ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

CHARACTERIZATION OF CARRAGEENAN FILMS INCORPORATED WITH FLAXSEED OIL

M.Sc. THESIS

Nil Işık YEŞİL

Department of Chemistry

Chemistry Programme

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Thesis Advisor: Prof. Dr. F. Bedia ERİM BERKER

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

KETEN TOHUMU YAĞI İÇEREN KARRAGENAN FİLMLERİN KARAKTERİZASYONU

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TEMMUZ 2020

Nil Işık YEŞİL, a M.Sc. student of ITU Graduate School of Science Engineering and Technology student ID 509171236, successfully defended the thesis/dissertation entitled "CHARACTERIZATION OF CARRAGEENAN FILMS INCORPORATED WITH FLAXSEED OIL", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : 15 June 2020
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To my family and loved ones,

FOREWORD

This study was performed under supervision of Prof. Dr. F. Bedia ERİM BERKER to be presented as a master of science thesis in chemistry department of Istanbul Technical University. The study was also supported by Hyggefoods Gıda San. ve Tic. A.Ş..

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ABBREVIATIONS

ALA	**:** α-Linolenic acid
m_0	**:** Weight of films at time zero
m_t	**:** Weight of films at time t
SR	**:** Swelling ratio
WVP	**:** Water vapor permeability
WVTR	**:** Water vapor transmission rate
Op	**:** Opacity

SYMBOLS

κ	: Kappa
ι	: Iota
λ	: Lambda
T1	: Carrageenan film with 2.5% (v/v) Tween 80
T2	: Carrageenan film with 3.75% (v/v) Tween 80
T3	: Carrageenan film with 5% (v/v) Tween 80
T4	: Carrageenan film with 7.5% (v/v) Tween 80
YC	: Control carrageenan film without any flaxseed oil and KCl
Y0	: Carrageenan film without flaxseed oil
Y1	: Carrageenan film with 0.5% (v/v) flaxseed oil
Y2	: Carrageenan film with 1% (v/v) flaxseed oil
Y3	: Carrageenan film with 2% (v/v) flaxseed oil
Y4	: Carrageenan film with 3% (v/v) flaxseed oil

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CHARACTERIZATION OF CARRAGEENAN FILMS INCORPORATED WITH FLAXSEED OIL

SUMMARY

Food packaging industry is one of the major industry that has been developing each year with novel technology. Food packaging plays an important role in terms of purchasing by consumers and keeping food stable in their shelf life period. Therefore, packaging should be attractive by the consumers and also provide stable shelf life with high barrier properties. There are different types of materials used in food packaging such as glass, cardboards, metals or plastics.

In food packaging industry, plastics are major materials that have been used for centuries. Plastics with various features has been developed for the purpose of prolonging the shelf life, protecting specific food material, and/or meet consumer demands. For this reason, different types of plastics are being used. Plastics are advantageous in terms of lower cost, ease of use, diversity, and adaptability.

Biopolymers are one of the plastic type that are eco-friendly owing to its biodegredablity features. Biopolymers can be based on natural resources such as carbohydrates or proteins. Cellulose, chitosan, and carrageenan are carbohydrate based bioploymers while, gelatin, gluten, and whey are protein based biopolymers. In spite of natural based biopolymers, bioploymers can be produced synthetically such as polylactic acid, polyvinyl alcohol, and polyhydroxy-alkanoates. Natural sourced biopolymers have advantages as they are renewable, good for the environment, easier recycling, non-toxicity, and less energy to produce. Biopolymers based on natural resources are been studied for many application in food packaging industry.

Natural based biopolymers are being used for developing coatings and edible films as food packaging materials. Coatings and edible films are applied food stuff by immersion, spraying, coating, or wrapping with previously formed shaped. In general, coatings are liquid forms and films are solid forms. However, there are studies that film solution is both applied as coating and wraping as solid laminates. Researchers focus on edible films with higher performance of packaging features. By this reason, studies with edible films enriched with different materials are one of the popular area in packaging sector. Additional materials such as colorants, antioxidants, oils, plant extracts and nanoparticles are varies depending on the target food stuff and film property. To illustrate, films with lower oxygen permeability is preferred for coating fresh-cut fruits.

Carrageenan is one of the carbohydrate based biopolymers that are used in the food industry with different purposes. They are produced from red seaweeds and can be used as thickener, emulsifier and stabilizer in the food industry. In the purpose of usage, different types of carrageenans can be used. Carrageenan films with plant extracts, plant oils, and nanoparticles are investigated nowadays for novel edible film applications. With additional materials, carrageenan films' physical and mechanical properties are improved, thus its protective characterics are enhanced.

Flaxseed oil, also expressed as linseed oil, is plant-based oil originated from flaxseed. Flaxseeds contain approximately 40% fat in its compositions. More than half of its fat content is composed of polyunsaturated fatty acids. Further, flaxseed oil is stated as highest polyunsaturated fatty acid containg plant oil in common. Flaxseed oil usage in edible film developments is limited as in today. However, there are potential features of flaxseed oil that can be utilized for food packaging industry.

In this study, carrageenan films were incorporated with flaxseed oil and characterization of films were performed. This was the first report having the scope of investigation of physical and barrier properties of carrageenan films with flaxseed oil. Moreover, the effect of emulsifier, Tween 80, was also examined in this study. Films having a higher thickness and moisture content were obtained while Tween 80 amount was increased. As well as, swelling and opacity of films were reduced with higher emulsifier concentration. Then, flaxseed oil' concentration effect on carrageenan film was investigated. Increment of flaxseed oil concentration provided thicker and more opaque films which have lower moisture content, less swelling features, and less water vapor permeable. It has been seen that incorporation of carrageenan films with plant oil improved its film characteristics. Thus, carrageenan films containing flaxseed oil can be suggested for application of edible films for food stuff.

KETEN TOHUMU YAĞI İÇEREN KARRAGENAN FİLMLERİN KARAKTERİZASYONU

ÖZET

Gıda paketleme endüstrisi, her yıl yeni teknolojiler ile gelişen en büyük endüstrilerden biridir. Gıda ambalajları, tüketicilerin satın alma eğilimlerini ve gıdaların raf ömrü boyunca stabil olarak kalmasında önemli rol oynamaktadır. Bu nedenle, ambalajlar tüketiciler tarafından cazip olmalı ve yüksek bariyer özellikleri ile stabil raf ömrü sağlamalıdır. Gıda ambalajında; cam, karton, metal veya plastik gibi farklı malzeme türleri kullanılmaktadır.

Plastikler, gıda ambalajlama endüstrisinde yüzyıllardır kullanılan önemli malzemelerdir. Raf ömrünü uzatmak, belirli gıda malzemelerini korumak ve tüketici taleplerini karşılamak amacıyla çeşitli özelliklere sahip plastikler geliştirilmiştir. Bu nedenle farklı plastik türleri kullanılmaktadır. Plastikler düşük üretim maliyetleri, kullanım kolaylığı, çeşitliliği ve uyarlanabilirliği açısından avantajlıdır.

