DEVELOPMENT OF PEA FLOUR BASED FILMS CONTAINING ONION SKIN POWDER FOR ACTIVE FOOD PACKAGING

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ABSTRACT

DEVELOPMENT OF PEA FLOUR BASED FILMS CONTAINING ONION SKIN POWDER FOR ACTIVE FOOD PACKAGING

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The food industry has opportunities to add value as peas and pea products become worthwhile alternatives to conventional ingredients. Adding different bioactive substances, such as antioxidants and antimicrobials, into the edible films are important to improve microbial safety and nutritional and sensory aspects of foods. In addition, edible coatings can protect the bioactive compounds from environmental effects. In this study, the effects of pH (8, 11), onion skin powder extract content (0.5%, 1%), and homogenization techniques (high-speed homogenization, ultrasonication) on the characteristics of films (water vapor permeability, total phenolic content, solubility, moisture content, tensile strength, elastic modulus, and color) were determined. According to the results, low pH and high-speed homogenization was more effective for moisture, tensile strength, elastic modulus and yellowness values while ultrasonication was more effective for color properties. Films were not significantly different in terms of solubility and water vapor permeability. As pH was increased, the values for total color change, lightness and redness values increased. Ultrasonication was more effective on lightness rather than other color properties. High onion skin powder extract was significantly effective on

TPC and total color change. As long as mechanical properties, moisture content, yellowness values were considered, t film with pH of 8, containing 0.5% onion skin powder extract and treated with high-speed homogenization were selected as the best one This study showed that casting is a promising technique for making biopolymer-based active films that can be applied in the packaging industry.

Keywords: Biodegradable Film, Pea Flour, Edible Active Film, Casting Method

ÖZ

AKTİF GIDA AMBALAJLAMA İÇİN BEZELYE UNU BAZLI SOĞAN KABUĞU İÇEREN FİLMLERİN GELİŞTİRİLMESİ

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Bezelye ve bezelyeden elde edilen ürünler geleneksel bileşenler için değerli alternatifler haline geldikçe, gıda endüstrisine değer katma fırsatları olmaktadır. Antioksidanlar ve antimikrobiyaller gibi farklı biyoaktif maddelerin yenilebilir kaplama maddelerine eklenmeleri gıdaların mikrobiyal güvenliğinii, besine ve duyusal yönlerini iyileştirmeleri açısından önemlidir. Ayrıca, yenilebilir kaplama maddeleri biyoaktif bileşenleri çevresel etkilerden korumaktadırlar. Bu çalışmada pH (8, 11), soğan kabuğu tozu ekstrakt içeriği (%0.5, %1) ve homojenizasyon tekniklerinin (yüksek hızlı homojenizasyon, ultrasonikasyon) filmlerin karakterizasyonu (su buharı geçirgenliği, toplam fenolik içerik, çözünürlük, nem içeriği, gerilme mukavemeti, elastik modül ve renk) üzerine olan etkileri belirlenmiştir. Elde edilen sonuçlara göre nem, gerilme mukavemeti, elastik modülüs ve sarılık değerleri açısından düşük pH ve yüksek hızlı homojenizasyon daha etkili bulunurken, renk özellikleri için ultrasonikasyon daha etkili bulunmuştur. Filmler arasında öözünürlük ve su buharı geçirgenliği açısından önemli bir fark bulunmamıştır. pH arttıkça toplam renk değişimi, açıklık ve kırmızılık değerleri artmıştır. Ultrasonikasyon, diğer renk özelliklerinden ziyade açıklık üzerinde daha

etkili bulunmuştur. Diğer yandan, yüksek miktardaki soğan kabuğu tozu ekstraktı, toplam fenolik içerik ve toplam renk değişimini önemli derecede etkilemiştir. Mekanik özellikler, nem içeriği, sarılık değerleri düşünüldüğünde en ideal film, pH değeri 8 olan, %0,5 soğan kabuğu tozu ekstrakktı içeren ve yüksek hızlı homojenizasyon işlemi görmüş film en iyi film olarak seçilmiştir.. Bu çalışma, döküm metodunun ambalaj endüstrisinde uygulanabilen biyopolimer bazlı aktif filmler yapmak için umut verici bir teknik olduğunu göstermiştir.

Anahtar Kelimeler: Biyobozunur Film, Bezelye Unu, Yenilebilir Aktif Film, Döküm Metodu

To my family

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INTRODUCTION

1.1 Edible Coatings

The scientific challenge to create demand for large-scale biopolymer/biomaterial production will contribute to the long-term development of green packaging as an alternative to petroleum sources (Shalini et al., 2009). Modern food packaging is made from a variety of man-made and synthetic materials, including ceramics, glass, metal, paper, paperboard, wax, wood, and plastics. The most common materials used to make food packaging are glass, rigid plastic, paper, and paperboard (Muncke et al, 2012). Unfortunately, the majority of packaging is single-use and is either recycled or thrown away rather than being utilized again (Bodamer et al., 2016). According to the US Environmental Protection Agency, materials used in food packaging make up nearly half of all municipal solid waste (US Environmental Protection Agency, 2014). Only 35% (89 million tons) of the 258 million tons of municipal solid trash produced in the US in 2014 was recycled or composted, with more than 63% of it being food packaging (US Environmental Protection Agency, 2017). Although consumers are becoming more conscious of the adverse effects of plastic packaging on the environment, there is still a significant, although less obvious, ecological impact of wasted food inside the package. Recent estimates of the annual greenhouse gas emissions from food waste (4.4 Gt CO_2) in the world are higher than the emissions from the transportation (1.9 Gt CO_2) and electricity (1.8 Gt CO_2) sectors combined only in the USA (EPA, 2020). According to Avio et al. (2018), almost 10% of the annual production of plastics ends up in the oceans (about 35 million tons in 2017). Single-use plastic packaging has been banned as a result of the high proportion of plastic packaging in aquatic environments. Furthermore, the necessity to minimize environmental harm caused by packaging waste in China was

the driving force behind China's policy change in 2015 from accepting more than 48 million tons of unsorted plastic to a ban on specific materials and criteria and support for cleaner polymers. Food waste made up the majority of the 138 million tons of waste that were landfilled in the US in 2015, accounting for nearly 22% of the total. Paper and paperboard made up around 13% of the total, while plastics made up about 19%. (EPA, 2018).

Packaging problems are associated with the irrational use of material resources, energy waste, and low product competitiveness due to improper packaging. Furthermore, packaging materials are biodegradable according to the EU Standardization Committee's standards. These standards were introduced not to exclude products that are recently produced using non-renewable resources for practical and economic reasons, but which may be produced using renewable resources in the future. Three main groups can be stated;

- Group 1: Polymers that have been extracted directly from biomass. Examples are polysaccharides like starch and cellulose and proteins such as casein and gluten.
- Group 2: Polymers produced from renewable biobased monomers in a conventional chemical synthesis process. Polylactic acid, a biopolyester made from lactic acid monomers, is a nice example. The production of the monomers themselves may involve fermentation of the carbohydrate substrate.
- Group 3: Polymers made by bacteria which are genetically modified microorganisms. The majority of the polyhydroxyalkonoates in this group of biobased polymers. (Shalini et al., 2009).

Leading international research teams have spent the last few years developing "regulated life cycle materials," which are biodegradable and edible packaging made from renewable biological sources. Recent developments indicate that natural food

resources will continue to show a significant role in the production of industrial products. Biobased/green polymer food packaging is the way of the future. Researchers have been urged to produce novel bio-based and natural polymers appropriate for food packaging. The aim is to use natural materials such as edible films and coatings to increase the shelf-life of foods and to protect them from oxidation and microbial degradation while decreasing the packaging waste. Due to the fact that they have low cost, convenience of production, and availability compared to pure components such as starch and proteins, there has recently been a rise in interest in producing edible active coatings using agricultural crop flours (Nouraddini et al., 2018).

Edible active films can be considered as encapsulating matrices capable of providing protection against exterior factors, enhancing solubility, and controlling release of active compounds. This is especially applicable when considering the disadvantages of storage of bioactive compounds. Bioactive molecules are required to be protected from high temperatures, which is provided by edible coatings. Most food additives are very sensitive to temperature, however many food industry processes require these conditions. In order to prevent their loss, bioactive compounds (i.e. antioxidants and antimicrobials) are placed into a matrix or wall system (edible film) during encapsulation. This protects the additive by forming a strong barrier to the environment (Quirós-Sauceda, 2014). Kayaci and Uyar (2012) developed a functional capsule matrix having vanillin with extended shelf life and stability for high-temperature by cyclodextrin inclusion complexation. According to Milanovic et al. (2010), various temperatures are required for the ethyl vanillin release.

It is expected from an edible active coating to be an easily digestible product. The nutrient amount (such as proteins, carbohydrates, and minerals) absorbed when it is consumed is known as digestibility. The substance amount absorbed by the digestive tract determines its digestibility. It is influenced by a number of factors (Hall, 2008)

such as nutrient type and levels of antinutrient components, consumer health, food types consumed with peas and preparation of the product.

Packaging applications include edible films, active packaging, and intelligent packaging (He & Hwang, 2016). Lower WVP values are better for packaging purposes since lower values mean that less moisture is transferred to the environment. The thickness of the coating directly affects the WVP (Uygun, 2019). Although carbohydrate-based biodegradable films are cheap and prevalent, they are rarely used alone because of weak emulsifying properties. Lipid and protein combinations with carbohydrates might provide better core material protection and efficiency (Rosenberg & Sheu, 1995).

1.2 Active Packaging

According to European regulations, edible active packaging systems are designed to "deliberately contain components that would release or absorb substances into or from the packaged food or the environment surrounding the food." In the context of this study, the coating film acts as an active-releasing system since it provides antibacterial and antioxidant components from the onion skin powder to the packaged food. The food industry has shown a high level of interest in antimicrobial films, which have been the center of significant effort in recent years to develop active packaging and to improve the microbial safety of food. The increasing consumer expectation for foods that are minimally processed and additive-free is said to be the driving force behind this interest in antimicrobial packaging. Active packaging systems made up of polymer-based materials are a prevalent feature of modern food packaging technology. This packaging can enhance the nutritional and sensory qualities of food products as well as its shelf life through its interaction with the food (Bastarrachea et al., 2011).

