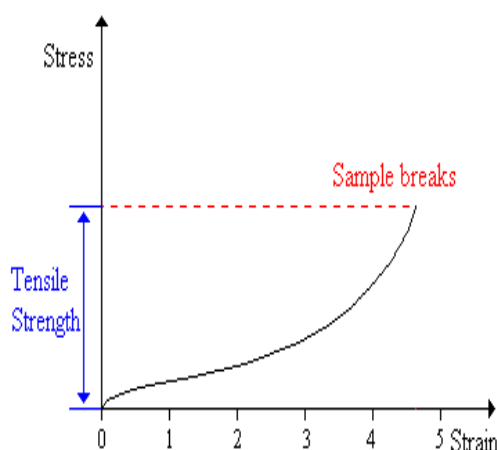


Figure 2.4 The graph of tensile strength

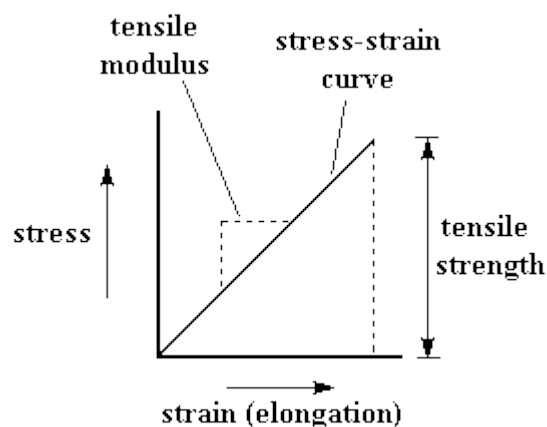


Figure 2.5 Stress-Strain Curve

Elongation is an indicator of the film's toughness and flexibility. Speed of the instrument and the measurement conditions effect the mechanical properties of the films (Gennadios et al., 1993).

Edible films act more brittle and stiff if the speed is higher or temperature lower so conditioning is necessary to test the mechanical properties of the films at controlled temperature and relative humidity in order to achieve good reproducibility.

Mechanical properties of edible films can be affected from a lot of factors, such as polymer structure, type and concentration of the plasticizer, molecular weight of the film forming materials, type of solvents, film thickness and film formulation

(Alyanak, 2004). The film forming conditions e.g. types of process and solvent, rate of cooling and evaporation, coating technique (spraying, spreading, etc.) generally affect the mechanical properties of edible films and coatings.

Table 2.1 WVP values for some of the edible films

Film	x ^a	Conditions	WVP ^b	References
WPI	0.13	25°C, 0/50%RH	11.92	Ramos et al. (2013)
WPI	0.13	25°C, 0/75%RH	2.93	McHugh et al. (1994)
SPI	0.08	25°C, 0/40%RH	5.06	Galus et al. (2012)
Carnauba wax	-	27°C, 0/100%RH	0.09	Shellhammer and Krochta (1997)
Beeswax	-	25°C, 0/100%RH	0.09	Shellhammer and Krochta (1997)
Tapioca starch	0.04	25°C, 0/75%RH	4.82	Maran et al. (2013)
LDPE	-	38°C, 90/0%RH	0.08	Kim, (2000)
HDPE	-	38°C, 90/0%RH	0.02	Kim, (2000)

^a: thickness in mm; ^b: WVP in (g.mm/m².d.kPa)

2.3.2 Barrier Properties

2.3.2.1 Water Vapor Permeability of Edible Films

ASTM E96-80 defines permeability as the rate of water vapor transmission through a unit area of flat material of unit thickness induced by a unit vapor pressure difference between two surfaces, under definite temperature and humidity conditions (ASTM, 1980).

Permeability consists of a process of solution and diffusion where the vapor dissolves on one side of the film and then diffuses through to the other side. WVP results provide us information about possible mass transfer mechanism and solute and polymer interactions in edible films. Protein and carbohydrate based edible films have hydrophilic nature therefore they do not provide good barriers to water conversely to lipid films. The WVP values of some of the edible films are given in Table 2.1. WVP of the edible films is affected from the composition of film forming materials, film thickness and plasticizer content.

The barrier properties of lipid films against oxygen have been found to be rather poor than protein and carbohydrate films. According to Fennema and Donhowe (1994), candelilla and carnauba wax films showed better oxygen barrier properties than beeswax (BW) films. The presence of unsaturated hydrocarbons is the main reason for high permeability of BW films.

2.3.2.2 Oxygen Permeability of Edible Films

OP is one of the most widely researched transport properties of edible films. Controlling oxygen related reactions improves food quality because oxygen has a deleterious effect on food products for example presence of oxygen causes lipid oxidation, growth of microorganism, and enzymatic browning and vitamin loss.

The higher the OP, the lower is the OP barrier ability of the films. Similar to water if it is allowed to enter packages freely it causes deterioration. If a coating which has suitable permeability is chosen, a controlled respiratory exchange can be obtained and so the preservation of food can be prolonged.

2.3.3 Physical Properties

The appearance (color, gloss and haze) of edible films and coatings is important to improve consumer acceptability (Zahedi et al., 2010). The measurement of the film color was done using a tristimulus colorimeter. Color can be specified with L (Lightness), a (redness) and b (yellowness) values.

Gloss is the property of a surface responsible for shiny appearance of a product. Physically, gloss originates from a geometrical light distribution reflected by the surface of an object, with an increased flux in the specular direction. A surface of high gloss will reflect most light in the specular direction while a surface with low gloss will reflect most of its light in directions other than specular (Mendoza et al., 2010).

2.4 Differential Scanning Calorimetry (DSC)

The studies of thermal deterioration of polymers are required as a lot of applications depend on their thermal stability. This technique is used to study what happens to polymers/samples upon heating. In order to obtain the required information different methods are applied. DSC (Differential Scanning Calorimetry) is one of them. This is a thermoanalytical technique.

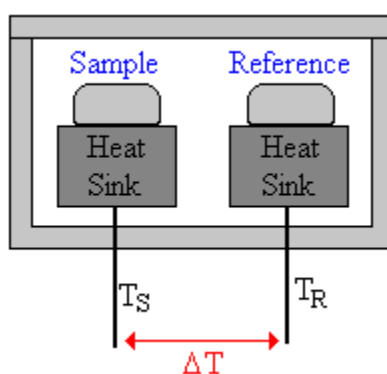


Figure 2.6 The diagram of the heat flux into DSC

In this technique the difference in the amount of heat needed to increase the temperature of a sample with respect to reference are measured as a function of temperature (Figure 2.7). During the experiment the temperature of both the sample and reference are maintained at nearly.

DSC is useful for measuring temperature of the thermal transitions such as glass transition, crystallization and melting of a polymer (Figure 2.7). Below glass transition temperature (T_g) the material is brittle, and above it becomes flexible. (Guilbert et al. 1996). Above the glass transition, the polymers have a lot of mobility. The measurement of the glass transition temperature of a polymer mixture is often used as criterion to found its miscibility. A miscible polymer mixture would display a single transition between T_g of the two components.

Because of the increasing immiscibility the transition may broaden. When polymers came into these crystalline arrangements, they give off heat. If heating was still kept, the polymer past its T_c , ultimately reached another thermal transition, melting (T_m). At the polymer's melting temperature, or T_m , those polymer crystals begin to fall apart, that is they melt.

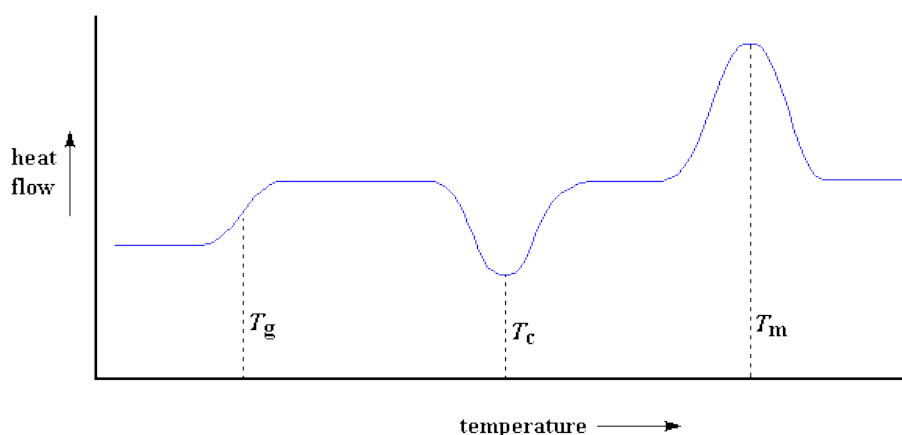


Figure 2.7 A representative thermogram of DSC

2.5 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is an analytical technique that applied on different substances such as liquid, gas and solid matter to determine the unknown materials. In infrared spectroscopy, when IR radiation is passed through a sample, some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum shows the molecular absorption and transmission, that to create a unique

molecular structure of the sample. This makes infrared spectroscopy useful for several types of analysis.

2.6 Moisture Absorption Isotherms

Water activity has long been considered as one of the most important quality factors in food systems especially for long-term storage, drying and roasting operations. Moisture absorption isotherms give the relationship between equilibrium moisture contents and water activities of the packed food material. However, the packaging material itself can absorb water vapor and this is important if the material having such high water vapor absorption capacity like edible polymers. Since moisture is absorbed at different rates by different materials or by the same material under different humidity conditions, it appeared interesting to characterize the whole sorption process and not only the equilibrium conditions. Appropriate selection of the plasticizer type and concentration will be helpful in controlling the moisture content and moisture absorption rate of a film, thereby improving the film stability under varying relative humidity conditions. Dynamic vapor sorption (DVS) techniques are currently used to determine the sorption characteristics of food products. When compared with the static gravimetric technique, extensively used as a classical method in the existing scientific literature, DVS techniques have shown many advantages, including shorter equilibration time.

CHAPTER 3

MATERIAL AND METHOD

3.1 Materials

The film forming materials WPI (protein content >90%) was obtained from Davisco Foods International Inc. (BiPro, Le Sueur, MN) and SPI (protein content >90%) is obtained from Gushen Biological Technology Group Co., Ltd. Sunflower oil was purchased from a local market from Gaziantep. CMC (Na-salt) and Glycerol (84-88%) were supplied from Sigma-Aldrich (Sigma-Aldrich Co, USA). Distilled water was used as a solvent throughout the experiment.