Gıda endüstrisinin gelişimiyle birlikte ambalajlı ürünlerin tüketimi artmıştır. Tüketim kaynaklı atık problemlerine çözüm olarak kullanılabilecek ve yüksek miktarda ithal edilen plastik hammaddeleri ikame edebilecek biyopolimer esaslı ambalaj materyallerinin geliştirilmesi önem kazanmıştır. Biyopolimerler, biyobozunur özellikleri sayesinde çevre dostu plastik çeşitlerindendir. Biyopolimerler karbonhidratlar veya proteinler gibi doğal kaynaklardan elde edilebilirler. Selüloz, kitosan ve karagenan, karbonhidrat bazlı biyoploymerler iken jelatin, gluten ve peynir altı suyu proteini protein bazlı biyopolimerlerdir. Doğal kaynaklı biyopolimere karşın biyopolimerler, polilaktik asit, polivinil alkol ve polihidroksi-alkanoatlar gibi sentetik olarak da üretilebilir. Yenilenebilir, çevreyle uyumlu ve daha kolay geri dönüştürülebilir olmalarının yanında ayrıca toksik olmamaları ve daha az enerji ile üretilebilinir olmaları doğal kaynaklı biyopolimerlerin avantajları arasında yer almaktadır. Doğal kaynaklara dayanan biyopolimerler, gıda ambalaj endüstrisinde birçok uygulama için incelenmiştir.

Doğal bazlı biyopolimerler, gıda ambalaj malzemeleri olarak kaplamalar ve yenilebilir filmler geliştirmek için kullanılmaktadır. Kaplamalar ve yenilebilir filmler, daldırma, püskürtme, kaplama veya daha önce oluşturulan şekil ile kaplama metotları ile gıda maddelerine uygulanabilir. Genel olarak kaplamalar sıvı; yenilebilir filmler ise katı formlardır. Bununla birlikte, film çözeltisinin hem katı lamine olarak sarılması hem de kaplama olarak uygulandığı literatür çalışmaları bulunmaktadır. Araştırmacılar, daha iyi paketleme özelliklerine sahip yenilebilir filmlere odaklanmaktadırlar.

Bu nedenle, farklı malzemelerle zenginleştirilmiş yenilebilir filmlerle yapılan çalışmalar, paketleme sektörü içinde popüler alanlardan biri haline gelmiştir. Bazı biyopolimerlerin mekanik ve bariyer özellikleri geleneksel olarak kullanılan plastiklere göre yetersiz kalabilmektedir. Bu sebeple biyopolimerlerin ticari kullanımı sınırlanmaktadır. Ancak, biyopolimere çeşitli ajanların eklenmesi ile bu özellikler uyarlanabilmektedir. Hedeflenen gıda maddelerine ve film özelliklerine

bağlı olarak eklenecek malzemeler, örneğin renklendiriciler, antioksidanlar, bitkisel yağlar, bitki ekstraktları ve nanopartiküller değişkenlik gösterebilmektedir. Örnek olarak, taze kesilmiş meyvelerin kaplanması için düşük oksijen geçirgenliğine sahip filmler tercih edilmektedir. Bu amaç doğrultusunda film formülasyonuna oksijen geçirgenliğini düşürücü ajanlar eklenmektedir. Bir diğer örnek ise antimikrobiyal ajan ilavesi ile biyopolimere bu özellik kazandırılarak gıdaların raf ömrünce bozulması önlenebilir.

Karragenan, gıda endüstrisinde çeşitli amaçlarla kullanılan karbonhidrat bazlı biyopolimerler çeşitlerinden biridir. Kırmızı deniz yosunlarından üretilirler ve gıdalarda kıvam arttırıcı, emülgatör ve stabilizatör olarak kullanılmaktadırlar. Yapısı sülfatlanmış galaktoz ünitelerinden oluşan polisakkaritlerden oluşmaktadır. Temel olarak üç çeşitte sınıflandırırlar; kappa, iota ve lambda. Karragenan çeşitlerinden hedeflenen amaca yönelik olarak kullanımı yapılmaktadır. Örneğin, karragenan çeşitleri arasında yüksek jelleşme kapasitesi ve stabil film dağılımı nedeniyle κ-karragenan, genellikle film formülasyonları geliştirme çalışmalarında sıklıkla kullanılmaktadır.

Karragenanın film oluşturma ve jelleşme özelliklerinin yüksek olduğu rapor edilmiştir. Sıcak suda çözünerek kuvvetli jel oluşumu sağlanmaktadır. Karragenanın film oluşturma mekanizması incelendiğinde uygun sıcaklıkta jelleşmenin ardınından kurutma işlemi uygulanmaktadır. Çözücünün buharlaştırılmasıyla karragenan yapısındaki polisakkaritlerin çift kat sarmal yapı oluşturarak film oluşumu gerçekleştirilir. Ayrıca film formülasyonuna ilave edilecek potasyum iyonları ile jelleşmesi desteklenebilmektedir. Bu durumda jelleşme ve erime sıcaklıklarının yükseldiği rapor edilmiştir. Bitki özleri, bitkisel yağlar ve nanopartiküller içeren karragenan filmleri günümüzde yeni yenilebilir film uygulamaları için araştırılmaktadır. İlave malzemelerle, karragenan filmlerinin fiziksel ve mekanik özellikleri iyileştirilir, böylece bariyer özellikleri de arttırılabilir. Mekanik ve bariyer özelliklerinin yanında film yapısının homojen dağılımı için plastikleştirici ve emülsifiye edici ajanların kullanımı da sıklıkla görülmektedir.

Keten tohumu yağı aynı zamanda farklı endüstriyel kullanımlarında bezir yağı olarak da adlandırılmaktadır. Keten tohumu yağı, keten tohumundan elde edilen bitki bazlı yağlardandır. Keten tohumu, kompozisyonunda yaklaşık %40 yağ içerir. Bu yağ içeriğinin yarısından fazlası çoklu doymamış yağ asitlerinden oluşur. Çoklu doymamış yağ asitleri bileşiminde yaklaşık %60'ı alfa-linolenik asit içermektedir. Ayrıca, keten tohumu yağı, genel olarak en yüksek çoklu doymamış yağ asidi içeren bitki yağı olarak da ifade edilir. Günümüzde yenilebilir film geliştirme çalışmalarında keten tohumu yağı kullanımı sınırlıdır. Ancak, keten tohumu yağı eklenerek yapılan çalışmalarda termal ve bariyer özelliklerin geliştirildiğini rapor edilen çalışmalar bulunmaktadır. Bu sebeple keten tohumu yağı ile katkılandırılan kaplamaların ve yenilebilir film malzemelerinin gıda ambalaj endüstrisinde kullanılabilme potansiyeli mevcuttur.