Active films have been extensively studied and used in the food industry for their effective functionality to prolong the shelf-life of products by controlling the exchange of gases, water vapor, and aroma compounds between the product and the environment. These films are designed to be permeable to specific gases while inhibiting the passage of others, which makes them ideal for the preservation of food products. On the other hand, the casting method is a popular technique used to manufacture active films. In this method, a polymer solution is cast onto a flat surface and dried to form a thin film. The resulting film can be modified with various additives. The choice of polymer and additives depends on the specific application of the film, as well as the desired properties (IFT, 2020).

Robertson (2006) defined active packaging as "packaging in which subsidiary constituents have been deliberately included in either the packaging material or the package headspace to enhance the performance of the package system." Both active and intelligent packaging are based upon the concept of controlled release with the aim of preserving the quality of packaged products. Due to its beneficial effects on food quality and storage durability, and the fact that it eliminates the need to add excessive amounts of preservatives, this approach has attracted a lot of customers' interest (Castro-Lopez et al., 2012). The interaction between the package, the product, and the environment is known as active packaging, and it is an innovative idea that aims to increase the product's safety or sensory qualities while extending its shelf life (Suppakul et al., 2003). According to Kou et al. (2017), edible coatings have the potential to preserve the quality and antioxidant capacity depending on the proper materials and concentration of the film coatings. It is found that the DPPH scavenging activities in the coating-treated jujube fruits were significantly higher than those in the control fruit. So, film coatings can enhance antioxidant activity in jujube during storage. In the study of Huertas et al. (2012), it is found that alginate films showed a positive impact on retaining higher concentrations of total phenolics and antioxidant activity in cherries. Another study stated that as a coating, chitosan interacts with components on the surface of food, improving its antioxidant capacity

in strawberries (Garcia et al. 1996). In another research by Kou et al. (2017), high levels of antioxidant activity in the skin and inner parts of pears (*Pyrus pyrifolia*) are maintained and the loss of antioxidant contents is inhibited with chitosan and pullulan coatings. Li et al. (2010) discovered that the chitosan film enhanced the activities of CAT, POD, SOD, ascorbateperoxidase, and glutathione reductase in pears.

Several studies have investigated the use of the casting method to fabricate active films for food packaging and preservation. For example, Sanchez-Gonzalez et al. (2016) used this method to fabricate active films containing oregano essential oil and chitosan. The resulting films exhibited antimicrobial activity against *S. aureus* and *E. coli*, demonstrating their potential as a natural alternative to synthetic preservatives. Similarly, Muriel-Galet et al. (2015) used the casting method to fabricate active coatings having chitosan and gallic acid. Resulting films exhibited antioxidant activity and were effective in delaying lipid oxidation in packaged meat products. One study investigated how various plasticizers affected the mechanical qualities of active coatings made from carboxymethyl cellulose (CMC) using the casting method (Santos et al., 2019). The results showed that the addition of glycerol as a plasticizer improved the flexibility of the film without compromising its barrier properties. In another study, Lim et al. (2018) used the casting method to fabricate active films containing silver nanoparticles and chitosan. The resulting films exhibited antimicrobial activity against various foodborne pathogens, including *Salmonella enterica* and *L. monocytogenes*. In conclusion, active films fabricated using the casting method have shown potential for various applications in the food industry.

1.3 Biopolymers Used In Active Films

Several biopolymers, including polysaccharides, proteins and lipids have been used as biodegradable active films. In general, protein-based films are effective lipid,

oxygen and aroma barriers at low relative humidity (RH) conditions, therefore, proteins are used widely to form edible films. Proteins such as gelatin (Arvanitoyannis, Psomia-dou, Nakayama, Aiba, & Yamamoto, 1997), whey protein (Fang, Tung, Britt, Yada, & Dalgleish, 2002), wheat and corn proteins (Gennadios & Weller, 1990) and soy protein (Pol, Dawson, Acton, & Ogale, 2002) have been extensively studied.

Limited information is available on the use of legume seeds protein for packaging applications; although, pea (Choi & Han, 2001) proteins along with soy protein have been studied for use as edible films. The value of legumes as a cheap source of high-quality protein products has long been known. Pea flour, a legume flour, is a popular alternative ingredient because it doesn't contain any allergens and gluten, has a low carbon footprint, high water binding and texturizing qualities, and is produced sustainably (Tulbek et al., 2017).

Like most synthetic polymers, edible film materials require property modifiers to improve the physical and mechanical properties of the film. As with synthetic plastics, plasticizers are incorporated into the edible coating/film materials which overcome the brittleness caused by the extensive intermolecular forces. The most common edible plasticizers are polyols, mono/di or oligosaccharides, lipids, and derivatives (Choi & Han, 2001). Lentil protein not only has a medium nutritional value but also a good source for edible film formation and, therefore, its usage in the film can be suggested. The characteristics of the lentil protein-based edible films were comparable with other edible protein films, such as soy, pea, and whey protein in terms of tensile strength, elongation, moisture barrier property and water solubility (Bamdad et al, 2006). Lentils are small legume seeds belonging to the *Lens culinaris* species and the Leguminosae family (Samaranayaka et al. 2017). They are gluten-free, low in fat, and high in dietary fiber and other nutrients, such as protein, vitamins, and minerals (Jarpa-Parra et al. 2016). Lentil protein films are relatively effective as moisture barriers and showed good mechanical properties is promising for lentil flour films (Aydoğdu, 2018). Approximately 80% of the nutritional content

of chickpea flour is carbohydrates. Protein, lipid and moisture have been stated as 14.63, 3.98, 9.15%, respectively. Also, it is an easily accessible legume, so, chickpea can be used as a good type of legume in studies (Kocakulak, 2019). According to Ozkahraman et al., beans, chickpeas, soybeans, lentils, peas and lupins are the most common legumes consumed in the meals and used in studies. They found that functional cakes containing lentil and chickpea flour had the softest structure and highest specific volume (2015).

On the other hand, due to their hydrophobic nature, lipids are a popular choice for making moisture barrier films that can prevent moisture migration. Additionally, lipids are known to have excellent oxygen barrier properties, making them useful for creating gas barrier films. Some examples of lipids used in active films include fatty acids, phospholipids, and triglycerides. Yang et al. (2019) investigated the effect of adding soybean oil, a lipid, to whey protein isolate-based films. The addition of soybean oil resulted in films with lower water vapor permeability and better thermal stability and improved the water resistance and thermal stability of the films compared to films without soybean oil, making them suitable for use in high-moisture food products.

Carbohydrates are another class of molecules commonly used in active films. They are known for their hydrophilic nature. This property makes carbohydrates useful for creating films that can absorb moisture and prevent food products from becoming too dry. Additionally, carbohydrates can be modified to have antimicrobial properties, making them useful for creating films that can help prevent the growth of bacteria and other microorganisms. Some examples of carbohydrates used in active films include chitosan, cellulose, and starch. Aloui et al. (2021) investigated the effect of adding chitosan, a carbohydrate, to cassava starch-based films. The addition of chitosan improved the mechanical and barrier properties of the films, making them suitable for food packaging applications with higher tensile strength and elongation at break, as well as lower water vapor permeability and oxygen permeability.

The combination of lipids and carbohydrates in active films can provide a wide range of functionalities. For example, films made from a combination of lipids and chitosan have been shown to have excellent antimicrobial properties, making them useful for preventing spoilage in food products (Militello et al., 2019). Similarly, films made from a combination of lipids and cellulose have been shown to have excellent moisture barrier properties, making them useful for preventing the loss of moisture in food products (Fang et al., 2020).

1.3.1 Onion and Onion Powder

As a common vegetable in the Allium genus, *Allium cepa* (onion) has been reported to have antimicrobial, anti-spasmodic, anti-cholesterolaemic, hypotensive, hypoglycemic, anti-asthmatic, anti-cancer, and antioxidant properties (Ashwini et al.,2013). In the human diet, onions contains high amount of flavonoids. Onions have significant antioxidant activity due to flavonoid contents such as quercetin, kaempferol, myricetin, and catechin. 80% flavonoids in onions come from two primary components: quercetin monoglucoside and quercetin diglucoside. They have much higher levels of quercetin glucosides than other vegetables. Onion, on the other hand, is grown fresh in every season or kept under the ground by allowing it to develop, and its strength is increased by drying it in a suitable environment after harvest (Ahmad 1996). Among the fresh materials, it was determined that only fresh onion and fresh garlic extracts prepared with ethyl alcohol showed inhibitory activity, and fresh onion was more effective than garlic (Irkin, 2007).

Fresh and dehydrated onions (*Allium cepa*) are commonly consumed as a source of nutrients, spicy garnish, and non-nutritive health promoting components. Onions have lots of functional phytochemicals, and they have been related to improve health and prevent diseases like heart diseases and cancers in different tissues, neurological disorders, and cataract development. In onions, large amounts of flavonoids, fructans, and organosulphur are thought to be vital among phytochemicals with

health benefits. The outermost skin of the onion bulb is a crispy, scaly skin that is commonly discarded. The onion bulb is made up of multiple layers. On the other hand, researches have manifested that the onion's skin contains a high concentration of flavonoids, mainly quercetin. They found that the onion peel extract had the highest total quercetin and antioxidant levels, then onion peel powder came, and then onion flesh extract.