3.2 Experimental Studies

3.2.1 Preparation of the Films

Table 3.1 The combinations of the compounds used in film formation in preliminary study

		Protein			Polysaccharide			Lipid
Main source		WPI	SPI	CMC	CS	WS	Pectin	SO
Protein	WPI	✓	-	NF	NF	NF	NF	✓
	SPI	-	✓	✓	-	-	-	✓
Polysaccharide	CMC	NF	✓	✓	-	-	-	NF
	CS	NF	-	-	NF	-	-	NF
	WS	NF	-	-	-	✓	-	NF
	Pectin	NF	-	-	-	-	NF	-
Lipid	SO	✓	✓	NF	NF	NF	NF	-

NF means "Not Formed" that the materials could not generate a film.

Different combinations (protein-polysaccharide-lipid) were formed (Table 3.1) in preliminary experiments in order to get applicable composite films.

The main idea during the selection of the components was to get as much as possible cheap, easily obtainable (reachable), and high film formation ability component.

Corn starch (CS), wheat starch (WS), pectin, CMC, and sunflower oil (SO) were the examples of them. However, some of them did not form any acceptable film having good mechanical properties in our study. Hence the expensive but having high film forming ability compounds (WPI and SPI) were also examined.

Table 3.1 gives all combinations of our preliminary studies with indication of “Formed (✓)” and “Not Formed (NF)” for tested films. Materials to be used were decided by these preliminary works.

3.2.1.1 WPI Based Edible Film: Aqueous solution of 8% (w/w) WPI were prepared by mixing WPI powder in distilled water and glycerol was added at 8% (w/w) as a plasticizer.

The resulting solutions were magnetically stirred using magnetic stirrer for 5 minutes. After complete dissolution of film forming materials, the solution was heated to a final temperature of 80°C for 25 minutes under continuous agitation (Ramos et al., 2012). Subsequently; the film forming solution was kept in an ultrasonic bath to remove dissolved air for about 1 hour. Nine mL of solution were poured onto per glass plate (10cm×10cm) and spread evenly with a glass rod and allowed to dry at room conditions for 24 hours. Afterward dried films were peeled from casting surface and they were stored in a desiccator at room conditions until the tests were applied.

3.2.1.2 SPI Based Edible Film: SPI (4%, w/w) and glycerol (3%, w/w) were dispersed in distilled water with magnetic stirrer. After complete dissolution of film forming materials the pH of the solution was adjusted to 10 using 1M NaOH solution and denatured by heating at 70°C for 20 minutes (Galus et al., 2012).

The alkaline solution was used because pH 10 has been found to convert protein molecules to a state of maximum unfolding.

Furthermore, the high pH encourages high lysine–alanine linkage formation (Su et al., 2010). After that the film forming solution was kept in an ultrasonic bath to remove air bubbles for about 1 hour. Six mL of solution were poured onto a plastic plate (9cm internal diameter) and spread equally. The spread films were dried at room conditions for 24 hours. Finally the films were detached from the plastic plate and maintained in desiccators at room conditions (20°C and 40% RH) before the films were tested.

3.2.1.3 WPI-SO Blend Edible Films: Film forming solution was prepared as follows: 8% (w/w) WPI, 8% (w/w) glycerol and different SO concentrations (0.05, 0.10 and 0.15%) were dispersed in distilled water by constant stirring.

The film samples were coded WS1, WS2, and WS3 for defining SO concentrations 0.05, 0.10, and 0.15%, respectively. The other steps were the same as described in section 3.2.1.1.

3.2.1.4 SPI-SO Blend Edible Film: The blend was prepared by mixing SPI (4%, w/w) and glycerol solution (3%, w/w) with SO 0.06% (w/w). All the other stages were carried out as described in the section 3.2.1.2 and coded as SPI-SO.

3.2.1.5 SPI-CMC Blend Edible Film: Aqueous solution of blend edible film were prepared by mixing 4% (w/w) SPI powder, 0.5% (w/w) CMC in distilled water, then glycerol (3%, w/w) was added. The same steps in the section of 3.2.1.2 was followed and coded as SPI-CMC.

3.2.1.6 CMC Based Edible Film: CMC (2.4%, w/w) was solubilized in distilled water while agitated by magnetic stirrer for 5 minutes. Glycerol (1.2%, w/w) was added as a plasticizer. The solution was heated at 75°C for 10 minutes after complete dissolution of film forming materials (Babak et al., 2011).

Furthermore the solution was kept in an ultrasonic bath to remove dissolved air for about 1 hour. Nine mL solution were poured onto glass plate (10 cm×10cm) and spread equally. The spread films were dried at room temperature for 24 hours. Dried films were peeled and stored in desiccator.

3.2.2 Characterization of Edible Films

3.2.2.1 Thickness

Film thickness was measured with a manual micrometer (Imperial, Sigma-Aldrich) with a sensitivity of 0.0025 mm. Five to ten random thickness measurements were applied for each film, the average values of them were taken.

3.2.2.2 Mechanical Properties

Texture Analyzer (Stable Microsystems, Godalming, UK, Model: TA-XT2i) was used to measure tensile strength and percent elongation of the samples according to ASTM Standard Method D 882 (ASTM, 1993). Prior to running mechanical tests, film samples were cut into rectangular strips of 25 mm width by 75 mm length and stored in desiccators at room conditions.

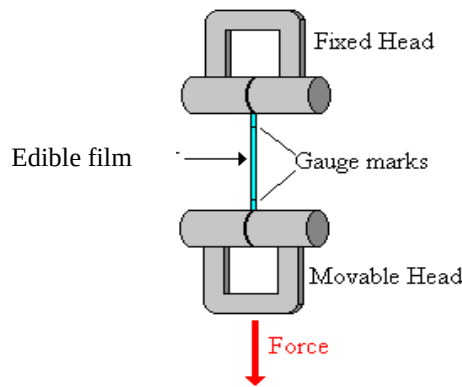


Figure 3.1 Representation of placing films on texture analyzer in a tensile test

Film samples were tested using two grips. Initial grip separation was set at 50 mm and crosshead speed at 0.8 mm s^{-1} . Pre-test speed was set to 3 mm/min and post-test speed was 10 mm/min. Forces and distances were used to calculate mechanical properties.

TS and E% values reported were the averages of at least three measurements for each type of film. The TS was expressed in MPa and was calculated according to below equation (equation 1).

$$\text{TS} = N / \text{mm}^2 \quad (1)$$

where, TS is the tensile strength in MPa; N is the force maximum at rupture of the film; mm² is the initial cross sectional area of the film. The %E (percent elongation at break) was calculated according to equation (Equation 2).

$$\%E = \frac{(D_f - D_i)}{D_i} \times 100 \quad (2)$$

Where %E is the percent elongation at break; D_f is the distance elongation at break (mm); D_i is the initial distance between the baselines (mm).

3.2.2.3 Barrier Properties

3.2.2.3.1 Water Vapor Permeability

WVP of the films was measured gravimetrically using procedures described by Kaya and Kaya(2000). Prior to test, the samples were conditioned at room conditions in desiccators for ten days. Circular glass test cups with internal diameter of 4.7 cm and depth of 2 cm were used. External and internal %RH of the measurement system shown in Figure 3.2 were adjusted using saturated NaCl solution (RH=75%) and silica gel (RH=0%), respectively.

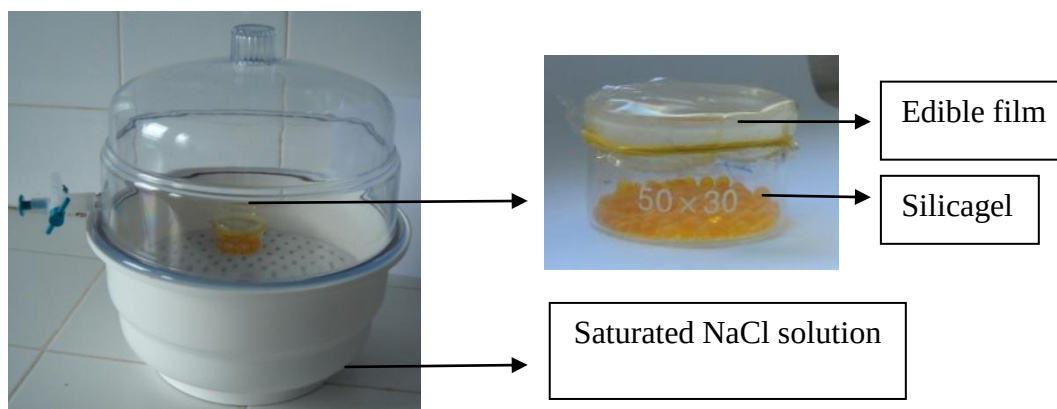


Figure 3.2 Schematic presentation of the experiment of water vapor permeability

Films were cut with a diameter slightly larger than the diameter of the cup and placed tightly over the cup containing silica gel (Figure 3.2). The test cups were weighted every two hours periodically to evaluate weight gain within a day. The given WVP values were calculated using Equation 3 from the average of the three replication results of the films.

$$\text{WVP} = \frac{\Delta m}{(\Delta t)(\Delta p)(A)} \times d \quad (3)$$

Where WVP is the water-vapor permeability in g mm/(m²)(d)(kPa); $\Delta m/\Delta t$ is the weight of moisture gain per unit of time in g/d; A is the film area in m²; ΔP is the water vapor pressure difference between the two sides of the film in kPa; d is the film thickness in mm.

3.2.2.3.2 Oxygen Permeability

Oxygen permeability (OP) of the film samples were measured using Mocon Oxygen Permeability ML-2/21 an Ox-Tran System (Mocon Inc., Minneapolis, MN, USA) at 23°C and 0% RH respect to Standard ASTM D3985-05(2010)e1. The method involve a flowing an oxygen gas stream on one side of the film and a nitrogen stream (carrier gas), to carry the transmitted oxygen gas to the analyzer, on the other side. A colorimetric sensor, an infrared sensor, a gas chromatograph or a dedicated oxygen analyzer may be used for monitoring. Film samples were cut as a shape of circular and placed between two aluminum masks that allowed an exposure area of 5 cm². Three replicates of each film were evaluated.

The OP was reported in cm³mm /(m²)(hour)(atm) for all films by converting the oxygen transmission rate values (OTR) obtained from the instrument (cm³/m²hour) with the use of the appropriate conversion units and the following equation:

$$P = (\text{OTR} \times d)/\Delta p \quad (4)$$

where d: thickness of the film; Δp : pressure differential acting on the film.