Bu çalışmada karragenan filmler, keten tohumu yağı ile zenginleştirildi ve filmlerin karakterizasyonu yapıldı. Yapılan çalışma keten tohumu yağı ile karagenan filmlerinin fiziksel ve bariyer özelliklerinin araştırılmıştır. Bu çalışmada karragenan filmler dökme metoduyla hazırlanmıştır. Yenilebilir film malzemesi olarak geliştirilmesi hedeflenmiştir. Filmin hazırlanmasında gliserol, plastikleştirici ajan olarak kullanmıştır. Film çözeltisine eklenen Tween 80 emülgatör ajan olarak kullanılmıştır. İlave olarak, film çözeltisine potasyum klorür eklenerek karragenanın jel oluşturması desteklenmiştir. Elde edilen filmlerin karakterizasyon çalışmalarında filmin kalınlığı, nem içeriği, şişme yüzdesi, su buharı geçirgenliği ve opasitesi incelenmiştir. Ayrıca bu çalışmada, emülgatör olan Tween 80 konsantrasyonunun etkisi de incelenmiştir. Yapılan karakterizasyon çalışmalarının sonuçlarına göre Tween 80 miktarı artırıldıkça daha yüksek kalınlıkta ve nem içeriğinde filmler elde edilmiştir. Bunun yanı sıra, yüksek emülgatör konsantrasyonu ile filmlerin şişmesi ve opaklığı azaltılmıştır. Daha sonra, keten tohumu yağı konsantrasyonunun karagenan filmlerinin üzerindeki etkisi araştırılmıştır. Filmdeki keten tohumu yağı konsantrasyonunun artması, daha düşük nem içeriğine, daha az şişme özelliklerine ve daha az su buharı geçirgenliğine sahip olan, daha kalın ve daha opak filmlerin oluşmasını sağlamıştır. Karrageenan filmlerin bitki yağı ile birleştirilmesinin filmlerin karakteristik özelliklerini geliştirdiği görülmüştür. Bu nedenle, keten tohumu yağı içeren karagenan filmler, gıda maddeleri için yenilebilir filmlerin uygulanma çalışmaları için potansiyel malzeme olarak önerilebilir.

1. INTRODUCTION

Novel technology brings out new featured packaging materials today. Increase of developing intelligent and smart packaging, which are able to give information, alter safety, and improve quality of foods at storage, transportation or distribution stage, lead to the usage of new materials in packaging industry [1]. Biopolymers are new polymer materials that replace synthetic polymers, which have been used for many years and cause of major waste-disposal problem since they have poor biodegradability [2]. Natural biopolymers containing proteins such as whey, gluten, casein etc. and polysaccharides such as starch, carrageenan, chitosan etc. They have being used for developing novel biodegradable packing materials [3].

Natural sourced biopolymers are used to create coatings and edible films as packaging purpose. Coatings and edible films form a barrier to food stuffs and retard the environmental impacts during its life. Some of them also has protective features depending on material properties. Apart from their functions and benefits, coatings and edible film application on foodstuffs still have limited usage in industry. Even though total replacement of conventional non-biodegradable packing material with biodegradable coatings and edible films are not expected, these biopolymers can reduce the demand of synthetic polymers [4]. By reducing the usage of synthetic polymers, waste and recycling problem can be decreased as well. Consequently, sustainable, biodegradable, and edible materials can be adapted in food packaging to enhance productivity, food quality, freshen, and support food safety [5].

Edible packaging can be applied to fruits and vegetables, dairy products, meat and poultry. By decreasing the change of moisture, lipid, volatiles, and gasses in the medium of food and the surrounding, coatings and edible film applications aim to extend food shelf life [4]. Edible films and coatings have been studied by researchers to obtain alternative food packaging materials. Commonly, they focus on multicomponent films and coatings which can be based on polysaccharides, lipids or proteins.

Polysaccharide-based edible films naturally sourced from animals, plants, marine or microbial origin [5]. Polysaccharides are advantageous in terms of availability, non-toxicity, and selective permeability to oxygen and carbon dioxide [6].

Carrageenans are one of the marine originated polysaccharides that are frequently used as gelling, emulsifying, and stabilizing agents in food, dairy, pharmaceutical industries. Carrageenans are extracted from red seaweeds and classified as biopolymers that consist of water-soluble galactose units. There are several studies have been published about developing edible films based on carrageenan [7].

With the aim of improving functional properties, plant extracts, antioxidants, antimicrobials, colorants, flavorings, and essential oils can be added to edible film composition. In studies of developing new edible films different agents have been added into carrageenan. Flaxseed oil is one of the omega-rich plant-based oil, used for multiple purpose in the food industry. Flaxseeds' oil content change between 20-40% depending on cultivation [8].

The objective of this study is to prepare carrageenan films incorporated with flaxseed oil. The purpose of flaxseed oil addition into carrageenan films is to make use of hydrophobic features of the oil. Since higher hydrophobicity of edible films can support to decrease the moisture penetration and water vapour permeability of edible films. To sum above all, aim of this study is to produce carrageenan films incorporated with flaxseed oil and investigate its characteristics in terms of mechanical properties and moisture barrier properties.

2. LITERATURE STUDIES

Food packaging industry evolves with novel technology, consumer demands and economy. This brings out new methods or materials to the field. Coatings and edible films based on biopolymers, are one of this new application methods that packaging industry has interested in recent years. Generally, coatings and edible films are mix each other. While coatings are used as liquid forms, edible films applied to foods in the form of solid lamites [1]. Representative scheme of the methods is given in Appendix A section as Figure A.1.

Application method of coatings and edible films depend on the type of target foodstuff. Target food' surface, shelf life period, and moisture content significantly affect the application method. Besides, an application can be made in order to prevent specific features such as protection from sunlight or environmental humidity. As an example, foods that are oxidation sensitive can be coated with biopolymers having low oxygen permeability. Low oxygen permeability can prolong the oxidation period; thus food quality might be maintained until the consumption.

In addition, coatings and edible film application have benefits in supporting food quality during its shelf life and in reducing food losses after production. Especially foods such as fruits and vegetables, application of coatings and edible films have great impact during the postharvest management where significant losses occur because of microbes, insects, respiration, and transpiration.

Coatings and edible films have been functionalized with additional compounds. Additional compounds have functions such as antioxidant or antimicrobial. Synergistic effects of such functional compounds in polymer matrix directly affect mechanical and barrier properties of films [5]. Accordingly, schematic representation of edible packaging and functionalities of films given in Figure A.2.

2.1 Carrageenan

Carrageenan, polysaccharide-based biopolymer, can be found in different types depending on their structure. Several carrageenan types differ in terms of sulfation patterns and distributions of 3,6-anhydro-D-galactose units [9]. Owing to pattern and distribution characteristics carragenans are generally divided into three groups: kappa (κ), iota (ι), lambda (λ) [10]. These three types of carrageenans have different glycosidic linkage as seen in Figure 2.1.