With 2-10 g/kg of flavonoids, onion peels are much higher in flavonoids than the edible portion. Despite the high quantities of flavonoids of onion's outer layers, they are removed and go to waste before cooking. Exterior layers of onions have derivatives of quercetin, which account for more than 80% of the total flavonoids (Albishi et al., 2013). Skrede et al. (2000) found that onion powder contained a significant amount of quercetin, a flavonoid with antioxidant properties. The study showed that the quercetin content in onion powder was higher than that was found in fresh onions, due to the concentration of compounds during the drying process. Moreover, Kadhirvel et al. (2010) found that onion powder contains high levels of anthocyanins, the pigments that give onions their red color and have antioxidant properties. In addition to antioxidant properties, phenolic compounds found in onions have been shown to have anti-inflammatory, anti-hypertensive and anti-cancer properties. Also it is found that quercetin found in onions can inhibit the growth of colon cancer cells.

It is reported that onion was used as the most important vegetable and medicinal plant of Central Asia 6000 years ago. Today, it is a very common vegetable used in Pakistan, India and Mediterranean countries. Onion consists of 8% sugar, which consists of glucose and sucrose, and 90% water. It also contains protein, calcium, sulfur, fluorine, provitamin A, B and C vitamins (Sharma et al. 1979, Elnima et al. 1983, Block 1985, Minakshi et al. 1999, Griffiths et al. 2002). Onion contains compounds gathered under two main groups, namely flavonoids and alk(en)yl cysteine sulfoxides. There are 2 important groups of flavonoids in onions, these

groups are flavonols and anthocyanins. Flavones are phenolic structures containing a carbonyl group. Flavonols are formed as a result of the addition of 3 hydroxyl groups. Their activity is due to the formation of complexes with proteins in the bacterial cell wall, as with quinones. The flavonols found in onions mostly cause the inner skins of the onions to acquire a yellow-cream color. Concentrations of phenolics are mostly increased in the outer layers of red onions. 16 different flavonols, including aglycones, quercetin glycoside derivatives, isohamnetin and campferol, were detected in onion species. Alk(en)yl cysteine sulfoxides, proteins, saponins, phenolic compounds in onions have antifungal and antibacterial properties. (Pruthi, 1980; Elnima et al., 1983, Breu and Dorsch, 1994; Zohri et al., 1995; Fossen et al., 1998; Cowan, 1999; Griffiths et al., 2002).

It is stated that *Aspergillus niger, Penicillium italicum, Tryptophyton gypseum* and *Microsporon naudouini* can be inhibited by thiosulfinate compounds found in onions. In addition, it was determined that the onion extract boiled in water was effective against *Alternaria tenuis, Helminthosporium spp*. and *Curvuluria perniseta*, and compounds with structurally similar formulas such as thiuram sulfide and di-thiocarbamate had fungistatic effects on *Rhizopus nigricans* (Wei et al. 1967, Sharma et al. 1979, Pruthi 1980, Phay et al. 1999).

Encapsulation and packaging applications of onion skin powder have been studied to enhance shelf life and protect the product from oxidation and moisture. Encapsulation of onion skin powder using spray drying technology was investigated by Jayawardena et al. (2020). The effects of various coating ingredients on the encapsulation of phenolic substances extracted from onion skin have been evaluated by Akdeniz et al. (2017). The results showed that protein addition to the film decreased the particle size of capsules significantly. Also, with its greater efficiency values, the combination of maltodextrin and casein proved successful in maintaining the phenolic compounds inside the capsule. Chen et al. (2019) researched the effect of onion skin powder on the physicochemical and sensory properties of fresh pork

sausages during refrigerated storage. It was found that onion skin powder could effectively delay lipid oxidation and improve the sensory attributes of sausages. Another study found that encapsulated onion skin powder had improved stability and increased antioxidant activity compared to non-encapsulated powder. Li et al. (2021) developed a novel active packaging film using chitosan and onion skin powder. The film exhibited antimicrobial activity and was effective in conserving the nutritional and microbial quality of fresh-cut cucumber during storage. Finally, a research by Oms-Oliu et al. (2019) evaluated the effects of modified atmosphere packaging (MAP) on the quality of fresh-cut lettuce coated with onion skin powder. The study found that MAP combined with onion skin powder coating improved the sensory quality and extended the shelf life of lettuce.

1.3.2 Pea Flour

There is an increasing interest about using flours in industries since they are naturally digestible and a source of fibers, proteins, carbohydrates, and lipids (Mendes, Stephansen, & Chronakis, 2017). It's a common biopolymer with a lot of starch composition in it. Amylose makes up the linear chain and amorphous section of starch granules, while amylopectin makes up the branched and crystalline component. Amylopectin and amylose are appealing molecules with barrier qualities that can be used as packaging components. To conclude, various mechanical performances and physical structures are possible (Woranuch et al., 2017). Starch can be combined with various protein types to increase their packaging characteristics. The combination of starch and proteins reduced water vapor permeability while increasing tensile strength (Jagannath, Nanjappa, Das Gupta, & Bawa, 2003).

Peas have been used in cereal and oilseed crops to better control disease, add organic matter to the soil, fix nitrogen, improve soil aggregation, and conserve water (Biederbeck, Zenter, & Campbell, 2005; Chen et al., 2006; Lupwayi, Rice, &

Clayton, 1998). For the last thirty years in the USA and Canada, peas have been an important crop due to the benefits of nitrogen fixation and being a protein source for the world's nutritional requirements (Pavek, 2012). A comparatively limited amount of dried peas is produced in North America for domestic use, with the majority (about 70%) destined for export.

Peas are harvested when they reach maturity, usually when the green scale turned yellow. If the plants are not dry enough, harvesting could be challenging, but if farmers wait until the peas are too dry and brittle, seed damage might result (Pavek, 2012). Pea components can affect glycemic index and insulin resistance, gastrointestinal and cardiovascular health, and weight control, according to epidemiological, in vitro, and interventional research. The effect of fiber and indigestible carbohydrates was seen in experimental and clinical trials, according to the literature review (Dahl et al., 2012).

Depending on the method of extraction, the chemicals used, retention time, the method of separation (such as centrifugation or filtration), and the method of drying, proteins and starch fractions exhibit different functional properties (i.e. spray-drying).All of these factors affect the properties of the ingredients, including their ability to emulsify, foam, whip, gel, and bind water and oil. The effects of peas on satiety, food intake, and blood glucose may be responsible for the possible advantages of regular consumption (Dahl et al., 2012). Peas are able to reduce hunger and this could be attributed to their high levels of protein and fiber that can slow down digestion in stomach, reduce glucose absorption, and promote the hormones that control digestion and appetite.

Coming to pea production processes, impurities can be eliminated with equipments like indent cleaners. Dehulling and splitting, which separate the outer layer of whole peas and separates the pea seed, after cleaning. The main purpose of these processes was to reduce the process of cooking and gelatinization time and prepare to split

peas. Split peas were then further processed using wet milling, dry milling, and air classification methods to separate them into protein, flour and starch sections. A clean label milling and separation method called dry milling and air classification can be applied to make products like fine and coarse flour, protein and starch concentrates. Nutritive value of peas is briefly explained at the table below;

Table 1. Proximate Composition Analysis of Pea (Tulbek, 2014)

Component	**Composition (%)**
Protein	23.4
Fat	1.2
Carbohydrate	60.1
Total dietary fiber	21.2
Sugar	6.5
Starch	49

Wheat and other cereals contain more starch than peas do, although pea seeds contain more protein, total dietary fiber, and sugar than whole wheat. This phenomenon emphasizes how important peas are for developing and poor countries as a source of dietary fiber and protein. Contrary to animal proteins, pea proteins have all the necessary essential amino acids. According to the Osborne classification, pea proteins can be divided into five different protein fractions: albumins, globulins, prolamins, glutelins, and residue protein (Osborne, 1924). The vicilin and legumin proteins make up the most common protein class in peas.

Globulins and albumins are the two major protein parts that contribute to the functionality of pea proteins in foods including drinks, sauces, and custards. The majority of the carbohydrates found in pea seeds, 60-65% of them, are made up of polysaccharides, oligosaccharides, disaccharides and monosaccharides. Starch is the

primary storage carbohydrate in the cotyledons, and makes up the majority of the pea's carbohydrate composition. Amylopectin (55-75%) and amylose (25-45%) make up starch (Hoover, Hughes, Chung, & Liu,2010). Depending on the cultivar, pea seeds have a fat content that ranges from 1.2% to 1.8%. As with other pulse crops, roughly 25% of the fatty acids in pea seeds are made up of oleic acid (18:1) and 50% of linoleic acid (18:2). Pea types and its components don't suffer lipid oxidation or shelf-life problems because peas have a low fat content. On the other hand, minerals and vitamins are abundant in pea seeds. Moreover, peas have higher concentrations of calcium, magnesium, phosphorus, iron, and copper when compared to wheat and other cereals.

Pea flour is made from whole or split peas in various granulations for snacks, baked products, batters, pasta, extruded pet food, depending on the final product. In addition to grain flour, pea flour is frequently used and consumed as a protein ingredient (Huisman & van der Poel, 1994). Water and oil binding, gelation, emulsification, and texturizing are among pea flour's main attributes, and it can be specifically used in cereal, pulse, meat, and gluten-free applications. Pea flour does not break down when cooked for extended periods of time and can sustain higher shear and temperature levels. Thus, pea flour is an ideal material for edible coatings. Protein-based materials are great for packaging because of their strong gas barrier characteristics. However, due to their hydrophilic nature, the mechanical and gas properties of starch plastics are affected by relative humidity. Chemical modification is one method of altering protein characteristics (Shalini et al., 2009).

Due to their high carbohydrate and protein content, flours of legumes are a suitable source for film formation; some of them also contain a large number of lipids. Film-forming materials have been investigated with lentils, defatted soy, and grass pea flours (Diaz et al., 2019). Peas are also a legume protein source which is essential in the food industry because of their different functional properties and high nutritional quality. Heat denaturation at 95°C for 25 min increases the tensile strength of pea

protein concentrate coatings, as well as with other edible protein coatings. As proteins and carbohydrates are the primary film forming molecules, pea seems to be a feasible alternative for edible and biodegradable films (Oğur et al., 2009). However, on the surface of flour film microstructures, as a result of the presence and interaction of macromolecules (proteins, lipids, starch) in the film, irregularities and pores are frequently found.