3.2.2.4 Physical Properties

3.2.2.4.1 Gloss and Haze

Gloss and haze of the films were measured with BYK Gardner micro-gloss 45 and diffusion systems M57D with respect to ASTM D1003-61 and ASTM D2457-70. The gloss and haze values were determined six and three measurements per sample, respectively.

3.2.2.4.2 Color

The color values of edible films were measured by the L, a, b, YI system using a colorimeter (Hunter Lab ColorFlex, Hunter Associates Laboratory Inc., Reston, VA, USA). The instrument was standardized each time with white and black ceramic plates. Later the color measurements were performed by placing the film sample over colorimeter. The Hunter L, a, b and YI color values were measured.

Lightness (L) refers to the relation between reflected and absorbed light, without regard to a specific wavelength. Redness (a) and yellowness (b) results are obtained from differences in absorption of radiant energy at various wavelengths. Instrumental color readings were lightness or L (0=black, 100=white) and chromaticity parameters or a (-60=greenness to +60=redness), and b (-60=blueness to +60=yellowness). A white tile (L: 93.41; a: -1.12; b: 1.07) was used as reference. For each sample, three measurements were taken and averaged. The results were expressed as total color difference (ΔE) between the samples and reference (white tile) to the Equation (5).

$$\Delta E = \sqrt{(L_{\text{Ref}} - L_{\text{sample}})^2 + (a_{\text{Ref}} - a_{\text{sample}})^2 + (b_{\text{Ref}} - b_{\text{sample}})^2} \quad (5)$$

3.2.3 Thermal Properties

Thermal properties of the edible films were determined using DSC (Perkin Elmer, DSC 4000). Prior to the experiment, the DSC was calibrated using indium samples that the phase transition temperatures are known. The sample weight ranged between 10 and 15 mg were weighted into aluminum sample pans (inner volume: ca. 50 μL), and pans were hermetically sealed. Prior to the analysis of samples, the baseline was defined with an empty, hermetically sealed inert aluminum pan. The WPI based film samples were subjected to the following program: Holding for 3 min at 0°C for stabilizing, and heating from 0°C to 120°C at a rate of 10°C min⁻¹, holding for 5 min at 120°C, cooling from 120°C to 0°C at a rate of 10°C min⁻¹. The SPI based film samples were subjected to the same program with WPI based films but there were only one difference that instead of heating from 0°C to 120°C, heating from 0°C to 170°C were applied.

Thermal transitions such as glass transition, crystallization and melting of a polymer were defined in terms of peak or transition temperature. Heat of transition or

enthalpy ΔH (J g^{-1}) was qualified from peak areas and results expressed per weight (g) of sample.

3.2.4 FTIR

The measurements were carried on a Perkin–Elmer Spectrum 100, FT-IR spectrophotometer at the resolution of 4 cm^{-1} in the wave number region $650\text{--}4000 \text{ cm}^{-1}$ at room temperature to investigate the expected changes in the main characteristic groups of studied edible films. Films were cut about 1 cm^2 to prepare suitable samples for the measurement.

3.2.5 Moisture Absorption

Moisture absorption of WPI, SPI and CMC based film samples were measured in the range of 0–80% RH at 25°C using the instrument of DVS (DVS Intrinsic, Surface Management Systems, London, UK). The sample was weighted ranged between 5–15 mg with 0.0001 sensitivity. The sample was exposed to increasing steps in humidity from 0% RH to 30, 50, and 80% RH, such that absorption values were measured for each RH%. The dry nitrogen flow rate used was 200 scc and the dm/dt (weight gain/time) was set at 0.008%/minute. All measurements were performed in two replicates. The moisture absorption of the samples was calculated as follows:

$$\text{Moisture absorption} = \frac{(W_t - W_o)}{W_o} \times 100 \quad (6)$$

where W_o : Initial weight of the sample; W_t : The weights of the sample after t time at target % RH.

3.2.6 Statistical Analysis

ANOVA analysis was performed on all results using a statistical program SPSS V. 10.0 at a confidence interval of 95% to determine the significant difference between group samples.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Mechanical Properties

Mechanical properties of the edible films based on WPI, SPI, and CMC were investigated to determine the physical resistance, flexibility and reliability of the films. They are important to give information about the applicability of the film as a packaging material.

Table 4.1 Mechanical properties of edible films

Film Type	Percent Elongation (%E)	Tensile Strength (MPa)
WPI	10.54	4.17
WS1	21.75	1.72
WS2	32.37	1.29
WS3	39.90	1.01
SPI	28.59	0.32
SPI-CMC	94.62	0.77
CMC	3.39	11.79
SPI-SO	66.99	0.63

The main mechanical properties of almost all packaging films were the percent elongation and tensile strength (Ahmadi et al., 2012) values of the films were determined and shown in Table 4.1. The %E value of the WPI film was lower than the other films studied. However, it is possible to state that it was found to be one of the most resistant films presenting the second highest tensile strength of 4.17MPa among the studied films.

The effect of SO concentration on the tensile strength of WPI films were found to be significant ($p < 0.05$). Addition of SO improved %E (Figure 4.1) but decrease tensile strength of WPI films possibly the presence of oil particles between protein structure cause weaknesses of the film (Figure 4.2). Increasing SO improved elongation and this means that extensibility of the films was enhanced.

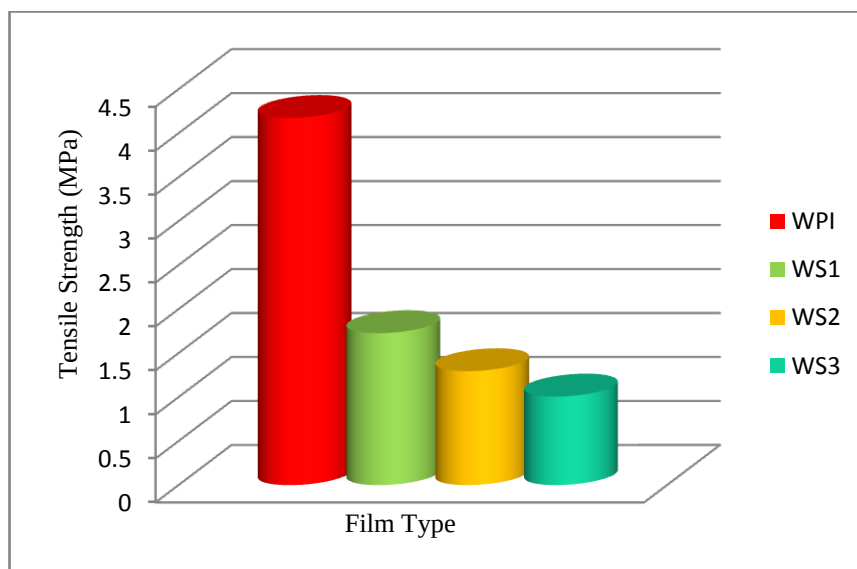


Figure 4.1 Tensile strength of WPI and WPI-SO blend films

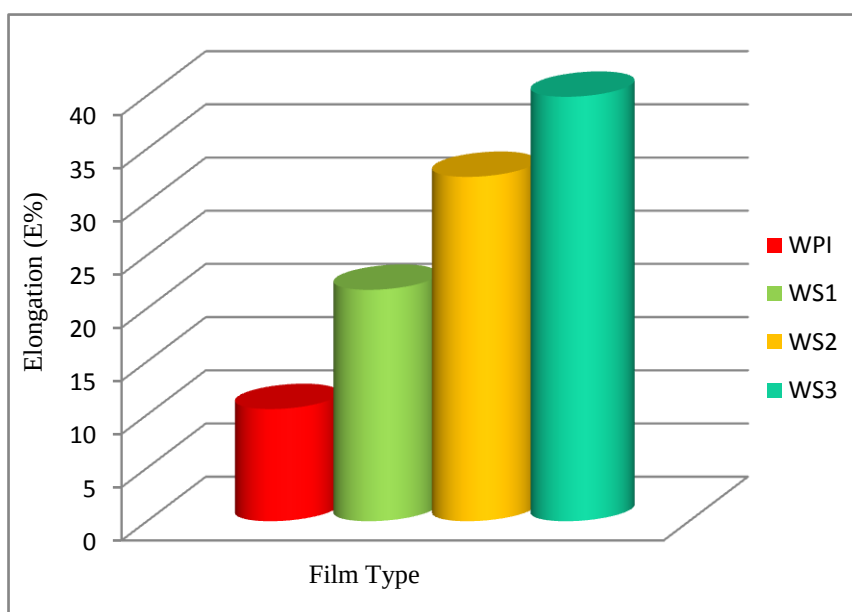


Figure 4.2 Elongation of WPI and WPI-SO blend films.

The effect of lipid or essential oil addition in films has been studied and in all the cases significant decreases in tensile strength has been reported (Zinoviadou et al., 2009). Similar observation was also reported by Shaw et al (2002). They suggested that the increasing soy oil concentration and glycerol content caused decreasing in TS and increasing %E since the presence of soy oil decreased film strength. It was reported that lipid addition encourages the development of a heterogeneous film structure, featuring discontinuities. The latter may affect the stretching ability of the film based on the characteristics of the lipids added. Since sunflower oil is liquid at room temperature, it will be present in the film in the form of oil droplets that can easily be deformed, enhancing the film's extensibility. Krochta (1997) reported decreases in strength with increasing lipid concentration for a variety of WPI/lipid composite films.

SPI based film showed the lowest tensile strength of 0.32 MPa and CMC had the highest tensile strength of 11.79 MPa among the all other studied edible films (Table 4.1).