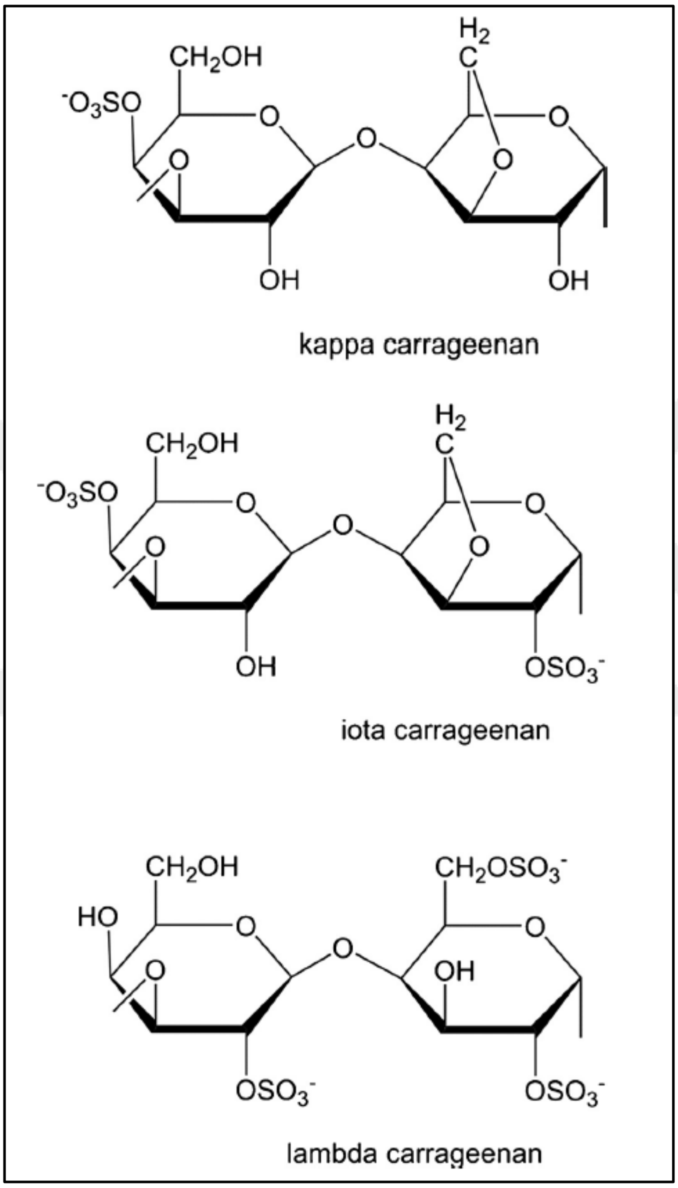

Figure 2.1: Structures of kappa, iota, and lambda carrageenans [10].

Structural difference results in diverse properties in carrageenan groups. For instance, in case of gel texture properties λ- types are non-gelling, while ι- and κ- types form gels. Further, ι- types form soft and elastic gels whereas κ- types form firm and brittle gels [11]. Such functional differences of carrageenans play significant role in the development of edible films. List and comparison of carrageenan properties are given in Figure A.3.

In the studies of developing edible films with improved functions κ-carrageenan based films are incorporated with various substances such as plant extracts, oils, nanoparticles [12-14]. Different active agents results in different κ-carrageenan films that differs in mechanical and barrier properties. To illustrate, in a study addition of grapefruit seed extract improved antimicrobial activity of carragenan films and resulted in inhibition of tested pathogens [12]. Furthermore, κ-Carrageenans which have one negative charge per disaccharide unit, able to form great gels and filming properties. Such properties lead to κ-carrageenans having the highest tensile strength compared to λ- and ι- carrageenans [7].

2.2 Flaxseed/Lineseed Oil

Flax originated from Linaceae family as Linum usitatissimum. After processing flaxseeds, they usually used for food and feed. Flaxseed is also called as linseed through out the time due to its purpose of use. In North America, "flaxseed" is used when flax is consumed by humans, whereas "lineseed" used when flax utilized for industrial purposes. Brown flaxseeds contain 41% fat, 20% protein, 28% total dietary fiber, 7.7% moisture, and 3.4% ash in average [16].

Flaxseed oil contains different amounts of polyunsaturated fatty acids. It is rich in omega-3 fatty acid and α-linolenic acid (ALA) [16]. These polyunsaturated fatty acids cannot be produced by humans, they are said to be essential components for humans. Researches reported that flaxseed oil consists of nearly 59% ALA and in total nearly 91% polyunsaturated fatty acids (Figure 2.2) [17].

Moreover, comparing animal based and plant based fats and oils, flaxseed oils contain the most ALA among others, which makes them valuable source for essential oils [15]. Comparison of saturated and unsaturated fatty acid compositions of different oils and fats is given in Figure A.4.

Flaxseed oil fatty acids		Percentage (%)
C16:0	Palmitic	5.76 ± 0.09
C16:1 n7	Palmitoleic	0.11 ± 0.02
C18:0	Stearic	2.70 ± 0.18
C18:1n9c	Oleic	15.13 ± 0.41
C18:2n6t	Linolelaidic	0.67 ± 011
C18:2n6c	Linoleic	16.20 ± 0.68
C18:3n6	gamma-Linolenic (cis 6, 9, 12)	0.23 ± 0.16
C18:3n3	alpha-Linolenic (cis 9, 12, 15)	58.84 ± 0.91
C20:0	Arachidic	0.37 ± 0.02
Unsaturated fatty acids		91.18 ± 0.39

Figure 2.2: Fatty acid composition of flaxseed oil [17].

3. MATERIALS AND METHODS

3.1 Materials

Carrageenan was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glycerol ($\geq$99.5%), Tween 80, and potassium chloride were Merck (Darmstadt, Germany). Flaxseed oil supplied from Talya Bitkisel (Antalya, Turkey). All solutions were prepared with deionized water.

3.2 Preparation of Films

Films were prepared by casting method reported in the study of Shojaee-Aliabadi with some modifications. It has been stated that films solutions containing 50% (w/w) glycerol (based on carrageenan weight) can be taken off easily from the plate [18].

Film solutions were prepared as follows, 1% (w/v) of ccarrageenan was dissolved in deionized water at 80^{o}C for 15 min under magnetic stirring. Then, glycerol was added as plasticizer at a concentration of 50% (w/w, based on carrageenan weight) while stirring. To investigate the effect of the emulsifier Tween 80 amount, it was added in different concentrations (2.5, 3.75, 5 and 7.5%, v/v) while oil content kept constant (1%, v/v) named as T1, T2, T3, and T4 respectively. Similarly, to investigate the effect of flaxseed oil amount, it was added to reach final concentrations of 0.5, 1, 2 and 3% (v/v) while Tween 80 amount kept constant (3.75%, v/v) named as Y1, Y2, Y3, and Y4. After flaxseed oil addition, film solutions were mixed with portable high-speed mixer for 5 min at 80^{o}C. High speed mixing resulted in foaming in films solutions. In order to remove air bubbles from emulsions, magnetic stirring was applied for another 10 min at 65^{o}C. Then, 2.5 mL of KCl solution (1 M) was added to support the gelation of carrageenan [19]. Film solutions were stirred 45 min in total. Carrageenan film without any flaxseed oil and KCl (YC) and carrageenan film without any flaxseed oil, but with KCl (Y0) were prepared for later control.