Due to the presence of more water in glycerol, the interactions between macromolecules can be changed by adding glycerol, and the pea flour film matrix can be more homogenous. As the tendency of consumers to consume animal proteins decreases, people try to include more plant proteins in their diet. This demand is also motivated by sustainability concerns, low manufacturing costs, and great nutritional value. As a result of its nutritional characteristics, pea protein is frequently used in protein-enhanced diets. Glutamic acid, aspartic acid, tryptophan, threonine, cysteine, methionine, phenylalanine, arginine, leucine, and lysine are among the amino acids found in pea protein (Banaszek et al., 2019; J. Boye et al., 2010). All of these different protein groups and amino acid types affect the functional qualities of pea proteins, such as solubility, water binding capacity, foam stability, gelation, emulsion activity, and stability (Lu et al., 2019).

When considering the effect of pH on protein solubility, the isoelectric point comes to mind first because changes in the electrostatic repulsive forces above, below, and at the isoelectric point (pI) influence solubility. This means that depending on whether the protein is at or below the isoelectric point, its net charge can be negative or positive, promoting hydrophilic interactions and consequently solubility. However, since the net charge at the pI is zero, the repulsive forces are reduced to a minimum, resulting in protein aggregation due to the increased hydrophobic interactions between proteins (Fernández-Quintela et al., 1997). Due to structural changes in the legumin section of the protein, alkaline pH increases pea protein solubility. Under alkaline conditions, legumin hexamers in pea protein tend to break

down to monomers. The solubility increases as a result of partial denaturation (Estevinho & Rocha, 2018). However, due to their limited functional characteristics, integrating these proteins into food formulations poses a difficulty. Several modification techniques or combinations with carbohydrates or fibers, on the other hand, can be used to improve their effectiveness.

According to Oğuz et al., (2018) increase of pH yields a considerable increase in consistency coefficient for all pea flour concentrations. The consistency coefficient similarly increased for alkali conditions as pea flour concentration did. Pea flour, obtained by the whole pea or split pea, has become a significant optional ingredient by providing opportunities to add value and contribute to the food industry as a result of maintenance of production, allergen-free, low carbon footprint, GMO free, single-ingredient, and clean label trends in the global market (Tulbek, Lam, Wang, Asavajaru, & Lam, 2017).

The protein content of pea flour is high enough to be affected by variation of pH. Hydrophobic and hydrophilic parts of protein can change at various pH levels, causing cross-link and chain-chain interactions of molecules to act different (Xu et al., 2015). The molecular net charge of protein molecules also fluctuates with solution pH because they are zwitterions with free amino and carboxyl groups. The isoelectric point, where the molecular net charge approaches zero, is a specific pH value for each protein (Ulaganathan et al., 2015). According to Bara et al. (2015), pea proteins have an isoelectric point of 4,5. The protein will have a tendency to aggregate at this pI level. The formation of negative charge induced by the interaction of polysaccharides and protein molecules can result in electrostatic repulsion, which can prevent further interactions. Both solubility and viscosity are altered by increasing the pH over the isoelectric point. The pH increase had a positive effect on film morphology. Solutions with higher alkaline conditions formed more homogeneous films (Lin et al., 1990; Tang et al., 2012).

Recent literature studies have investigated the use of pea flour for packaging applications, employing methods such as casting and electrospinning. Casting involves mixing pea flour with a binding agent, and pouring the mixture into a mold to form a solid film. In a study by Bejo et al. (2020), pea flour/chitosan films were found to exhibit antimicrobial properties and good mechanical strength, making them suitable for food packaging applications. In another study by M'hemdi et al. (2018), pea flour was combined with potato starch and glycerol to produce biodegradable films that demonstrated promising oxygen and water vapor barrier properties. Datta et al. (2019) used pea protein isolate to produce electrospun nanofibers that exhibited excellent barrier properties, suggesting their potential use in food packaging. Additionally, the study found that incorporating plant extracts into the nanofibers could further enhance their antimicrobial activity. Overall, these studies demonstrate the potential of pea flour-based packaging materials to enhance the shelf life and quality of food products, while also contributing to a more sustainable and environmentally friendly packaging industry.

1.3.3. Plasticizers

By reducing intermolecular tensions between polymer chains, plasticizers are frequently added in film solutions to promote flexibility, elongation, and ductility. The type and amount of plasticizers can alter the thermal and barrier properties of films having a protein or starch base. Glycerol is frequently used as a plasticizer because of its high efficiency, fast polymer dispersion, and interaction; however, other substances including lipids and water may be useful in this manner. Edible film materials, like the rest of synthetic polymers, need property modifiers to enhance the mechanical and physical characteristics of the coating. The edible coating/film materials contain plasticizers, just like synthetic plastics, to overcome the brittleness caused by strong intermolecular interactions. Polyols, mono/di or oligosaccharides, lipids, and their derivatives are the most widely used edible plasticizers (Choi & Han, 2001). Plasticizers improve the adhesiveness of the film while also increasing the

flexibility of the polymer chains and their mechanical resistance, minimizing the possibility of discontinuities and brittle zones. They are added to biopolymers to reduce their brittleness and increase their toughness, and to obtain a filmogenic solution. (Lin et al, 2000).

According to Farhan and Hani (2017), plasticizer usage improved the films' thermo-plasticity but decreased their resistance. Turbiane and Kieckbusch (2011) assert that higher amounts of hydrophilic plasticizers can be used to enhance elongation. However, this plasticization of the film also results in an increase in hydrophilicity and water vapor permeability and a decrease in tensile strength. The solubility was confirmed to be controlled by the type of plasticizer by Müller et al (2008). Since glycerol interacts better with the film matrix and exhibits greater water resistance in its interactions with the network, the authors were able to confirm that it increased the solubility of the films by expanding the free volume between the chains and making it simpler for water to diffuse The film is still acceptable despite the increased solubility because the product's application determines if the solubility is adequate or not.

Plasticizers are well known to increase the free volume, and make the film solution more thinner, as well as enhance water molecule mobility across the matrix at high concentrations, enhancing WVP (water vapor permeability). Glycerol is also hydrophilic, which makes it easier to absorb water. On the other hand, it's also possible that an antiplasticization effect can occur. Low concentrations of glycerol reduced moisture migration rates in starch films having low amylose. This effect is due to the plasticizer's strong hygroscopicity and hydrophilic properties, which hold water and prevented its diffusion through the film (Diaz et al., 2019). Higher glycerol concentrations, however, could yield a reduction in tensile strength and elastic modulus while increasing elongation (Kocakulak et al., 2009).

1.4. Phenolic Compounds

Phenolic compounds are found in a wide range of plants, both edible and non-edible, and have been shown to have different biological effects, including antioxidant activity. The antioxidant components of plant materials have recently attracted the attention of researchers, food producers, and consumers as a potential future trend for the preservation of health and fight against cancer and coronary heart disease. The most prevalent antioxidants in the human diet are polyphenols. Phenolic compounds, as antioxidants, limit the free radical formation, which have harmful consequences and are thus significant in the reduction of disease risk. They have been shown to be effective against certain types of cancer.

Other parameters affecting phenolic extraction include particle size, solvent, extraction method, storage duration, and presence of other molecules (Naczk and Shahidi, 2004). Many extraction solvents used for phenolics include methanol, ethanol, acetone, water, propanol, and ethyl acetate, as well as their different mixtures. By altering the sample-to-solvent ratio, improvent of the phenolic extraction can be seen. Polyphenolic extracts always contain a mixture of phenolic and non-phenolic components. In order to isolate the necessary phenolic components from the crude extract, additional purification may be required. There are several challenges because there is no common method for isolating all types of phenolics. Flavonoids, which are the most common type, are made up of flavones, flavonols, flavanones, flavanols, isoflavonoids, and anthocyanins, all of which consist of the same basic molecule structure. To adsorb target phenolics, harmful gas-solid and liquid-solid phase adsorption methods are used (Albishi et al., 2013). Terpenoids, phenolics, and alkaloids are types of compounds that have an aromatic ring with hydroxyl groups. (Robards, Prenzler, Tucker, Swatsitang, and Glover,1999).

The physiology of plants and cellular metabolism are significantly influenced by phenolic compounds. They provide many purposes in plants, like sensory qualities (astringency, taste), pollination, physical structure, resistance to pests and predators,

UV light, and protection from oxidative damage (Fraga, 2010). Major classes of phenolic substances can be seen in Table 2. According to their distribution in nature, phenolic compounds are classified as simple phenols, aldehydes, hydroquinone, pyrocatechol, resorcinol, flavonoids, phenolic acids, and coumarins which are widely distributed molecules, and lignin and tannin which are polymers (Vermerris & Nicholson, 2008). Moreover, phenolic compounds have physiological properties such as antimicrobial, anti-inflammatory, anticarcinogenic, antiallergenic, and antimutagenic effects. By scavenging reactive oxygen species, they can protect DNA from oxidative damage (Balasundram et al., 2006; Robards et al., 1999). Few studies have been done about phenolic compounds of onion skin powder in literature, where antioxidant and antimicrobial effects are studied.

Antioxidants are bioactive phenolic compounds that greatly slow down or prevent the oxidation of a substrate when they are present in low concentrations in comparison to the substrate. Phenolic compounds like butylated hydroxyanisole, butylated hydroxytoluene, tertbutylhyroquinone, propyl gallate, and ascorbic acid, erythrrobic acid, and ascorbyl palmitate are approved for usage in studies and food. Tocopherols and their derivatives, carotenoids, antioxidant enzymes, and a wide variety of phenolic components, mostly from plants, are examples of natural antioxidants. It is widely understood how significant food-based antioxidants are for both food stability and for providing crucial antioxidants. However, the usage of these compounds comes with a number of drawbacks, including sensitivity to high temperatures and light, high volatility, restricted solubility, and undesired flavor and odor. As a result of these features, their functionality is limited (Ayala Zavala et al 2008; Fang and Bhandari 2010). Some encapsulation technologies can therefore successfully alleviate these disadvantages.