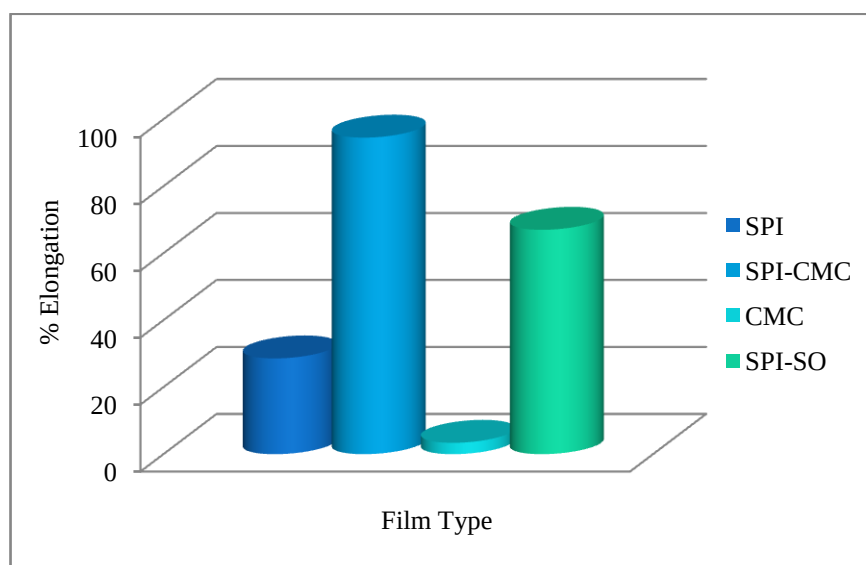


Figure 4.3 Comparison the elongation values of SPI and CMC films

Their blend of SPI-CMC had the tensile strength of 0.77 MPa and higher elongation of 94.62% than both SPI (28.59%) and CMC (3.39%). It was suggested that addition of CMC to the SPI films caused the production of more flexible films and CMC played a role in weakening or reduction of the number of hydrogen bonds, acting as a plasticizer (Figure 4.3). Similar observations were evaluated by Su et al. (2010) that

mechanical properties of SPI-CMC blend films improved with addition of CMC to SPI film. It was suggested that CMC molecules have a lot of hydroxyl groups that they might generate strong intermolecular interactions such as hydrogen bonding with protein molecules.

Besides, it was also suggested that variations in mechanical strength (decreasing the tensile strength and increasing %E) of soy protein based films were due to the type and concentration of soy protein and plasticizer used.

4.2 Barrier Properties

4.2.1 Water Vapor Permeability

WVP studies were carried out to measure the water vapor barrier efficiencies of the films. Water has a deteriorative effect on foods, therefore water vapor barrier property of edible films is an important factor in the packaging area. All experimental permeability values were given in Table 4.2 for each kind of films.

Table 4.2 Water vapor permeability of edible films

Film Type	WVP (gmm/m ² hourkPa)	Thickness (mm)	Permeance (g/m ² hour kPa)
WPI	2.57	0.23	11.38
WS1	0.62	0.15	4.19
WS2	1.66	0.21	7.87
WS3	1.42	0.16	8.72
SPI	0.94	0.12	7.83
SPI-CMC	0.06	0.14	0.40
CMC	0.84	0.06	13.25
SPI-SO	0.63	0.14	4.46

Figure 4.4 shows WVP values of sunflower oil added WPI films as a function of SO concentration. From this Figure, it can be understood that WVP values of WPI films decreased when SO was added. Similar observations were detected by McHugh and Krochta (1994); Ozdemir and Floros (2008). It was suggested that incorporation of lipids into the whey protein film matrix were significantly improved the water barrier properties.

It has been generally known that the addition of lipids to hydrophilic protein-based films decreased WVP of the films (Atarés et al., 2010). However, the addition of small amounts of lipids or oils sometimes showed reverse affect as shown in Table 4.2. This could be due to the interactions of oil components with some protein tails that could promote the decrease in the hydrophobic character of the protein matrix (Perez-Gago and Krochta, 1999). Similar results were observed by Srinivasa et al. (2007) when fatty acids were added to chitosan films, WVP of them were found higher than that of pure chitosan film.

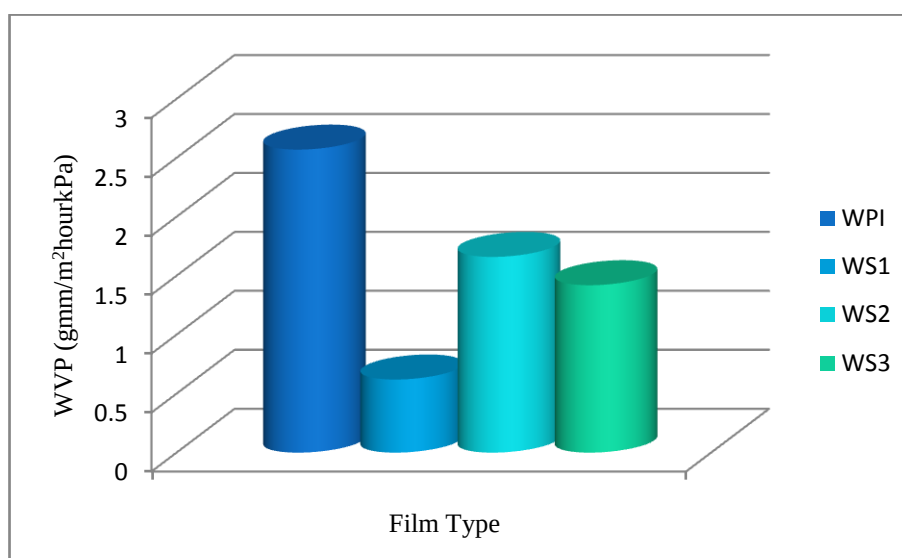


Figure 4.4 Effect of SO concentration on the WVP of WPI films

The WVP values were lower for all WPI-SO blend films compared with WPI film due to the non-polar character of the SO component. The results given in Table 4.2 shows that WVP is affected by SO addition on WPI. The influence of sunflower oil was found to be significant ($p < 0.05$), ANOVA results were given in Appendix Table A5.

There were statistically significant differences between WPI and SO added WPI based films. The lower WVP values obtained with SO addition can be explained by the formation of an interconnecting lipid network within the film matrix, which provides high hydrophobicity and thus reduces the absorption of water molecules (Valenzuela et al., 2013).

The presence of SO and CMC in the composite SPI films decreased WVP compared with the SPI film (Figure 4.5, Table A2). The WVP of the emulsion film was about half value of WVP of SPI film. The blend of them improved barrier properties significantly ($p < 0.05$).

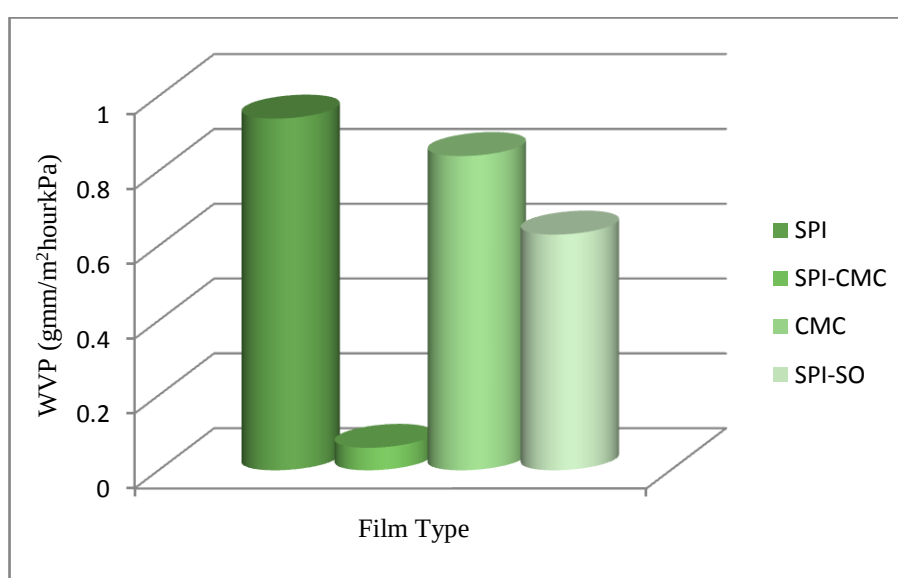


Figure 4.5 Effect of CMC and SO incorporation into SPI films on WVP.

4.2.2 Oxygen Permeability

The OP of the studied films were tabulated in Table 4.3. It was determined that the OP of WPI based film was the highest among the films studied. Nevertheless addition of SO to the WPI film produced the films that had better barrier properties.

Similar results were observed by Gennadios et al. (1993). They investigated the effect of lipid (acetylated monoacylglycerols) on OP of wheat gluten/lipid emulsion films. They suggested that acetylated monoacylglycerols lowered OP of the films by approximately 30% than that of the control films.

A significant decrease had been observed in OP with the addition of lipids Candelilla wax and Butter fat to WPI films (Kim S., 2000).

In the case of SPI films, OP was lower than that of WPI. Cho et al. (2010) found that OP of SPI film had been $0.21 \text{ (cm}^3\text{/mm)/(m}^2\text{)(hour)(atm)}$ at 25°C and 50% RH. Since there was a difference in RH in the measurements, the difference in those values was reasonable. Because of the presence of moisture in measurement condition may affect the OP.

Table 4.3 Oxygen permeability of edible films

Film Type	Thickness (mm)	OP ($\text{cm}^3\text{mm /m}^2 \text{ hour atm}$)
WPI	0.226	1.17
WS1	0.148	0.41
WS2	0.212	1.15
WS3	0.163	0.62
SPI	0.137	0.52
SPI-CMC	0.142	0.79
CMC	0.064	0.30
SPI-SO	0.142	0.50

Protein based films are good oxygen barriers because of their polar nature. Polar polymers exhibit high degrees of hydrogen bonding, which creates limited polymer chain motion, resulting in low gas permeability (McHugh and Krochta, 1994a).

Addition of SO to SPI based film was not very affective on the OP. These findings are in contrary to previous WPI results. However addition of CMC slightly increased the OP of SPI films ($p < 0.05$), probably due to new interactions that are generated in the network between soy protein and cellulose, which can disorder the structure.

4.3 Physical Properties

4.3.1 Gloss and Haze

Gloss is considered to be one of the most important visual attributes of many foods, which is difficult to measure objectively. Since the complexity of food surfaces due to the inhomogeneous distribution of gloss makes it more difficult to characterize this feature. Gloss values could be related with lightness of WPI and CMC films were higher than those of the other type of films (Figure 4.6).

Generally it was expected that incorporation of oil increased the gloss of the films but addition of SO slightly decreased the gloss and lightness of WPI and SPI films that probably because of mixing SO to WPI (upto a level) and SPI caused discontinuous and heterogeneous film structure.

The measurement of haze provides some information on the homogeneity of the surface and internal defects. There were not significant differences among the studied edible films. But also SPI-CMC blend film had the highest and worst haze value probably due to their incompatible interactions.