Film solutions were placed on clean petri dishes with a diameter of 9 cm, then films were dried for 24 hours at 40ºC. After drying, the films were stored in a test cabinet at 25ºC and 50% relative humidity until evaluation. Films were peeled off the casting surfaces before the analysis. Representative image of film preparation is given in Figure A.5 and image of Y1 film sample given in Figure A.6.

3.3 Characterization of Films

3.3.1 Thickness

Thickness of films was measured with a digital micrometer (Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan) having a sensitivity of 0.001 mm. Five thickness values were taken at different positions randomly from all films.

3.3.2 Moisture content

Moisture content of the films was measured with Shimadzu MOC63U Unibloc Moisture Analyzer (Kyoto, Japan). Analysis was done by heating films to 110ºC until samples reach stable mass. Then, moisture content evaluations of films were reported. Four replications were made for each film.

3.3.3 Swelling ratio (SR)

Stored samples were taken from the cabinet and cut as square shapes (1x1 cm^2) in order to investigate swelling properties of the films. Then, cut pieces were weighed quickly. Film pieces were placed in tubes containing 50 mL of deionized water and tubes were put into Nüve ST-402 shaking water bath (Ankara, Turkey) at 25ºC having 20 rpm shaking rate. The swelled film pieces were taken from tubes at individual time intervals (5, 10, 20, 30, and 60 minutes), dried carefully with filter paper and weighed. Four replications of measurements were performed.

Swelling ratio (SR) of the films was evaluated gravimetrically. SR was calculated using the following equation 3.1:

$$\mathbf{SR}\ \% = \frac{\mathbf{m_t - m_0}}{\mathbf{m_0}}\ \mathbf{x\ 100} \tag{3.1}$$

3.3.4 Water vapor permeability (WVP)

Analysis of water vapor permeability of films was performed based on the ASTM E96-96M "Desiccant Method" with some modifications [20]. By using test cabinet that enables temperature and humidity control (Nüve TK-120, Ankara, Turkey), test conditions were set as 25ºC and 50% humidity. Plastic test cups were filled with distilled water until 2 cm below the top of the cups. Film samples were stretched onto the mouth of cups. Area of vapor permeation was 0.00159 m^2 in average for each film. The mass of cups was recorded by using weighing balance (Mettler Toledo, tare range: 0-100 g; sensitivity: 0.00001, Ohio, USA). Single weighing measurements were done in a 10 days period. Water vapor transmission rate (WVTR) in g/day.m^2 of films was evaluated gravimetrically with following eqution 3.2:

$$\mathbf{WVTR} = \frac{\mathbf{m}}{\mathbf{A}} \tag{3.2}$$

where m is slope that found by simple linear regression analysis of weight change-time curve and A is the water permeation area of films. Because of unit (mmHg) vapor pressure difference, water vapor permeance (g.mm/day.m^2.mmHg) of films were steady (g) per unit (m^2) area of surface in unit (day) time. Permeance was evaluated as following equation 3.3 depending on WVTR and ΔP:

$$\mathbf{Permeance} = \frac{\mathbf{WVTR}}{\mathbf{\Delta P}} \tag{3.3}$$

Lastly, water vapor permeability (WVP) was evaluated according to Equation 3.4:

$$\mathbf{WVP = Permeance\ x\ Film\ Thickness} \tag{3.4}$$

Two parallel measurements were conducted for water vapor permeability tests.

3.3.5 Optical properties

The sample films' transmittance was measured with Shimadzu UV-1800 model UV-VIS spectrophotometer (Kyoto, Japan). Films were cut in rectangular shapes (1.5x2.5 cm) and placed in the film holder apparatus that attaches to the standard sample unit of the instrument. Films' transmission was scanned for each film between 200 and 800 nm wavelength range and recorded. The opacities of the films were evaluated by measuring the absorbance at 600 nm and calculated with equation 3.5. An empty test cell was used as the reference. Opacity (Op) was calculated using the following equation:

$$\mathbf{Opacity} = \frac{\mathbf{Abs_{600}}}{x} \tag{3.5}$$

where Abs_{600} is the value of absorbance at 600 nm and x is the film thickness (mm). Based on this equation lower the Op values, higher the film transparency. Four replicates of each film were performed.

4. RESULTS AND DISCUSSION

Films were analyzed into two groups: effect of Tween 80 and effect of flaxseed oil on film samples. Then, the results of both groups were compared with each other in overall section.

4.1 Effect of Tween 80 on Carrageenan Films

In order to investigate effect of the emulsifier, Tween 80, on film formulation, film thickness, moisture content, swelling ratio, and opacity properties were analyzed. Characteristic properties of films with different amount of Tween 80 was reported (Table 4.1).

4.1.1 Thickness of films

Based on the results given in Table 4.1, it can be seen that thickness of films was increased accordingly to Tween 80 amount in the formulation. Thus, T4 films have a highest thickness since they contain highest amount of Tween 80 (7.5%, v/v). In a study investigating Tween types of emulsifiers, similar results were reported [21]. They have stated that higher emulsifier amount results in higher thickness.

4.1.2 Moisture content

Moisture content of films with different concentration of Tween 80 was also given in Table 4.1. Data shows that, the increase in Tween 80 concentration results in films with higher moisture content. A similar study reported that carrageenan films had higher moisture content owing to higher Tween 80 content in film formulation [22]. At the same study, they stated that films with higher moisture content were obtained due to higher amounts of emulsifier Tween concentration because, Tween has hydrophilic structure which brings along greater attraction from film solutions to moisture.

Table 4.1: Characteristic properties of films with different amount of Tween 80.

Sample	Tween 80 Amount (%, v/v)	Thickness (mm)	Moisture Content (%)	Swelling Ratio[a] (%)	Opacity
T1	2.5	0.07 ± 0.00	11.8 ± 0.65	2289 ± 2.09	0.43 ± 0.07
T2	3.75	0.08 ± 0.00	14.3 ± 1.76	2273 ± 0.22	0.28 ± 0.01
T3	5	0.10 ± 0.00	14.9 ± 1.34	1506 ± 6.72	0.30 ± 0.01
T4	7.5	0.14 ± 0.00	15.1 ± 0.14	1143 ± 0.48	0.21 ± 0.07

[a]Reported values were calculated SR% at 20 min.

4.1.3 Swelling ratio

The swelling ratio of T-series films results indicated that the addition of Tween 80 in film formulation cause films with lower swelling behaviour. In other words, T1 films, containing the lowest amount of Tween 80, swollen more water from media, whereas T4 films, containing the highest amount of Tween 80, swollen less water from media. Also, owing to the higher swelling behavior of T1 films, they started to break apart after 20 minutes of experiment (Figure 4.1). In case of T4 films, higher emulsifier concentration in film formulation resulted in higher hydrophobic behavior. Thus, T4 films had the lowest swelling ratio.

In other study investigating effect of Tween type and its concentration on carrageenan films reported that after 30 min of immersion all films were dissolved in distilled water. It was also mentioned that Tweens are hydrophilic emulsifiers, therefore the complete interaction of carrageenan and Tween molecules yields greater water-soluble films [22]. These results support the swelling behavior of carrageenan films with changing Tween concentration since all films had swelling ratio above 1000% in common.