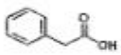
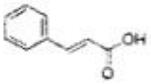
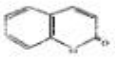
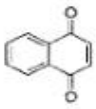
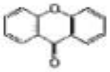
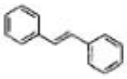
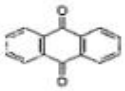
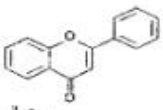
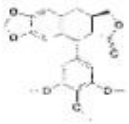
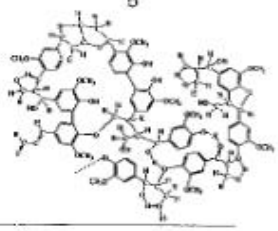
According to Saucedo-Pompa et al. (2009), adding ellagic acid to a candelilla wax matrix has a significant impact on enhancing the quality and shelf life of avocados. The correlation coefficient between total phenolic content and antioxidant activity is found 0.837. Other researchers have also found a correlation between phenolic

compounds and antioxidant activity (Cordenunsi et al., 2005; Nile & Park, 2016; Somawathi et al., 2014). It was found that the amount of antioxidants in microcapsules and the active component of total phenolics were closely linked (Settharaksa et al., 2012).

On the other hand, using food-grade materials, such as onion skin, an edible, antimicrobial film could be produced that is also biodegradable and reduces the use of synthetic polymers (Padgett et al., 1998). *L. monocytogenes, B. cereus,* and *E. coli* O157:H7 have been recognized as the most potent pathogens related to food related diseases, and their contamination of food products has become a severe public concern. One possible strategy to stop pathogen contamination and the growth of microorganisms that cause food spoilage on the surface of the product is antimicrobial packaging (Kandasamy et al, 2005). As a result, the utilization of agricultural biopolymers that are fully biodegradable and eco-friendly, has a big potential role in the food supply chain.

Antimicrobials have been used in edible films to prevent the growth of bacteria, yeasts, and molds on various food surfaces. These antimicrobials include benzoic acid, propionic acid, nisin, and lysozyme. Antimicrobial edible films have been produced using zein (corn) and wheat gluten. Due to their high hydrophobicity, these proteins are soluble in aqueous ethanol (Cagrı et al, 2004). Antimicrobial diffusion through an edible film is affected by the film (type, production procedure), food (pH, water activity), hydrophilic properties, and storage conditions. Controlling antimicrobial release from edible films is essential.

Table 2. Major classes of phenolic compounds **(**Fraga, 2010)

Class	Basic skeleton	Basic structure
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarins, isocoumarins	C_6-C_3	
Chromones	C_6-C_3	
Naphthoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	
Lignans and neolignans	$(C_6$-$C_3)_2$	
Lignins	$(C_6$-$C_3)_n$	

Diseases like high blood pressure, diabetes, and obesity have become abundant in recent years. People are keen to eat more functional foods made from natural supplements since they help avoid certain diseases. Foods containing phenolic and antioxidant components (such as fruits and vegatables) enable this. With its

antimicrobial, anticancer, and antioxidant properties, as well as a high phenolic content, onion is a functional food. According to studies, onion skin contains a high amount of antioxidant and phenolic compounds. So, onion skin can be advised to be used for its antioxidant activity. Furthermore, onion skin contains a significant color pigments derived from phenolic compounds, so rather than throwing away onion skin, these pigments can be used in the cosmetic products (Akdeniz, 2017).

The composition of onion varies depending on its maturation, habitat, storage duration, and cultivar (Abayomi & Terry, 2009). It is composed of water (89.1%), carbohydrates (9.3%), protein (1.1%), fat (0.1%), vitamins and minerals (Nile & Park, 2013). Onion's flavor is because of substances containing sulphur that are formed by the cleavage of sulphoxides (Griffiths et al., 2002). Furthermore, onion contains a significant percentage of dietary fiber and a good soluble to insoluble dietary fiber ratio, both of which are significantly linked to physical and metabolic effects. Flavonoids and alkenyl cysteine sulphoxides are the two main chemical groups. Onion flavonoids are divided into two groups: flavonols and anthocyanins (giving onions their red color)(Benítez et al., 2011; Griffiths et al., 2002).

Due to the rapid growth of phytopathogenic agents like *Sclerotium cepivorum*, wastes are inappropriate for animal feed or landfill suppression (Roldánet al., 2008). As a result, waste processing and utilization could be a feasible option. According to a recent research, sulfhydryl groups can be utilized to inhibit the polyphenol oxidase (PPO) enzyme, causing enzymatic browning and can negatively affect the organoleptic and visual characteristics of food (Kim, Kim, & Park, 2005).

Flavonoids are the most abundant phenolic compound in plants. They have a low molecular weight since they are made up of 15 carbon atoms with a C6-C3-C6 skeleton. Flavonoids are divided into six subgroups based on the type of heterocycle (Figure 1), which are flavones, flavanols (cathechins), flavanones, flavonols, anthocyanidins and isoflavonoids (Balasundram et al., 2006).

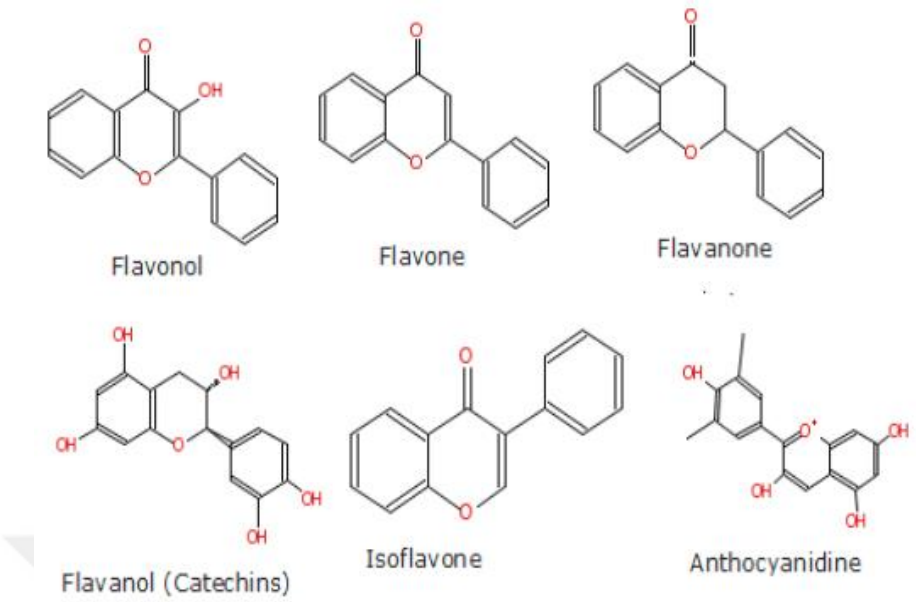


Figure 1. Chemical composition of the main groups of flavonoids (Spencer & Crozier, 2012)

As phenolic compounds are susceptible to environmental factors, they may degrade during the heating processes. Various studies that studied how the total phenolic content of grape seed flour, apple pomace, and 6 onion types changed over temperature and time found fluctuations (Candrawinata et al., 2014; Ross et al., 2011; Sharma et al., 2015). Some types may increase with heat application, while others may decrease. As a result, it is not possible to generalize the change of total phenolic content by thermal treatment. Cleavage of esterified and glycosylated bonds caused an increase in total phenolic content during heating. As a result, the bonded phenolics were released, resulting in an increase in total phenolic content (Sharma et al., 2015). Furthermore, the interconversion of phenolic compounds was another reason for the increase in total phenolic content (Soong & Barlow, 2004). However, as they were sensitive to environmental factors, heating might have degraded the phenolic compounds after a certain time (Cavalcanti et al., 2011). The degradation temperature and time of phenolic compounds can differ depending on the type.

1.5. Homogenization Techniques

Homogenization techniques are crucial in the preparation of casted edible biofilms. This is important for both the structural integrity of the biofilm and its functional properties, such as its mechanical strength and its ability to act as a barrier to water and gases.

One common method of homogenization is mechanical disruption, which uses physical means to break up the biofilm. This can be done using a variety of tools, such as a sonicator, a homogenizer, or a bead mill. The choice of tool depends on the specific properties of the biofilm, as well as the desired level of homogenization. For example, sonication is often used for homogenizing low-viscosity biofilms, while homogenizers are used for high-viscosity biofilms. High-speed homogenizers are the most abundant and cost-effective method for homogenizing oil and aqueous phase mixtures among the food industry. According to one study, to achieve a smaller droplet size, a high-pressure homogenizer with its additional shear force should be used in addition to the high-speed homogenizer (Karthik & Anandharamakrishnan, 2016). Ultrasonic emulsification is a dispersing technique used for solid-liquid nanodispersion. On the other hand, high-intensity ultrasonic waves with frequencies higher than 20 kHz are used in ultrasonic homogenizers. These ultrasonic waves generate high pressure and shear, causing droplet disruption via the turbulent effect and cavitation mechanisms (McClements, 2005). Cavitation is the working principle of ultrasound. Cavitation is the vapor cavity formation and collapse in a liquid mixture (Jafari, He, and Bhandari (2016). Due to the change in local velocity, local pressure is reduced to the vapor pressure of the liquid. After that, with collapse of cavities, ultrasonic waves propagate through film matrix, breaking up the droplets into small sizes (He et al., 2016; Jafari et al., 2007).

Another homogenization method is chemical disruption, which uses chemicals to dissolve or weaken the biofilm matrix. This can be done using enzymes, such as

lysozyme or chemicals such as EDTA or Triton X-100. The choice of chemical depends on the biofilm matrix's composition and the desired level of homogenization. For example, enzymes such as lysozyme can be used to break down the cell walls of bacteria in the biofilm, while chemicals such as EDTA can be used to chelate metal ions that are involved in the formation of the biofilm matrix. A third homogenization method is enzymatic disruption, which uses enzymes to break down the biofilm matrix. This can be done using enzymes such as collagenase or chitinase, which degrade specific components of the biofilm matrix. The choice of enzyme depends on the biofilm matrix's composition and the desired level of homogenization (Hwang et al., 2017; Li et al., 2020; Sanchez-Garcia et al., 2018).