Table 4.4 Gloss and haze of edible films

Film Type	Gloss	Haze
WPI	78.43	13.06
WS1	64.61	13.67
WS2	56.88	23.00
WS3	77.90	17.13
SPI	40.00	-
SPI-CMC	7.88	94.73
CMC	78.60	2.12
SPI-SO	30.01	-

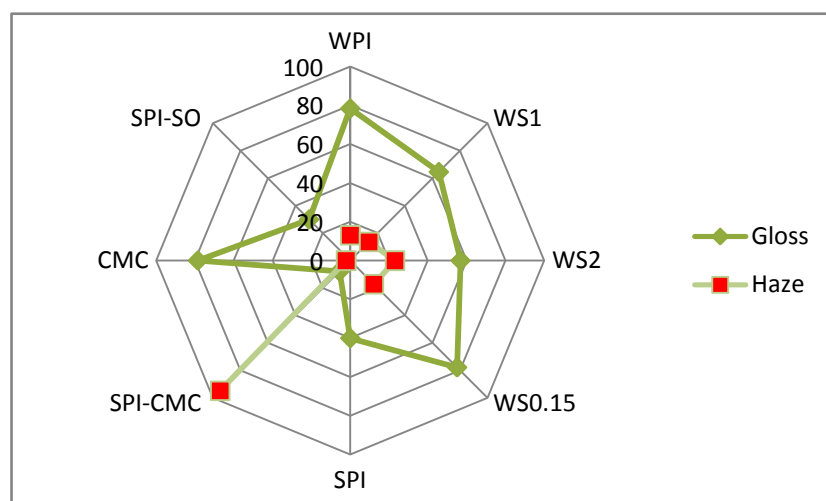


Figure 4.6 Gloss and haze results for studied films

4.3.2 Color

The color of edible film is important and should not give any additional color to food products or should not change the original color of them.

Table 4.5 Color parameters of the studied edible films

Film Type	L	A	b	YI	ΔE
WPI	80.42	-0.07	-1.04	-7.72	80.42
WS1	13.40	0.12	-1.06	-7.28	80.05
WS2	15.93	-0.27	-0.14	-4.47	77.49
WS3	27.98	0.12	1.36	6.92	65.44
SPI	11.08	1.19	1.72	-55.64	82.37
SPI-CMC	38.06	-0.73	4.31	13.75	55.45
CMC	10.60	-0.303	-0.42	-2.18	82.83
SPI-SO	17.81	-0.62	-0.21	-2.74	75.62

Therefore the variation of color in each edible film was studied. The L, a, b values of the edible films are given in Table 4.5. The lightness of WPI was so higher than the those of other type studied films. The presence of SO decreased the L-values of WPI films around 83% while it increased the SPI films. It was determined that the addition of SO did not change significantly ($p>0.05$) ΔE (total color) of the films.

4.4 Thermal Properties

The thermal properties of WPI, SPI, CMC and their composites were analyzed via DSC. The thermogram of DSC showed one thermal transition for the studied films that was a glass transition for the amorphous fraction. The glass transition temperature (T_g) and the enthalpy (ΔH) values are summarized in Table 4.6. Change in T_g and heat of fusion values of the WPI and SO incorporated WPI films were analyzed and tabulated in Table 4.6. T_g of WPI was found to be 50°C and heat of fusion was 0.023 kJ/kg. Osés et al. (2009) reported that T_g of WPI should be between 50-20°C. Johnston (1999) had also reported that T_g of films based on β – lactoglobulin and WPI were found as 45°C, he suggested that both could have the same value since the main component of WPI was β –lactoglobulin.

Table 4.6 Glass transition temperatures and heat of fusions for films as determined by DSC

Film samples	T_g (°C)	Heat of fusion (ΔH) (J/g)
WPI	50	0.023
WS1	69	0.078
WS2	71	0.073
WS3	93	0.079
SPI	68	0.015
SPI-CMC	116	0.019
CMC	51	0.045
SPI-SO	50	0.086

Water content, type and amount of plasticizers used and film forming processes applied might affect the thermal properties of the films. Incorporation of SO into WPI films caused increasing in T_g and heat of fusion. The T_g values of WS1 and WS2 were the same but WS3 was found to be the highest among them. It should be accepted that there are some difficulties in getting homogenous film and sample amount for the DSC scan was only about 10 mg, they may have contributed to the variability in the results (Johnston C., 1999).

SPI had T_g approximately 68°C and T_g of CMC was found about 51°C. Similar results were obtained with Su et al (2010). They found T_g values as 60°C and 55°C, respectively for the mentioned films. SPI-CMC blend film showed single T_g at 116°C. Generally, in a DSC scan a single T_g for blended polymers indicates good compatibility of the component polymers. Probably due to the presence of crosslinks between the blend components, T_g value of SPI-CMC blend films was higher than those of pure ones. Since the formation of the crosslinks inevitably increases T_g (Su et al., 2010).

4.5 FTIR

Figure 4.7 and 4.8 present the FTIR spectra of the studied films. The absorption peaks 3500-3200 cm^{-1} in the spectra of the films are sign of the O-H vibration; these groups can form hydrogen bonds by the electrostatic interaction between the cationic groups of CH and the anionic groups of protein (Abugoch et al., 2011). The peak at 2930 cm^{-1} , which was obtained in the FTIR spectrum of the SO added films, probably corresponded with the oleic acid (Muik et al., 2005), the main component of SO. The main absorption peaks of the lipid added films occurred at 2929-2875 cm^{-1} (C-H stretching), which are related with hydrophobic interactions. The absorption bands which were close the mentioned wavelength have been reported for other emulsified films (Andreuccetti et al., 2010; Valenzuela et al., 2013). In contrast, the FTIR spectrum of the films that not contain SO showed less absorption in this region due to the hydrophilic characteristics of compounds.

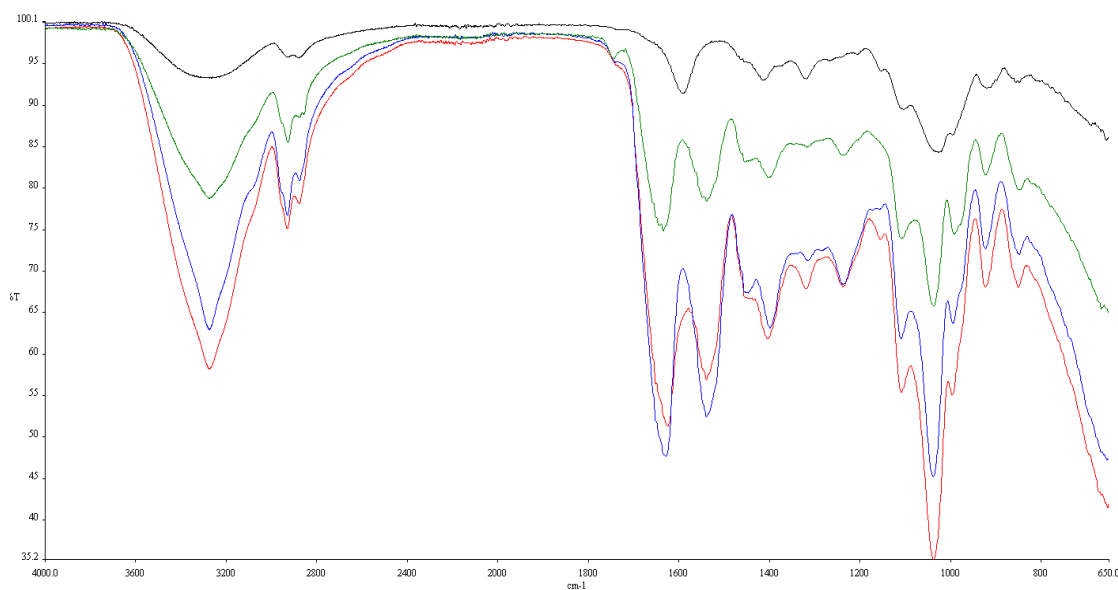


Figure 4.7 Spectra of FTIR spectroscopy of SPI (green line), SPI-SO (blue line), SPI-CMC (red line), CMC films (black line)

The peak at $1627\text{--}1591\text{ cm}^{-1}$, common to all films, were corresponded to the carbonyl group (C=O). This band was higher for SO added films than those for the other films because it was characteristic of the ester bonds from the triglyceride molecules (Velayutham et al., 2009). The other peaks that were less important and common to all films are related to C=O stretching at $1697\text{--}1630\text{ cm}^{-1}$ (amide I) and N–H bending at $1545\text{--}1531\text{ cm}^{-1}$ (amide II).

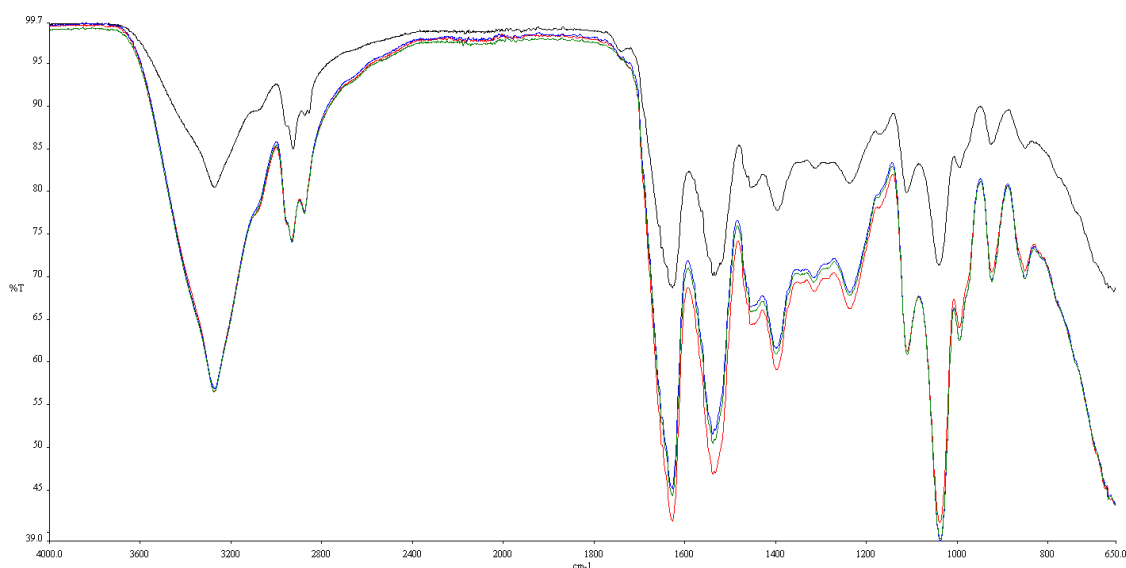


Figure 4.8 Spectra of FTIR spectroscopy of WPI (black line), WS1 (red line), WS2 (blue line), and WS3 (green line)

4.6 Moisture absorption

Figures of 4.9-4.11 show the moisture absorption of the studied pure films as a function of the %RH. It was observed that the moisture absorption of the films was proportional to the RH%. These results indicated that the decreasing the RH improve the water resistance of the film matrix. With the increase of the RH from 30% to 50%, the moisture absorption increased approximately twofold for the films and also when RH reached to 80%, absorption increased about seven-times. According to the results given in Figures of 4.9-4.11, at all %RH studied, CMC had the highest moisture absorption among the other protein based films. It is known that cellulose and its derivatives are typical hydrophilic polymers, therefore moisture uptake was increased with increasing %RH. The water absorption, permeability and diffusivity all are related to each other, so studying only permeability of water vapor was not enough to estimate exact permeability later work will be planned to study their relation in detail.