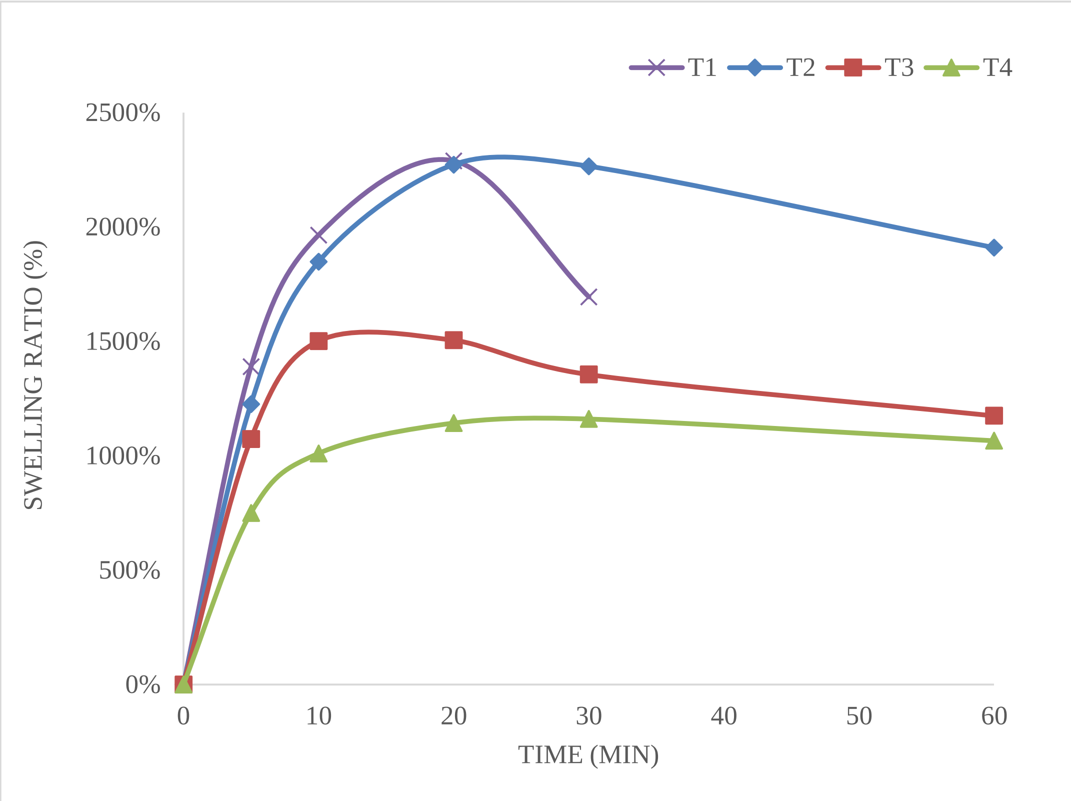

Figure 4.1: Swelling behavior of carrageenan films with different amount of Tween 80.

4.1.4 Opacity

The films' optical properties evaluated as opacity values based on absorption at 600 nm. Opacity results (Table 4.1) show that increase in Tween 80 concentration makes opacity values decrease. There were not any significant difference between T2 and T3 films. However, lower opacity values also mean higher transparency. Higher Tween 80 concentration produced better emulsified film solution, therefore T4 films were more transparent than all T-series films. In a study that investigates the effect of the different Tween type of emulsifiers on carrageenan films reported similar results [22]. They also added that, irregular opacity values might be obtained due to the moisture content of films, because moisture content in film structure can cause reflection of light from the film surface.

4.2 Effect of Flaxseed Oil on Carrageenan Films

Preliminary studies point out that films containing 3.75% (v/v) Tween 80 yielded better emulsion and film structure. Therefore, for the purpose of investigating flaxseed oil concentration on carrageenan films, Tween 80 concentration was fixed as 3.75% (v/v) based on preliminary analysis with T-series films. Then, Y-series of carrageenan films were examined from thickness, moisture content, swelling ratio, water vapor permeability, and opacity aspects.

4.2.1 Thickness of films

Thickness measurements (Table 4.2) show that carrageenan films tend to be thicker with the addition of flaxseed oil. Higher oil concentration yields thicker carrageenan films. However, there are not any significant difference between Y0 and Y1 films' thickness, which shows that 0.5% (v/v) flaxseed oil concentration does not quite affect the overall film thickness. Results were comparable with a study that carrageenan films containing Satureja hortensis essential oil [23]. They stated that carrageenan films' thickness increases as oil concentration increases. Besides, when film thickness results are compared, carrageenan films containing flaxseed oil are thinner than those containing Satureja hortensis essential oil.

4.2.2 Moisture Content

Results of moisture content analysis were given at Table 4.2. Moisture content of films varied from 8.0-16.0 based on results. Since oils are hydrophobic and repel water molecules, higher oil concentration causes films' hydrophobicity increase. Thus, 3% (v/v) flaxseed oil containing Y4 films had the lowest moisture content. Similar results were also seen in a study of carrageenan films with Zataria multiflora Boiss and Mentha pulegium essential oils [18]. Higher the oil concentration, lower the moisture content of films.

Table 4.2: Characteristic properties of films with different amount of flaxseed oil.

Samples	Flaxseed Amount (%, v/v)	Thickness (mm)	Moisture Content (%)	Swelling Ratio[a] (%)	WVP (g.mm/day.m^2. mmHg x10^{-8})	Opacity
YC	0	0.052 ± 0.004	16.2 ± 4.24	3067 ± 1.68	5.34 ± 0.12	0.19 ± 0.03
Y0[b]	0	0.072 ± 0.004	16.7 ± 1.88	2777 ± 2.38	5.59 ± 0.01	0.18 ± 0.03
Y1	0.5	0.083 ± 0.006	14.6 ± 4.47	2190 ± 0.77	4.74 ± 0.14	0.23 ± 0.06
Y2	1	0.100 ± 0.001	11.5 ± 1.69	1587 ± 1.19	4.85 ± 0.29	0.23 ± 0.05
Y3	2	0.117 ± 0.008	9.4 ± 3.83	1583 ± 0.57	4.60 ± 0.12	0.39 ± 0.14
Y4	3	0.135 ± 0.012	8.4 ± 2.66	1494 ± 0.47	3.52 ± 0.26	0.41 ± 0.00

[a]Reported values were recorded at 20 min during experiment. [b]Whereas YC does not contain KCl, Y0 contains KCl in film formulation.

4.2.3 Swelling ratio

Swelling tests indicate how much water is swollen by the film. Test results present that swelling ratio of Y-series films varied between 3066-1494%. YC films' swollen highest amount of water from media. Besides, YC films began to decompose after 20 min because of over swelling, therefore its swelling ratio decreased until the end of the experiment (Figure 4.2.). Further, film composition was not able to provide excessive hydration during swelling [16].