A study by Salgado et al. (2020) compared these techniques for the preparation of active films with thyme essential oil. According to this study, both techniques resulted in films with good antimicrobial activity, but high-speed homogenization was more effective in reducing the particle size of the essential oil, resulting in improved film properties. In another study, researchers compared the effect of high-speed homogenizer and ultrasonic treatment on the properties of chitosan-based edible films. The study found that both treatments improved the mechanical and barrier properties of the films, but ultrasonic treatment was more effective in improving the water vapor permeability of the coatings (Sánchez-González et al., 2013). In another study the effect of high-speed homogenizer and ultrasonic treatment on the cassava starch based edible biodegradable films were investigated (López-García et al., 2020). It was found that both treatments improved the tensile strength and elongation at the break of the films, but ultrasonic treatment was more effective in reducing the water solubility of the films In conclusion, both high-speed homogenizer and ultrasonic treatment can improve the properties of edible films, but their effectiveness may vary depending on the type of film-forming material and the specific properties that need to be improved.

1.6. Objectives of the study

The development of new sustainable packaging solutions is becoming increasingly important in today's world, where environmental concerns are at the forefront of many people's minds. Pea flour-based biofilms have shown to be a promising a sustainable packaging material due to their biodegradability and ability to act as a barrier against oxygen and moisture. Additionally, onion skin powder has been shown to have phenolic compounds, and antioxidant and antimicrobial properties, making it a potential candidate for incorporation into films to create active packaging. By combining these two materials, it may be possible to develop a novel biopolymer based active film that offers improved functionality and sustainability compared to traditional packaging materials. Therefore, this thesis aims to develop and to characterize pea flour-based films containing onion skin powder to be used as active packaging.

By using the casting method, it is possible to produce films with the desired properties for use in various applications, including food packaging. Additionally, the incorporation of active compounds into biopolymer based films can offer improved functionality, which can increase the shelf life, stability and safety of products. This can be especially significant in food industry, where spoilage and contamination can lead to economic losses and negative impacts on public health. The use of active biopolymer based films can also reduce the need for synthetic preservatives and other additives, which can decrease costs associated with their use and reduce environmental impacts. Therefore, the development of cost-effective and sustainable biopolymer based films using the casting method and incorporating active compounds has a significant impact on the food industry by improving food safety, reducing waste, and lowering production costs.

The main objective of the study is to develop and characterize an active edible coating containing pea flour and onion skin powder extract. Moreover, the effects of different homogenization methods, pH, and the onion skin powder extract amount on the properties of active films were also investigated.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Onion skin was bought from the market. In order to get rid of the stones and dust, the skin was washed, then put down to a baking tray and dried at 105 °C oven for 24 hours. Dried onion skin was stored at 25 °C, then ground by a grinder (Pulverisette 16, Fritsch GmbH Milling and Sizing, Germany) and sieved by a sieve having an opening of 200 micrometer.

The chemicals used in the experiments, which were acetic acid, ethanol, gallic acid, sodium hydroxide, sodium carbonate, and Folin-Ciocalteau's phenol reagent were all bought from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2 Preparation of solution and the film

The pea flour content was kept constant at 5% in all samples. Firstly, pea flour is mixed with deionized water and mixed by a magnetic stirrer (Daihan Scientific Co, KR) at 1000 rpm for 20 minutes to ensure total dissolution. Then, pH of film solutions was set to 8 and 11 by using 2M NaOH solution with a pH Meter (SG2 SevenGoTM, Mettler, Toledo, USA). Then, they were heated to 85°C in a water bath for 2 h. Then, they were cooled down to 40 °C. Then, glycerol (5% content in all samples) is added to the solutions. In order to extract phenolic compounds from onion skin powder, 10 g of ground onion skin powder was weighed and mixed with 100 ml of (70:30 v/v) ethanol and water. Then the pH adjustment is done by sodium hydroxide. After 1 hour, the extract is added to the film solution through microfilter

(0.45 μm Gema Medical Filter, Spain). This stage is done simultaneously while the other process are being done mentioned above. Then, the onion skin powder extract is homogenized by either high-speed homogenizer (HSH) for 3 min at 7000 rpm (IKA T25 Digital Ultra-Turrax; IKA®-Werke GmbH & CO. KG, Staufen, Germany) or by ultrasonic homogenizer (Sonic Ruptor 400; OMNI International, Kennesaw, GA, USA) (U) for 10 min at 160 W power with 60% pulse. Next, the film solution is poured into petri dishes and kept in the oven for 13 h at 40 °C.

2.3. Characterization of films

2.3.1. Water vapor permeability (WVP) of films

According to Sothornvit and Krochta's modified ASTM method E96, the WVP of fibers was determined (Sothornvit & Krochta, 2000). Films were placed over the permeation vessels filled with water. Until steady-state was established, samples were weighed every two hours in dessicators containing silica gel. Two replicates of each experiment were undertaken. With the slope of the weight loss against time shown in the equation below, it was simple to calculate the water vapor permeation rate and permeability values of coatings (1).

$$WVP\ (kg / Pa \times s \times m) = \frac{G \times x}{t \times A \times S(R_1 - R_2)} \qquad (1)$$

where G = water vapor flow (kg), x = film thickness (m), t = time (s), A= test area (m^2), S = saturated water vapor pressure (Pa) at measured temperature, R1 = relative humidity in the cups, R2 = relative humidity in the desiccator.

Film thicknesses were found by using digital micrometer (LYK 5202, Loyka, Ankara, Turkey).

2.3.2. Total Phenolic Content Analysis

According to Beretta et al. (2005), the Folin-Ciocalteau method was used to calculate the total phenolic content (TPC). In this method, the Folin-Ciocalteau reagent was reduced with sodium carbonate in the presence of phenolic compounds, and as a result of this reduction, color change was seen (Beretta et al., 2005). 1 ml of onion skin powder extract is added to 20 ml of ethanol:water (70:30 v/v) mixed with Vortex (ZX3, VELP Scientifica, Usmate, MB, Italy) for 30 seconds.

500 µl of diluted sample and 2.5 ml of 0.2 N Folin-Ciocalteau (2N, SIGMAALDRICH F9252) reagent were mixed in tubes with a vortex for 30 seconds to determine the TPC. 2 ml of sodium carbonate solution (75g/L, SIGMAALDRICH S7795) was added to the mixture after it was left in a dark place for 5 minutes, and the mixture was then vortexed again for 5 seconds. For one hour, all samples were kept at 25°C in the dark. Then, the absorption values at 760 nm were measured by using a UV/VIS spectrophotometer T70 (PG Instruments LTD, UK). A calibration curve was prepared with gallic acid solution at different concentrations (20, 40, 60, 80, 100 ppm in ethanol:water (70:30 v/v) mixture). By the calibration curve, total phenolic content was defined as mg gallic acid equivalents (GAE)/g dry weight with the equation (2).

$$\text{Total phenolic content} = \left(\frac{\text{Milligrams of GAE}}{\text{Grams of dry film weight}}\right) = \frac{A \times \text{mL of solution} \times \text{dilution rate}}{\text{Grams of dry film weight}} \quad (2)$$

2.3.3. Moisture Content and Solubility analysis

The method used by Vargas et al. was modified to determine the moisture content of the films. Using rectangular sheets that were cut into 2 × 1 cm, moisture content was

measured gravimetrically. The films were dried in oven for 24 hours at 105 °C. The samples' initial and final weights were noted. For each film, measurements were made in triplicate. The equation (3) was used to determine the moisture content.

$$\text{Moisture content}\,(\%) = \frac{W_i - W_f}{W_i} \times 100 \qquad (1)$$

(3) where W_i was the initial weight of the films (g) and W_f was the final weight of the films after drying (g).

where W_i was the initial weight (g) and W_f (g) was the final weight of the films after drying.

Salgado et al. method was used to determine water solubility. Weighted films that measured 2 by 1 cm were mixed with 50 mL of pure water. The mixture was then stirred at a speed of 100 rpm. After 24 h, the undissolved part was filtered and brought to constant weight previously. The remaining component was put into the oven and kept at 105 °C for 24 hours. Experiments were made in triplicates. The equation below was used to determine water solubility.

$$\text{Water solubility} = \frac{W_i \times (100 - \text{MC}) - W_f}{W_i \times (100 - \text{MC})} \times 100 \qquad (2)$$

where W_i was the initial weight of the films (g), W_f was the final weight after drying (g), and MC was the moisture content of the films (%). (4)

where W_i was the initial weight (g), W_f was the final weight after drying (g), and MC (%) was the moisture content of the films.

2.3.4. Mechanical Analysis

Using a texture analyzer (Brookfield Texture Analyzer CT310K, Middleborough, USA), the tensile strength and Young's (Elastic) modulus were calculated. Measurements were carried out at a speed of 10 mm/min applying a 0.1 N preload.

Film samples of 20 mm wide by 70 mm long were prepared. The tests were carried out four times at room temperature.

2.3.5. Color of films

The color of films was measured as color coordinates in CIELAB color space (L*, a*, b*) and total color change (ΔE*) by Datacolor device with respect to the standard values which are 95.75, 0.16, and 1.28 for L,a and b values, respectively. Two replicates were used in each measurement. The total color change (ΔE*) was measured by the equation below.

$$\Delta E^* = \sqrt{(L^*_{\text{film}} - L^*_{\text{standard}})^2 + (a^*_{\text{film}} - a^*_{\text{standard}})^2 + (b^*_{\text{film}} - b^*_{\text{standard}})^2} \quad (5)$$

2.3.6 Fourier-transform infrared (FTIR) analysis

Film samples were placed under Fourier transform infrared (FTIR) spectroscopy using an IR-Affinity1 FTIR spectrometer with an Attenuated Total Reflectance (ATR) device from Shimadzu Corporation in Kyoto, Japan. Analysis was done at a frequency range of 600-4000 cm^{-1} with 32 scans and a resolution of 4 cm^{-1} resolutions.