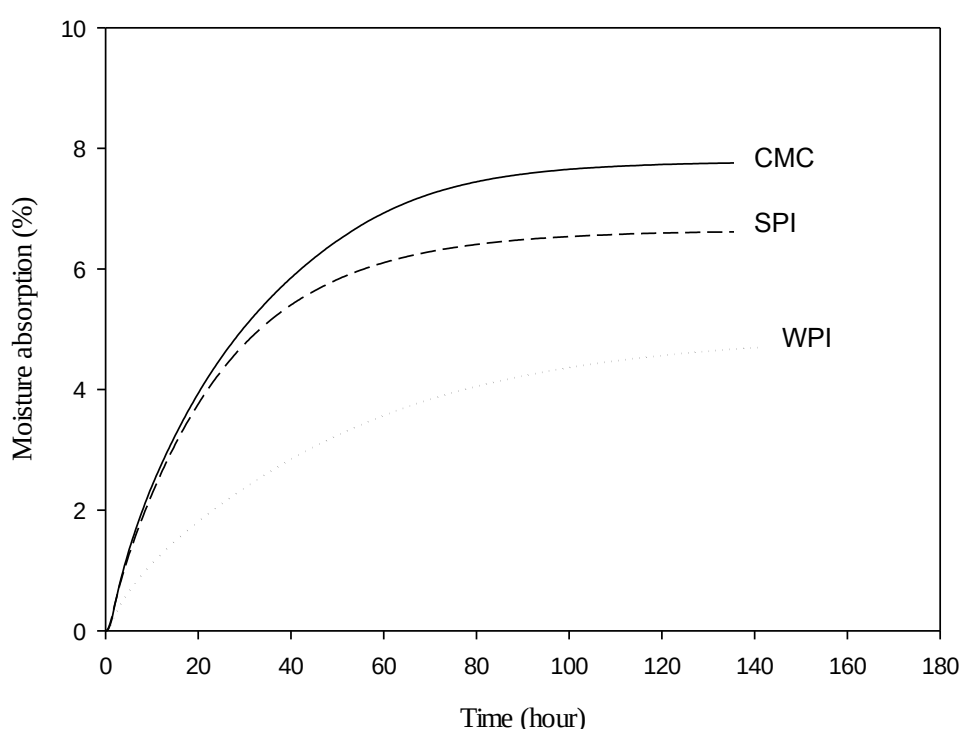


Figure 4.9 Moisture absorption of the films at 30%RH.

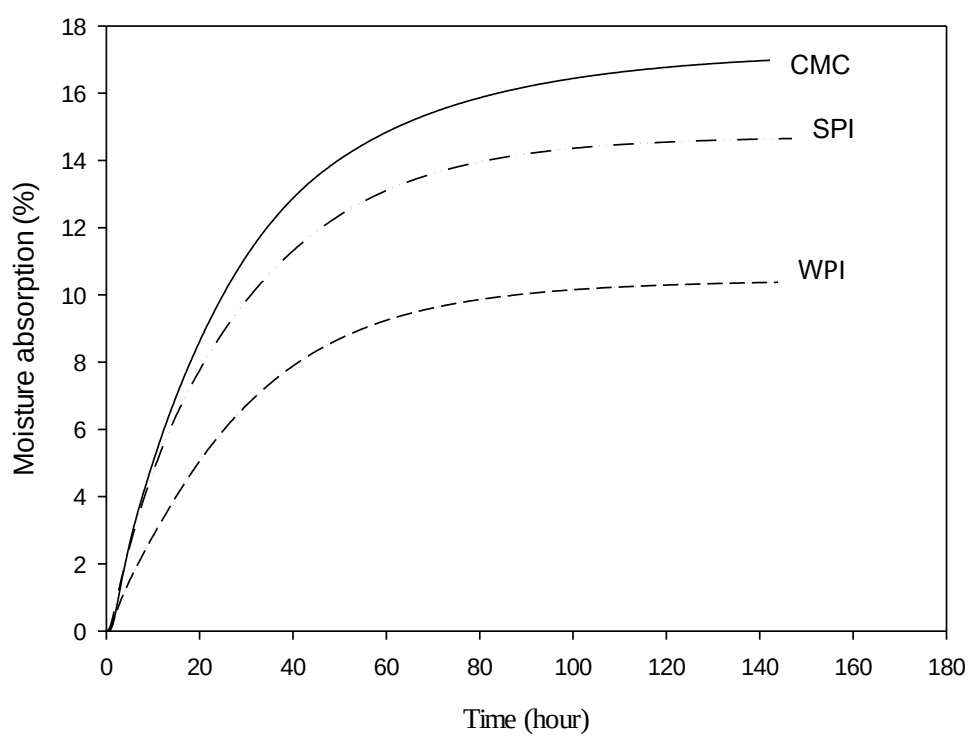


Figure 4.10 Moisture absorption of the films at 50%RH.

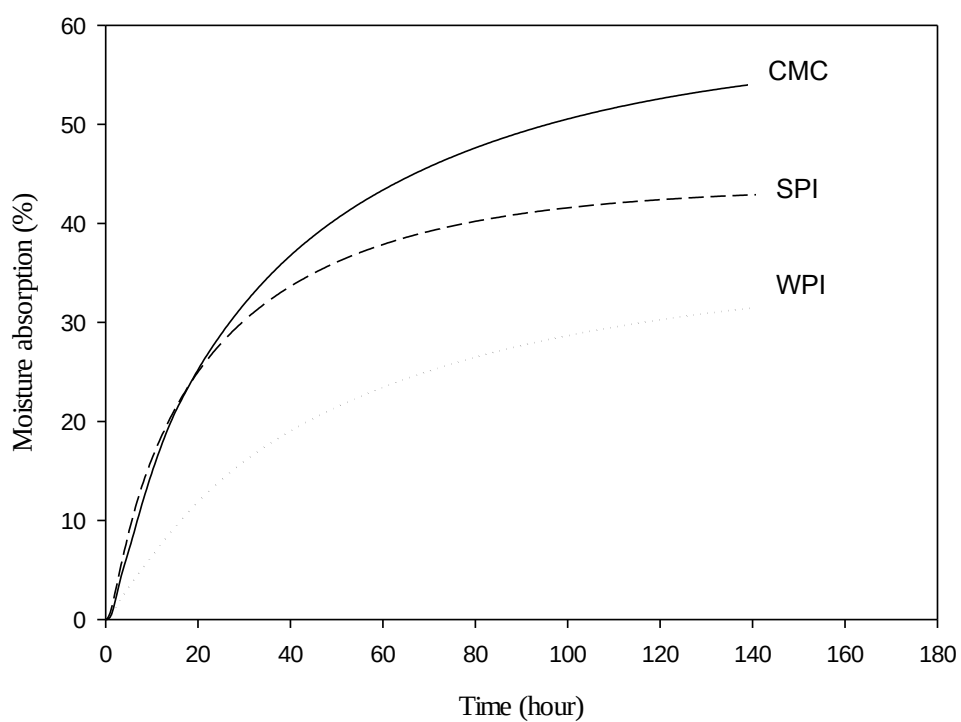


Figure 4.11 Moisture absorption of the films at 80%RH.

CONCLUSION

The study of forming and properties of composite edible films revealed the following conclusions:

1. Incorporation of SO into the WPI increased %E and decreased TS significantly ($p < 0.05$), and improved both WVP and OP. T_g of the film was increased proportionally with the SO content. The observations about the ΔE showed that addition of SO did not affect the total color of the films.
2. It was found that SPI-CMC and SPI-SO composite films had higher mechanical properties than pure SPI films. Incorporation of SO and particularly CMC improved the WVP property, but CMC increased the OP of the film also. A single and high T_g of the composite films indicated good compatibility of the components.
3. The WPI, SPI and CMC films showed similar results of %E, T_g and total color but their tensile strength values were found significantly different from each other ($p < 0.05$). WPI had the highest OP and WVP values as 1.17 $\text{cm}^3\text{mm} / \text{m}^2 \text{ hour atm}$ and 2.57 $\text{g mm} / \text{m}^2 \text{ hour kPa}$, respectively among the other mentioned films: SPI and CMC.
4. In the view of moisture absorption, CMC film had the highest moisture absorption among the others; WPI and SPI films. Also it was observed that the moisture absorption of the films was proportional to the RH%. These results indicated that the decreasing the RH improve the water resistance of the film matrix.
5. WS1 and SPI-CMC were the best two combinations with respect to all tests applied among the all studied composite films.

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APPENDICES

Table A1. Effect of sunflower oil concentration on tensile strength to WPI films

Descriptives								
TS								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WPI	2	4.17	0.29	0.21	1.50	6.83	3.96	4.38
WPI1	2	1.72	0.13	0.09	0.57	2.86	1.63	1.81
WPI2	2	1.29	0.09	0.07	0.46	2.12	1.23	1.36
WPI3	2	1.01	0.07	0.05	0.37	1.64	0.96	1.06
Total	8	2.04	1.34	0.48	0.92	3.17	0.96	4.38

ANOVA						
TS						
	Sum of Squares		df	Mean Square	F	Sig.
Between Groups	12.51		3	4.17	141.534	0.000
Within Groups	0.12		4	0.03		
Total	12.63		7			

Multiple Comparisons							
Dependent Variable: TS							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	WPI	WPI1	2.45*	0.17	0.001	1.68	3.21
		WPI2	2.870*	0.17	0.000	2.11	3.63
		WPI3	3.16*	0.17	0.000	2.39	3.92
	WPI1	WPI	-2.45*	0.17	0.001	-3.21	-1.68
		WPI2	0.42	0.17	0.250	-0.33	1.18
		WPI3	0.71	0.17	0.063	-0.05	1.47
	WPI2	WPI	-2.87*	0.17	0.000	-3.63	-2.11
		WPI1	-0.42	0.18	0.250	-1.18	0.33
		WPI3	0.28	0.17	0.508	-0.47	1.04
	WPI3	WPI	-3.16*	0.18	0.000	-3.92	-2.39
		WPI1	-0.71	0.19	0.063	-1.47	0.05
		WPI2	-0.28	0.17	0.508	-1.04	0.47

*, The mean difference is significant at the 0.05 level.