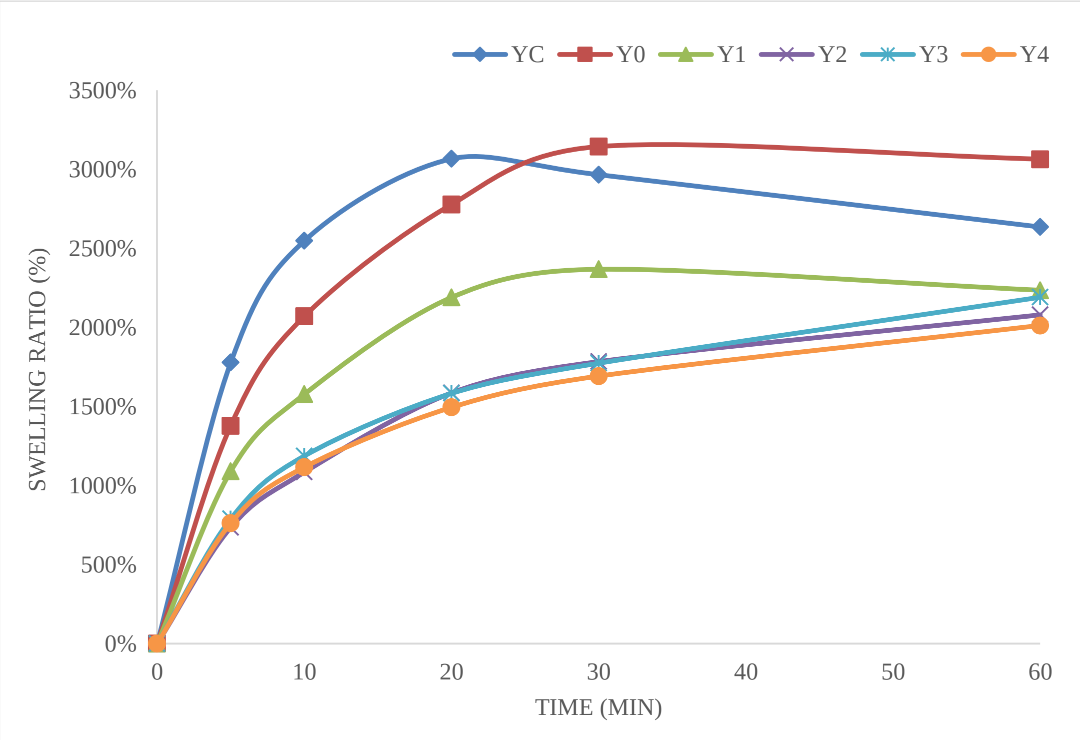

Figure 4.2: Swelling behavior of carrageenan films with different amount of flaxseed oil.

Films containing higher concentrations of flaxseed oil had lower swelling behaviour. Films with a lower swelling ratio also had lower moisture content. This results are related with films hydrophilicity which was reduced with flaxseed oil. In addition, due to interaction between lipid molecules and the hydroxly groups of carrageenan, carrageenan' attraction to water molecules could be reduced, thus higher water-resistant films can be obtained [23]. Similar results were obtained in other edible film studies which were stated that increase in hydrophobicity leads to decrease in water uptake by films [16, 24].

4.2.4 Water vapor permeability (WVP)

Water vapor permeability (WVP) properties are one of the key barrier parameters for edible films. This parameter indicates how much water vapor is permeable through film structure from medium to environment. Test results (Table 4.2) represent that oil addition to film formulation decreased water vapor permeability of carrageenan films. This can be explained with the reduction of affinity towards water by the addition of flaxseed oil. Also, oil droplets present in film structure may prevent water vapor shift between two media. Comparable results were obtained in a study of carrageenan films incorporated with pomegranate seed oil where WVP was nearly reduced by half when oil concentration was increased [25]. Besides, WVP decrease was detected with the oil addition to film composition [26]. However, edible films with lower WVP might be preferred for the packaging of high moisture containing foods.

4.2.5 Opacity

Opacity of films is one of the key features that is important for developing edible films. As it previously mentioned, greater opacity results point out less transparent films. According to results reported in Table 4.2, film opacity was increased as the flaxseed oil concentration was increased. Because of this nature, Y4 films ,which contains 3% flaxseed oil, had highest opacity value. Y4 films were least transparent film among the Y-series. Higher opacity also indicates greater light scattering from dispersed phase. Therefore, more droplets present in film structure, they results in higher light scattering and more opaque films [13]. Many of similar studies stated that oil concentration increment lead to higher opacity results and less transparent films [18, 22, 26].

5. CONCLUSION

In this study, characterization of carrageenan films incorporated with flaxseed oil was performed for the first time. Characterization was performed in two parts; from the point of emulsifier Tween concentration and flaxseed oil concentration. All films were prepared by casting method. Increase in emulsifier Tween 80 produced thicker films with higher moisture content. Also, higher Tween 80 concentration resulted in lower swelling behavior and more transparent films. At the second part, the effect of flaxseed oil concentration was examined which indicated that as the oil amount gets higher, thicker films having lower moisture content and less swelling behavior were obtained. Water vapor permeability of films was significantly decreased as the oil amounts get higher. Also, films were more opaque when oil concentration was higher. Oil incorporation into biopolymer based film alter its physical and barrier properties in overall. Therefore, films with flaxseed oil can be further explored for the potential usage as an edible food coating.

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APPENDICES

APPENDIX A: Supporting figures of the study.

APPENDIX A

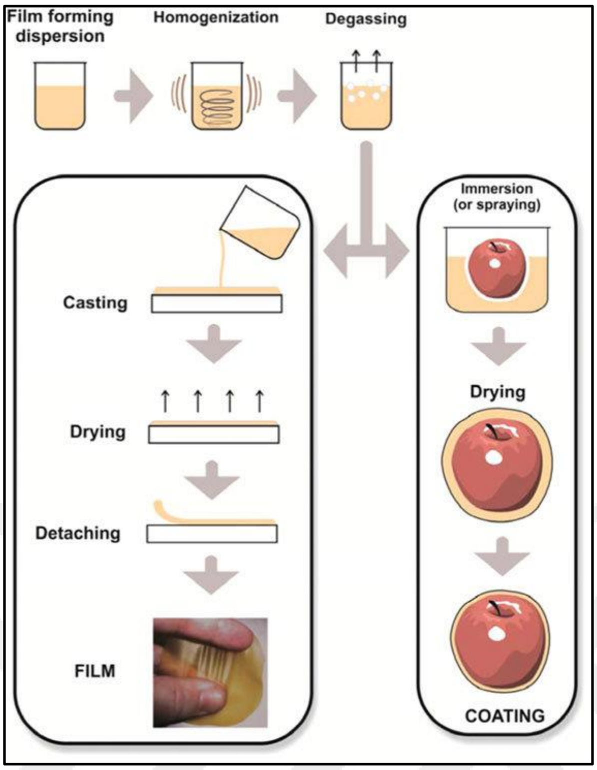

Figure A.1: Schematic representation of edible films and coatings application [4].