2.4. Statistical analysis

In the study, the independent variables used were pH (8,11), onion skin content (0.5%, 1%), and homogenization technique (HSH, U). These variables and pea flour and glycerol content were determined by preliminary studies to attain the most ideal comparison. To decide whether there was a significant difference between these variables on dependent variables (TPC, color, water vapor permeability, moisture content, solubility, mechanical strength, Fourier-transform infrared (FTIR)

analysis), the analysis of variance (ANOVA) was performed by MINITAB (Version 18). Tukey's Multiple Comparison Test was applied for comparisons of samples ($p \leq 0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Water vapor permeability (WVP) of the films

Edible films had substantially higher water vapor permeabilities than plastic films (Bamdad et al, 2006). Amylose and amylopectin are excellent biomaterials with barrier qualities. Starch can be combined with various protein types to increase their packaging characteristics. The combination of starch and proteins reduced water vapor permeability while increasing tensile strength (Jagannath et al., 2003). According to Padgett et al., hydrophobic amino acids (leucine, proline, and alanine) in zein protein provide good moisture barrier properties (1998). Additionally, plasticizers enhance water molecule mobility across the matrix at high concentrations, so do WVP (Diaz et al, 2019). One study emphasized the beneficial effects of adding lipid to starch based films on the barrier qualities of microcapsules. Gas and water vapor permeability was dramatically reduced as a result of the hydrophobicity property of lipid molecules (Garcia, Martino, & Zaritzky, 2000). Like other edible films, the pea protein concentrate film's ability to act as a moisture barrier was reduced as plasticizer content increased. Overall, relative humidity has a significant effect on the WVP of pea protein concentrate film (Choi and Han, 2001).

The WVP results are shown in Table 3. According to the results, none of the factors (pH, homogenization technique, and onion skin powder extract) had a significant effect on solubility ($p>0.05$) overall. Both homogenization techniques applied high shear and weakened the film matrix, especially the molecules of amylose and amylopectin (Cheng et al., 2010). In this study WVP of films varied in 1.82-4.74 x 10^{-11} $kgPa^{-1}s^{-1}m^{-1}$ are comparable with other films in the literature. For example, edible coatings of pea protein isolate, peanut protein films and pea starch films showed 6.3-

$12.5x10^{-11}$ $kgPa^{-1}s^{-1}m^{-1}$ (Kowalczyk & Baraniak, 2011), 7.79-$9.15x10^{-11}$ $kgPa^{-1}s^{-1}m^{-1}$ (Jangchud et al., 1999), and 2.99-3.33 $kgPa^{-1}s^{-1}m^{-1}$ (Choi et al., 2016), respectively.

Table 3. Effect of pH, onion skin powder extract (%) and homogenization method on WVP, solubility and moisture of films

pH	Onion skin powder extract (%)	Homogenization method	WVP$x10^{-11}$ ($kgPa^{-1}s^{-1}m^{-1}$)	Solubility (%)	Moisture (%)
8	0.5	HSH	4.74±0.15[a]	78.76±5.09[a]	10.13±0.40[a]
8	0.5	U	1.82±0.05[a]	69.76±4.64[a]	6.54±0.25[c]
8	1	HSH	3.51±0.27[a]	70.75±2.70[a]	9.76±0.66[ab]
8	1	U	4.16±0.14[a]	76.76±4.36[a]	9.17±0.34[ab]
11	0.5	HSH	3.10±0.18[a]	72.52±4.64[a]	6.46±0.40[c]
11	0.5	U	4.07±0.08[a]	62.28±4.29[a]	9.71±0.99[b]
11	1	HSH	2.67±0.14[a]	80.48±2.97[a]	7.09±0.53[c]
11	1	U	2.41±0.09[a]	75.66±3.13[a]	6.97±0.21[c]

*The significant difference between samples is shown by different letters in the same column ($p \leq 0.05$)

3.2. Solubility of the films

Being water-resistant and having a long shelf life, coatings with low water solubility are appropriate for packaging foods that are high in moisture. However, in order to

achieve efficient biodegradability, high solubility is also essential (Yildiz et al, 2021). By strengthening the interaction between water molecules, the hydrophilic groups in glycerol contribute to hydrophilicity, enabling better solubility of the coatings (Rodsamran & Sothornvit, 2019). Choi and Han (2001) found that films made of heat-denatured whey protein isolate, which contained lactoglobulin (whey protein) molecules, had stronger intramolecular interactions in aqueous conditions.

The solubility results of samples are shown in Table 3. According to the results, none of the factors (pH, homogenization technique, and onion skin powder extract) had a significant effect on solubility ($p>0.05$). Both homogenization techniques applied high shear and weakened the film matrix, especially the molecules of amylose and amylopectin (Cheng et al., 2010). In addition, the increased pressure in ultrasonication can result in the implosion of small gas bubbles and cause a localized high shear in the polymer, so depolymerization may occur (Borah et al., 2017). The weakened matrix might have reduced the positive effect of having more homogenous films (Kocakulak et al, 2019) and enhanced solubility of proteins (Aydoğdu et al, 2019) at alkali pH. On the other hand, in this study the onion skin powder extract content might not be enough to make more interactions with water, which could directly affect solubility.

3.3. Moisture Content

Moisture content results of the triplicates of samples are shown in Table 3. The pH and homogenization method showed a significant effect on moisture ($p<0.05$). The pH could change the homogeneity of the solution which could affect the paths of water molecules evaporating during the drying of the solution (Yildiz et al, 2021). Moreover, when the protein's net charge changed, the interaction between water and protein molecules was also changed (Aydoğdu et al, 2019). Ultrasound treatment can contribute to the breakage of the molecule bonds because of the high pressure caused by the collapse of the cavitation bubbles (Cheng, Chen, Liu, Ye, & Ke, 2010). So,

during drying the evaporation of moisture through paths might become easier byultrasonication (Eylül, 2021). According to literature, ultrasonication was shown to alter the hydrophobicity of the proteins (Chandrapala et al., 2011), which directly impacted how water and protein molecules could interact. This reduces the free volume and decreases the evaporation.

3.4. Total Phenolic Content (TPC) of the films

The average TPC of the onion skin powder in 70% EtOH/water diluted solution (v/v) in different pH values (8 and 11) were found as 68 and 76 (mg GAE/g sample), respectively. This is the indication that at higher pH, phenolics compounds are more stabilized (Krungkri et al., 2019). Singh et al. (2009) studied the polyphenolic compounds from different extracts of red onion skin to find TPC and potent antioxidant activities. It was documented that higher TPC (384.7 ± 5.0 mg GAE/g) of red onion were found for the ethyl acetate extract than dichloromethane extract (23.1 ± 0.9 mgGAE/g) and n-butanol (102.1 ± 1.8 mgGAE/g). This is related to the polarity of the solvent (Santas et al, 2008). Phenolics can break down at different temperatures and times, depending on their nature (Akdeniz et al., 2017).

The TPC results for samples are shown in Table 4. According to the Table, the onion skin powder extract had a significant effect on TPC ($p<0,05$). The reason could be that onion skin contained a significant amount of phenolic compounds (quercetin, myricetin and other flavonols) so when the extract content was increased the overall phenolic content was also increased (Akdeniz et al., 2017). On the other hand, pH had no significant effect ($p>0,05$). Quinones and other unstable intermediates, i.e., resonance structures, are formed as the pH increases. The phenolics in the film become less stable as a result. Thus, autoxidation triggered by an increase in pH causes the phenolic compounds to degrade (Pinho, Soares, & Henriques, 2015). In addition, phenolic acid can hydrolyze in alkaline and strongly acidic conditions, which will result in a reduction in the amount of phenolic (Medina et al, 2007). These

factors may counteract the increased total stabilized phenolics as the pH increases.

Table 4. Effect of pH, onion skin powder extract (%) and homogenization method on total phenolic content (TPC)

pH	Onion skin powder extract (%)	Homogenization method	TPC mg GAE/g film
8	0.5	HSH	29.04±0,89[b]
8	0.5	U	27.13±0.68[b]
8	1	HSH	39.22±0,67[a]
8	1	U	28.47±0.71[a]
11	0.5	HSH	30.97±2.70[b]
11	0.5	U	34.01±2.56[b]
11	1	HSH	31.27±2.87[a]
11	1	U	38.87±3.88[a]

*The significant difference between samples is shown by different letters in the same column ($p \leq 0.05$)

3.5. Mechanical Properties

Higher tensile properties result from covalent cross-linking in proteins of film solution that are caused by the denaturation of proteins by heat (Bamdad et al, 2005). It is evident that proteins heated to an alkaline pH have an impact on the film's physical properties because it makes intramolecular and intermolecular cross-links more likely to be formed (Kim, Weller, Hanna, & Gennadios, 2002). Marcos et al. (2010) stated that the measurement of the mechanical properties manifests vital information on the ability of packaging materials to maintain their integrity under the influence of different stresses occurring at the processing, handling, and storage steps. Elastic modulus is also a property of a material that gives hints about its strength and durability. Mechanical tests are vital when using different materials for various industrial applications (Han et al, 2013). Elastic Modulus results are shown in Table 5. Results are comparable with nanofibers which are microwave treated, containing 5% carob flour (Uygun, 2019).