Table A2. Effect of sunflower oil concentration on elongation to WPI films

Descriptives								
Elongation								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WPI	2	10.50	0.71	0.505	4.08	16.92	10.00	11.01
WPI1	2	21.70	1.56	1.10	7.72	35.67	20.60	22.80
WPI2	2	32.37	2.29	1.63	11.72	53.02	30.75	34.00
WPI3	2	39.90	2.83	2.00	14.48	65.31	37.90	41.90
Total	8	26.12	11.96	4.23	16.12	36.11	10.00	41.90

ANOVA					
Elongation					
		Sum of Squares	df	Mean Square	Sig.
Between Groups		984.75	3	328.252	80.993
Within Groups		16.21	4	4.053	0.000
Total		1000.96	7		

Multiple Comparisons							
Dependent Variable: Elongation							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	WPI	WPI1	-11.19*	2.01	0.024	-20.14	-2.24
		WPI2	-21.87*	2.01	0.002	-30.82	-12.91
		WPI3	-29.39*	2.02	0.001	-38.34	-20.44
	WPI1	WPI	11.19*	2.02	0.024	2.24	20.14
		WPI2	-10.67*	2.02	0.028	-19.62	-1.72
		WPI3	-18.20*	2.01	0.004	-27.15	-9.24
	WPI2	WPI	21.87*	2.01	0.002	12.91	30.82
		WPI1	10.67*	2.01	0.028	1.72	19.62
		WPI3	-7.52	2.01	0.086	-16.47	1.42
	WPI3	WPI	29.39*	2.02	0.001	20.44	38.34
		WPI1	18.20*	2.01	0.004	9.24	27.15
		WPI2	7.5	2.01	0.086	-1.42	16.47

*. The mean difference is significant at the 0.05 level.

Table A3. Effect of sunflower oil and carboxymethyl methyl cellulose including on tensile strength to SPI films

Descriptives								
TS								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
SPI	2	0.32	0.03	0.02	0.06	0.57	0.30	0.34
SPI-SO	2	0.62	0.05	0.04	0.18	1.06	0.59	0.66
SPI-CMC	2	0.77	0.05	0.04	0.26	1.27	0.73	0.81
Total	6	0.57	0.21	0.08	0.35	0.79	0.30	0.81

ANOVA						
TS						
		Sum of Squares	df	Mean Square	F	Sig.
Between Groups		0.21	2	0.106	49.078	0.005
Within Groups		0.01	3	0.002		
Total		0.21	5			

Multiple Comparisons							
Dependent Variable: TS							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	SPI	SPI-SO	-0.30 [*]	0.047	0.017	-0.50	-0.10
		SPI-CMC	-0.45 [*]	0.047	0.005	-0.65	-0.24
	SPI-SO	SPI	0.30 [*]	0.047	0.017	0.10	0.50
		SPI-CMC	-0.14	0.047	0.114	-0.34	0.05
	SPI-CMC	SPI	0.45 [*]	0.047	0.005	0.24	0.65
		SPI-SO	0.1	0.047	0.114	-0.05	0.35

*. The mean difference is significant at the 0.05 level.

Table A4. Effect of sunflower oil and carboxymethyl methyl cellulose including on elongation to SPI films

Descriptives							
Elongation							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min Max
					Lower Bound	Upper Bound	
SPI	2	28.50	2.12	1.50	9.44	47.55	27.00 30.00
SPI-SO	2	66.80	4.52	3.20	26.14	107.45	63.60 70.00
SPI-CMC	2	94.40	6.50	4.60	35.95	152.84	89.80 99.00
Total	6	63.23	29.82	12.17	31.93	94.53	27.00 99.00

ANOVA					
Elongation					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4380.97	2	2190.48	97.644	0.002
Within Groups	67.30	3	22.43		
Total	4448.27	5			

Multiple Comparisons							
Dependent Variable: Elongation							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	SPI	SPI-SO	-38.30 [*]	4.73	0.009	-59.00	-17.59
		SPI-CMC	-65.90 [*]	4.74	0.002	-86.60	-45.19
	SPI-SO	SPI	38.30 [*]	4.73	0.009	17.59	59.00
		SPI-CMC	-27.60 [*]	4.75	0.023	-48.30	-6.89
	SPI-CMC	SPI	65.90 [*]	4.73	0.002	45.19	86.60
		SPI-SO	27.60 [*]	4.73	0.023	6.89	48.30

*. The mean difference is significant at the 0.05 level.

Table A5. Effect of sunflower oil concentration on water vapor permeability to WPI films

Descriptives								
WVP								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WPI	3	2.56	0.12	0.07	2.25	2.87	2.44	2.69
WPI1	3	0.61	0.03	0.02	0.54	0.69	0.59	0.65
WPI2	3	1.66	0.08	0.04	1.45	1.86	1.58	1.74
WPI3	3	1.42	0.07	0.04	1.24	1.59	1.35	1.49
Total	12	1.56	0.73	0.21	1.10	2.02	0.59	2.69

ANOVA						
WVP						
		Sum of Squares	df	Mean Square	F	Sig.
Between Groups		5.78	3	1.927	270.521	0.000
Within Groups		0.05	8	0.007		
Total		5.83	11			

Multiple Comparisons

Dependent Variable: WVP

	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	WPI	WPI1	1.94*	0.061	0.00	1.70	2.18
		WPI2	0.90*	0.061	0.00	0.66	1.14
		WPI3	1.14*	0.061	0.00	0.90	1.387
	WPI1	WPI	-1.94*	0.060	0.00	-2.187	-1.70
		WPI2	-1.04*	0.060	0.00	-1.28	-0.79
		WPI3	-0.80*	0.061	0.00	-1.04	-0.55
	WPI2	WPI	-0.90*	0.061	0.00	-1.14	-0.66
		WPI1	1.04*	0.060	0.00	0.79	1.28
		WPI3	0.24	0.061	0.05	-0.00	0.48
	WPI3	WPI	-1.14*	0.060	0.00	-1.38	-0.90
		WPI1	0.80*	0.060	0.00	0.55	1.04
		WPI2	-0.24000	0.060	0.05	-0.48	0.00

*. The mean difference is significant at the 0.05 level.

Table A6. Effect of sunflower and carboxymethyl methyl cellulose including on water vapor permeability to SPI films

Descriptives								
WVP								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
SPI	3	0.94	0.047	0.027	0.82	1.05	0.89	0.99
SPI-SO	3	0.63	0.031	0.018	0.55	0.70	0.60	0.66
SPI-CMC	3	0.06	0.003	0.001	0.05	0.06	0.06	0.06
Total	9	0.54	0.387	0.129	0.24	0.84	0.06	0.99

ANOVA						
WVP						
		Sum of Squares	df	Mean Square	F	Sig.
Between Groups		1.19	2	0.598	558.555	0.000
Within Groups		0.01	6	0.001		
Total		1.20	8			

Multiple Comparisons							
Dependent Variable: WVP							
	(I)	(J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
	SO&CMC	SO&CMC				Lower Bound	Upper Bound
Scheffe	SPI	SPI-SO	0.31 [*]	0.03	0.00	0.22	0.39
		SPI-CMC	0.88 [*]	0.02	0.00	0.79	0.96
	SPI-SO	SPI	-0.31 [*]	0.02	0.00	-0.39	-0.22
		SPI-CMC	0.57 [*]	0.03	0.00	0.48	0.65
	SPI-CMC	SPI	-0.88 [*]	0.02	0.00	-0.96	-0.79
		SPI-SO	-0.57 [*]	0.02	0.00	-0.65	-0.48

*. The mean difference is significant at the 0.05 level.

Table A7. Effect of sunflower oil concentration on oxygen permeability to WPI films

ANOVA					
OP					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.88	3	0.295	256.63	0.000
Within Groups	0.01	4	0.001		
Total	0.89	7			

Descriptives							
OP							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min Max
					Lower Bound	Upper Bound	
WPI	2	1.14	0.042	0.030	0.75	1.52	1.11 1.17
WPI1	2	0.40	0.014	0.010	0.27	0.52	0.39 0.41
WPI2	2	1.16	0.042	0.030	0.77	1.54	1.13 1.19
WPI3	2	0.60	0.028	0.020	0.34	0.85	0.58 0.62
Total	8	0.82	0.356	0.126	0.52	1.12	0.39 1.19

Multiple Comparisons							
Dependent Variable: OP							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	WPI	WPI1	0.74*	0.034	0.000	0.58	0.89
		WPI2	-0.02	0.033	0.946	-0.17	0.13
		WPI3	0.54*	0.033	0.000	0.38	0.69
	WPI1	WPI	-0.74*	0.033	0.000	-0.89	-0.58
		WPI2	-0.76*	0.034	0.000	-0.91	-0.60
		WPI3	-0.20*	0.034	0.019	-0.35	-0.04
	WPI2	WPI	0.02	0.033	0.946	-0.13	0.17
		WPI1	0.76*	0.033	0.000	0.60	0.91
		WPI3	0.56*	0.034	0.000	0.40	0.71
	WPI3	WPI	-0.54*	0.033	0.000	-0.69	-0.38
		WPI1	0.20*	0.033	0.019	0.04	0.35
		WPI2	-0.56*	0.033	0.000	-0.71	-0.40

*. The mean difference is significant at the 0.05 level.