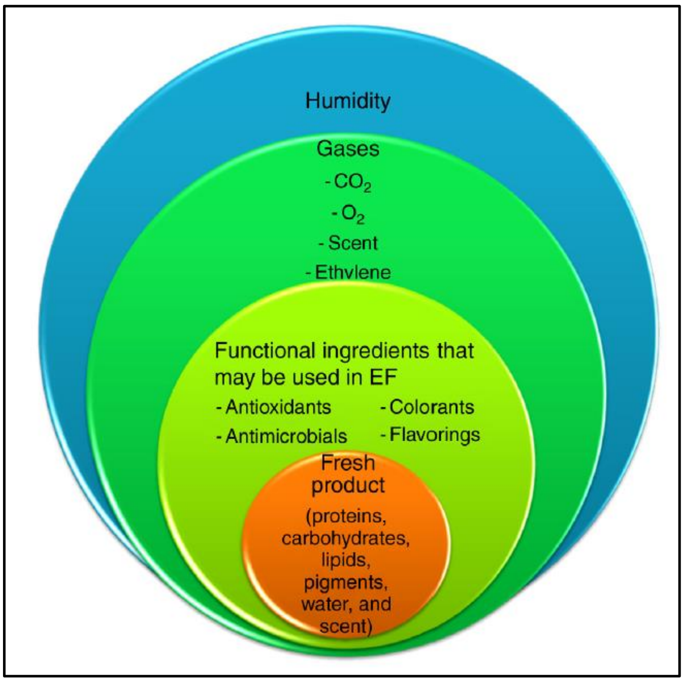

Figure A.2: Schematic representation of edible films and functionalities of films [5].

Carageenan type	Lambda	Iota	Kappa
Solubility			
Hot (80 °C) water	Soluble	Soluble	Soluble
Cold (20 °C) water	All salts soluble	Sodium salt soluble	Sodium salt soluble
Hot (80 °C) milk	Soluble	Soluble	Soluble
Cold (20 °C) milk	Thickens	Insoluble	Insoluble
Cold milk (TSPP added)	Increased thickening or gelling	Thickens or gels	Thickens or gels
50% sugar solutions	Soluble	Insoluble	Soluble hot
10% salt solutions	Soluble hot	Soluble hot	Insoluble
Gelation			
Effect of cations	Non-gelling	Strongest gels with calcium	Strongest gels with potassium
Gel texture	Non-gelling	Soft, elastic	Firm, brittle
Syneresis	Non-gelling	No	Yes
Hysteresis	Non-gelling	5–10 °C	10–20 °C
Freeze-thaw stability	Yes	Yes	No
Synergy with locust bean gum	No	No	Yes
Synergy with konjac glucomannan	No	No	Yes
Synergy with starch	No	Yes	No
Shear reversibility	Yes	Yes	No
Acid stability	Hydrolysis	Hydrolysis in solution, accelerated by heat; gels are stable	
Protein reactivity	Strong protein interaction in acid		Specific reaction with kappa-casein

Figure A.3: List and comparison of carrageenans properties [11].

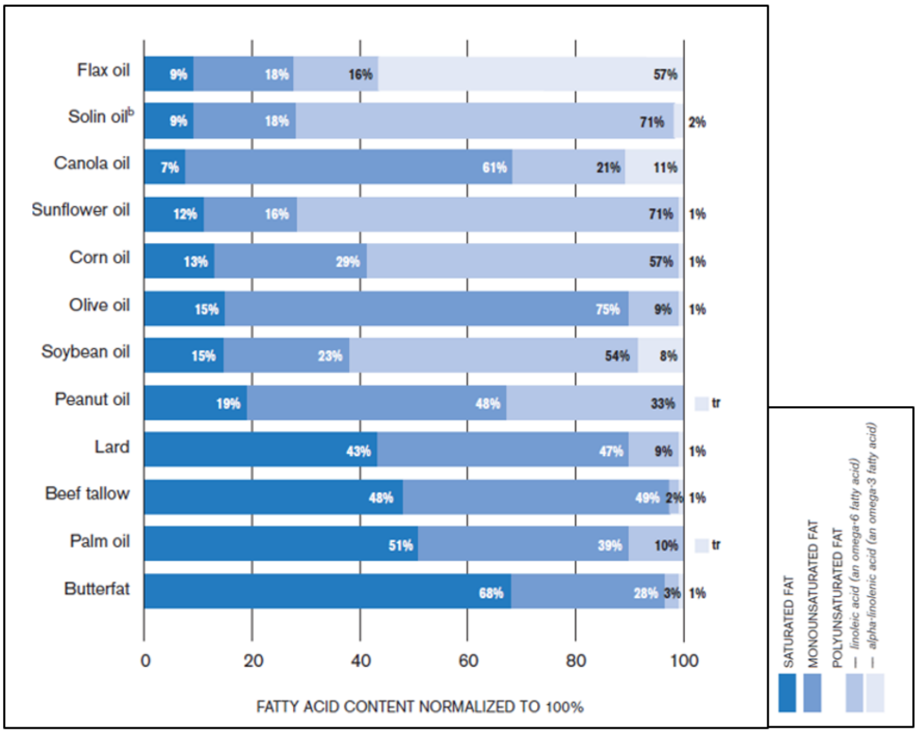

Figure A.4: Saturated and unsaturated fats composition of different oils and fats. [15].

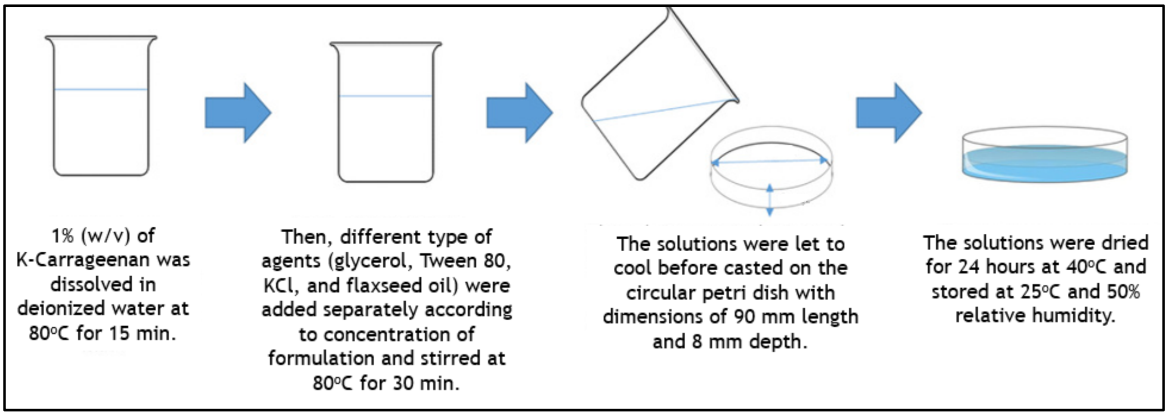

Figure A.5: Schematic representation of film preparation.

Figure A.6: Representation of Y1 film sample.

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