Table A8. Effect of sunflower oil and carboxymethyl methyl cellulose addition on oxygen permeability to SPI films

Descriptives							
OP							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min Max
					Lower Bound	Upper Bound	
SPI	2	0.53	0.014	0.01	0.40	0.65	0.52 0.54
SPI-SO	2	0.51	0.021	0.02	0.32	0.70	0.50 0.53
SPI-CMC	2	0.81	0.028	0.02	0.55	1.06	0.79 0.83
Total	6	0.61	0.149	0.06	0.46	0.77	0.50 0.83

ANOVA						
OP						
	Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	0.110	2	0.055	114.241	0.001	
Within Groups	0.001	3	0.000			
Total	0.112	5				

Multiple Comparisons							
Dependent Variable: OP							
	(I)	(J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
	SO&CMC	SO&CMC				Lower Bound	Upper Bound
Scheffe	SPI	SPI-SO	0.01	0.02	0.80	-0.08	0.11
		SPI-CMC	-0.28*	0.02	0.01	-0.37	-0.18
	SPI-SO	SPI	-0.01	0.02	0.81	-0.11	0.08
		SPI-CMC	-0.29*	0.02	0.01	-0.39	-0.19
	SPI-CMC	SPI	0.28*	0.02	0.00	0.18	0.37
		SPI-SO	0.29*	0.02	0.00	0.19	0.39

*, The mean difference is significant at the 0.05 level.

Table A9. Effect of sunflower oil addition on glass transition to WPI films

Descriptives								
Tg								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WPI	2	50.00	4.24	3.00	11.88	88.11	47.00	53.00
WPI1	2	69.00	5.65	4.00	18.17	119.82	65.00	73.00
WPI2	2	71.00	5.65	4.00	20.17	121.82	67.00	75.00
WPI3	2	93.00	7.07	5.00	29.46	156.53	88.00	98.00
Total	8	70.75	16.85	5.96	56.65	84.84	47.00	98.00

ANOVA						
Tg						
	Sum of Squares		df	Mean Square	F	Sig.
Between Groups	1857.50		3	619.16	18.763	0.008
Within Groups	132.00		4	33.00		
Total	1989.50		7			

Multiple Comparisons							
Dependent Variable: Tg							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	WPI	WPI1	-19.00	5.74	0.122	-44.54	6.54
		WPI2	-21.00	5.74	0.091	-46.54	4.54
		WPI3	-43.00*	5.74	0.008	-68.54	-17.45
	WPI1	WPI	19.00	5.74	0.122	-6.54	44.54
		WPI2	-2.00	5.74	0.988	-27.54	23.54
		WPI3	-24.00	5.74	0.061	-49.54	1.54
	WPI2	WPI	21.00	5.74	0.091	-4.54	46.54
		WPI1	2.00	5.74	0.988	-23.54	27.54
		WPI3	-22.00	5.74	0.080	-47.54	3.54
	WPI3	WPI	43.00*	5.74	0.008	17.45	68.54
		WPI1	24.00	5.74	0.061	-1.54	49.54
		WPI2	22.00	5.74	0.080	-3.54	47.54

*. The mean difference is significant at the 0.05 level.

Table A10. Effect of sunflower oil addition on heat of fusion to WPI films

Descriptives								
dh								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
WPI	2	0.022	0.003	0.002	-0.0034	0.0474	0.02	0.02
WPI1	2	0.075	0.007	0.005	0.0115	0.1385	0.07	0.08
WPI2	2	0.070	0.000	0.000	0.0700	0.0700	0.07	0.07
WPI3	2	0.075	0.007	0.005	0.0115	0.1385	0.07	0.08
Total	8	0.060	0.024	0.008	0.0403	0.0807	0.02	0.08

ANOVA						
dh						
		Sum of Squares	df	Mean Square	F	Sig.
Between Groups		0.004	3	0.001	49.21	0.001
Within Groups		0.000	4	0.000		
Total		0.004	7			

Multiple Comparisons							
Dependent Variable: dh							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Scheffe	WPI	WPI1	-0.05 [*]	0.005	0.003	-0.07	-0.02
		WPI2	-0.05 [*]	0.005	0.004	-0.07	-0.02
		WPI3	-0.05 [*]	0.005	0.003	-0.07	-0.02
	WPI1	WPI	0.05 [*]	0.005	0.003	0.02	0.07
		WPI2	0.01	0.005	0.819	-0.01	0.02
		WPI3	0.00	0.005	10.000	-0.02	0.02
	WPI2	WPI	0.05 [*]	0.005	0.004	0.02	0.07
		WPI1	-0.01	0.005	0.819	-0.02	0.01
		WPI3	-0.01	0.005	0.819	-0.02	0.01
	WPI3	WPI	0.05 [*]	0.005	0.003	0.02	0.07
		WPI1	0.00	0.005	10.000	-0.02	0.02
		WPI2	0.01	0.005	0.819	-0.01	0.02

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

Table A11. Effect of sunflower oil and carboxymethyl methyl cellulose addition on glass transition to SPI films

Tg	Descriptives							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
SPI	2	67.50	4.94	3.50	23.02	111.97	64.00	71.00
SPI-SO	2	50.00	4.24	3.00	11.88	88.11	47.00	53.00
SPI-CMC	2	115.50	7.77	5.50	45.61	185.38	110.0	121.0
Total	6	77.66	30.67	12.52	45.48	109.85	47.00	121.00

ANOVA						
Tg						
		Sum of Squares	df	Mean Square	F	Sig.
Between Groups		4600.33	2	2300.16	66.99	0.003
Within Groups		103.00	3	34.33		
Total		4703.33	5			

Multiple Comparisons							
Dependent Variable: Tg							
	(I)	(J)	Mean	Std.	Sig.	95% Confidence Interval	
	SOandCMC	SOandCMC	Difference (I-J)	Error		Lower Bound	Upper Bound
Scheffe	SPI	SPI-SO	17.50	5.85	0.126	-8.11	43.11
		SPI-CMC	-48.00*	5.85	0.009	-73.61	-22.38
	SPI-SO	SPI	-17.50	5.85	0.126	-43.11	8.11
		SPI-CMC	-65.50*	5.85	0.004	-91.11	-39.88
	SPI-CMC	SPI	48.00*	5.85	0.009	22.38	73.61
		SPI-SO	65.50*	5.85	0.004	39.88	91.11

*. The mean difference is significant at the 0.05 level.

Table A12. Effect of sunflower oil and carboxymethyl methyl cellulose addition on heat of fusion on SPI films

Descriptives								
dh								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
SPI	2	0.01	0.001	0.001	0.002	0.027	0.01	0.02
SPI-SO	2	0.08	0.006	0.004	0.028	0.142	0.08	0.09
SPI-CMC	2	0.01	0.001	0.000	0.012	0.024	0.02	0.02
Total	6	0.03	0.035	0.014	0.002	0.077	0.01	0.09

ANOVA						
dh						
		Sum of Squares	df	Mean Square	F	Sig.
Between Groups		0.01	2	0.003	220.267	0.001
Within Groups		0.01	3	0.000		
Total		0.01	5			

Multiple Comparisons							
Dependent Variable: dh							
	(I)	(J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
	SO&CMC	SO&CMC				Lower Bound	Upper Bound
Scheffe	SPI	SPI-SO	-0.07*	0.01	0.001	-0.08	-0.05
		SPI-CMC	-0.01	0.01	0.687	-0.02	0.01
	SPI-SO	SPI	0.07*	0.01	0.001	0.05	0.08
		SPI-CMC	0.06*	0.01	0.001	0.05	0.08
	SPI-CMC	SPI	0.01	0.01	0.687	-0.01	0.02
		SPI-SO	-0.06*	0.01	0.001	-0.08	-0.05

*, The mean difference is significant at the 0.05 level.

Table A13. Effect of sunflower oil addition on total color to WPI films

Descriptives							
color							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min Max
					Lower Bound	Upper Bound	
WPI	2	80.00	5.65685	4.00	29.17	130.82	76.00 84.00
WS1	2	78.00	2.82843	2.00	52.58	103.41	76.00 80.00
WS2	2	77.00	5.65685	4.00	26.17	127.82	73.00 81.00
WS3	2	65.00	4.24264	3.00	26.88	103.11	62.00 68.00
Total	8	75.00	7.23089	2.55	68.95	81.04	62.00 84.00

ANOVA						
color						
	Sum of Squares		df	Mean Square	F	Sig.
Between Groups	276.00		3	92.00	4.08	0.104
Within Groups	90.00		4	22.50		
Total	366.00		7			

Multiple Comparisons							
Dependent Variable: color							
	(I) SO	(J) SO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Scheffe	WPI	WS1	2.00	4.74	0.97	-19.09	23.09
		WS2	3.00	4.74	0.93	-18.09	24.09
		WS3	15.00	4.74	0.13	-6.09	36.09
	WS1	WPI	-2.00	4.74	0.97	-23.09	19.09
		WPI2	1.00	4.74	0.99	-20.09	22.09
		WPI3	13.00	4.74	0.19	-8.09	34.09
	WS2	WPI	-3.00	4.74	0.93	-24.09	18.09
		WPI1	-1.00	4.74	0.99	-22.09	20.09
		WPI3	12.00	4.74	0.23	-9.09	33.09
	WS3	WPI	-15.00	4.74	0.13	-36.09	6.09
		WPI1	-13.00	4.74	0.19	-34.09	8.09
		WPI2	-12.00	4.74	0.23	-33.09	9.09

Table A14. Effect of sunflower oil and carboxymethyl methyl cellulose addition on total color on SPI films

Descriptives								
color								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
SPI	2	82.00	5.65	4.00	31.17	132.8	78.00	86.00
SPI-SO	2	75.00	5.65	4.00	24.17	125.82	71.00	79.00
SPI-CMC	2	55.50	3.53	2.50	23.73	87.26	53.00	58.00
Total	6	70.83	12.89	5.26	57.30	84.36	53.00	86.00

ANOVA						
color						
		Sum of Squares	df	Mean Square	F	Sig.
Between Groups		754.33	2	377.16	14.79	0.028
Within Groups		76.50	3	25.50		
Total		830.83	5			

Multiple Comparisons							
Dependent Variable: color							
	(I)	(J)	Mean	Std.	Sig.	95% Confidence Interval	
	SO&CMC	SO&CMC	Difference (I-J)	Error		Lower Bound	Upper Bound
Scheffe	SPI	SPI-SO	7.00	5.05	0.47	-15.07	29.07
		SPI-CMC	26.50*	5.05	0.03	4.42	48.57
	SPI-SO	SPI	-7.00	5.05	0.47	-29.07	15.07
		SPI-CMC	19.50	5.05	0.06	-2.57	41.57
	SPI-CMC	SPI	-26.50*	5.05	0.03	-48.57	-4.42
		SPI-SO	-19.50	5.05	0.06	-41.57	2.57

*. The mean difference is significant at the 0.05 level.