The tensile strength results of the films shown in Table 5 are comparable with the pea protein concentrate films containing 50% glycerol of Choi and Han (2001), where film thickness (5.83 mm) is twenty times more than the film thickness in this study, and wheat gluten edible coatings having 10% gallic acid showed 0.53 MPa tensile strength (Hager et al., 2012). According to the results, onion skin powder extract and homogenization techniques had a significant effect on tensile strength, and homogenization method and pH had a significant effect on elastic modulus ($p<0.05$). For tensile strength, higher phenolic content might have formed more hydrogen bonds between water molecules, and polymer-polymer interactions are weakened so tensile strength is directly affected (Kocakulak, 2019). Ultrasonication could affect the brittleness by disintegrating the molecules because of the high pressure triggered by the collapse of the cavitation bubbles, so this affected the matrix of the films. As pH 11 was more far from the pI of proteins, hydrophobic interactions might have increased while ionic interactions reduced so unfolded

proteins might have been dissolved. Due to the same charged groups repelling each other, the polymer chain was stretched, and a tight structure was formed (Kocakulak et al, 2019). Cheftel et al. (1985) found that heating proteins at alkaline pH facilitates the development of intra- and intermolecular cross-links. These cross-links assisted heated SPI films become tougher (higher tensile strength) and less flexible (lower elastic modulus).

Table 5. Effect of pH, onion skin powder extract (%) and homogenization method on tensile strength and elastic modulus

pH	Onion skin powder extract (%)	Homogenization method	Tensile Strength (MPa)	Elastic Modulus (N/m^2)	Thickness (mm)
8	0.5	HSH	0.62 ± 0.08^{a}	28.01 ± 1.82^{a}	0.382 ± 0.007^{a}
8	0.5	U	0.38 ± 0.07^{abc}	16.92 ± 1.61^{ab}	0.341 ± 0.006^{ab}
8	1	HSH	0.26 ± 0.06^{bc}	14.17 ± 0.36^{a}	0.322 ± 0.008^{bc}
8	1	U	0.20 ± 0.05^{c}	13.08 ± 0.82^{ab}	0.359 ± 0.007^{ab}
11	0.5	HSH	0.46 ± 0.08^{ab}	22.21 ± 1.18^{ab}	0.261 ± 0.011^{de}
11	0.5	U	$0.21+0.03^{bc}$	$9.35+0.82^{b}$	0.295 ± 0.018^{cd}
11	1	HSH	$0.31+0.08^{abc}$	$16.80+0.47^{ab}$	0.222 ± 0.009^{e}
11	1	U	$0.27+0.07^{bc}$	$15.80+1.42^{b}$	0.245 ± 0.007^{e}

*The significant difference between samples is shown by different letters in the same column ($p \leq 0.05$)

3.6. Color Results

The results are shown in Table 6. The pH and homogenization technique had a significant effect on a* (redness), b* (yellowness) and all of the factors showed a significant effect on L* (lightness) and ΔE* (degree of total color change). At pH 11, films showed higher a*, b* and ΔE* values similar to the findings obtained with soy protein coatings (Mauri et al, 2008). The yellow color of films came from the natural color of pea. Changing the pH alters the interactions between proteins and phenolic substances so bond formation might become easier, so this made the film matrix more homogenous. As a result of this interaction, the L*, b* and ΔE* showed an increase. Since the homogeneity is also directly affected by the homogenization methods, the significant effect on L* and ΔE* can be explained by alteration of the light permeated which is related with homogeneity (Kocakulak et al, 2019).

Table 6. Effect of pH, onion skin powder extract (%) and homogenization method on lightness (L*) and total color change (ΔE*)

pH	Onion skin powder extract(%)	Homogenization method	L*	ΔE	a*	b*
8	0.5	HSH	36.11±0.75^{f}	60.34±0.08^{e}	19.53±1.08^{ab}	55.88±4.69^{a}
8	0.5	U	48.81+1.93^{b}	60.66±0.99^{f}	16.18±1.65^{b}	43.29±2.52^{a}
8	1	HSH	46.83+2.65^{d}	51.06±0.03^{c}	21.97±0.04^{ab}	40.13±0.28^{a}
8	1	U	50.89+0.63^{b}	50.81±0.78^{c}	17.67±0.29^{b}	41.68±3.97^{a}
11	0.5	HSH	38.47+3.08^{f}	44.20±0.21^{d}	24.21±0.21^{a}	34.70±2.33^{ab}
11	0.5	U	54.77+0.54^{a}	39.29±0.36^{d}	20.47±1.70^{ab}	22.62±0.88^{b}
11	1	HSH	42.49+2.22^{e}	57.74±0.35^{a}	23.90±0.34^{a}	35.21±1.86^{ab}
11	1	U	51.40+2.35^{c}	56.61±0.21^{b}	20.81±0.16^{ab}	21.50±0.39^{b}

*The significant difference between samples is shown by different letters in the same column ($p \leq 0.05$)

3.7. Fourier Transform Infrared Spectroscopy (FTIR)

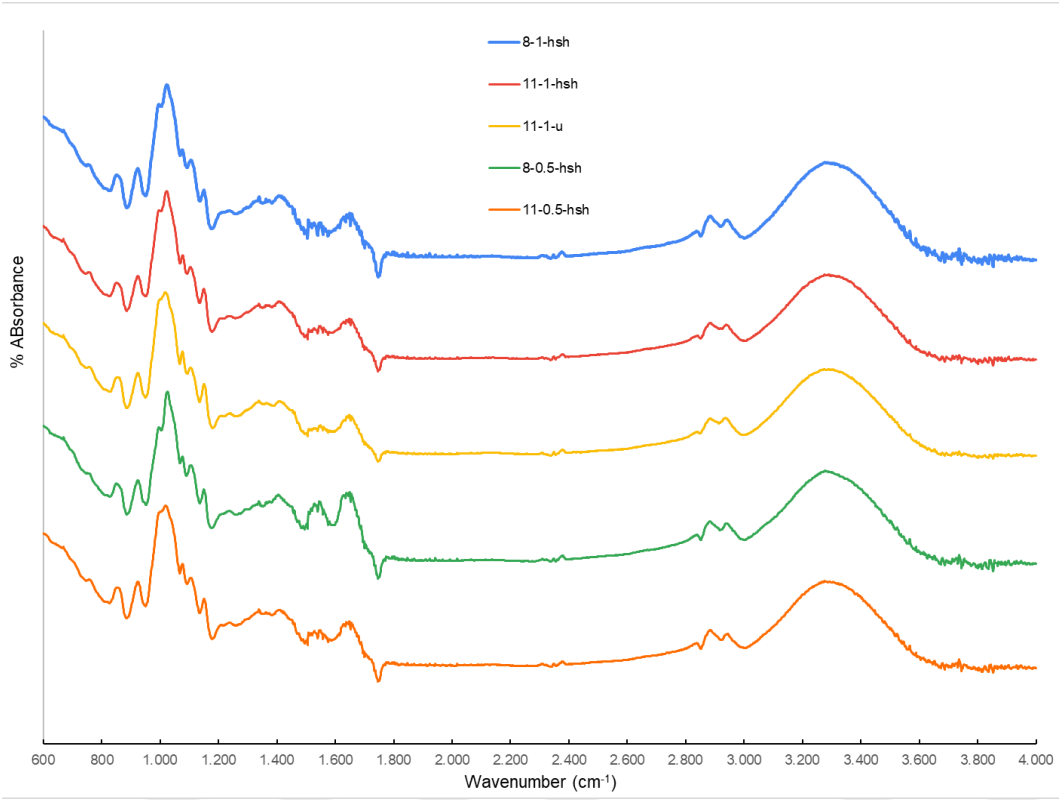


Figure 2. FTIR spectra of pea flour based films containing onion skin powder extract

As seen by the Figure 2, C–O bond stretchs around 900-980. Anhydroglucose ring (O–C) shows a peak around 1000. C–O–H bond stretchs around 1100-1200. These are indicative values for starch in pea flour. Shifting pH from 8 to 11 also shifts the peak from 1028 to 1024. The peak around 3000 is due to –CH. Around 1500 there is a C–N stretching and angular deformation of N–H. The peak around 3300 is due to O–H (Ahmed et al, 2010). Similar peaks are observed in chickpea flour based coatings including gallic acid (Kocakulak et al, 2019).

Shifting pH from 8 to 11 shifts the peak from 1646 to 1656. In addition, shifting occurs from 3314 to 3288. These are related to changes in the interaction of molecules in amides related to pea proteins. Also, there are shifts due to the onion skin powder extract content difference. Interaction of phenolics between starch,

protein, and water changes. For example, when onion skin powder extract content shifted from 0.5% to 1% the peak also shifted from 3293 to 3297, and 1649 to 1655. The peak around 1650 indicating C=O stretching within a carboxylic group is the evidence of phenolic substance interactions with the protein. The band around 670 cm^{-1} was characteristic of the presence of aromatic compounds (Pelissari et al, 2013). This could have been linked with aromatic amino acids (e.g., phenylalanine, tryptophan) found in peas and lentils (Samarayanaka et al, 2017) or to the phenolic compounds of which sinapic acid as the phenolic acid and (+)-catechin and (-)-epicatechin as flavonoids constitute a major part (Xu et al, 2010).

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

In this study, to determine the ideal coating application for pea flour based films containing onion skin powder extract, different combinations were applied. Water vapor permeability, tensile strength, elastic modulus, solubility, total phenolic content, moisture, color, and FTIR analysis were evaluated. Research on this topic is needed to produce novel edible active film applications with developed functionality and superior film characteristics focusing both on varying onion skin powder extract, pH, with different homogenization techniques.

This study revealed that casting is a trustworthy technique for biodegradable active film development to be applied in the packaging industry. The effects of pH, onion powder extract and different homogenization methods on pea flour-based edible films were evaluated. According to the results, low pH and high-speed homogenization was better for moisture, tensile strength, elastic modulus and yellowness values while ultrasonication was better for color properties. Solubility and water vapor permeability didn't show any significant effect on films. As pH was increased, the values for total color change, lightness and redness values increased. Ultrasonication was more effective on lightness rather than other color properties and high onion skin powder extract was better for TPC and total color change. The ideal composition of the film in terms of mechanical properties, moisture content, yellowness values is the one with pH of 8, containing 0,5% onion skin powder extract and treated with high-speed homogenization. To conclude, the casting technique proved to be an efficient method to protect bioactive compounds suggesting a great potential for active packaging material. Future research can be combining bioactive ingredients such as phenolics, enzymes, amino acids, gallic acid, flavors into the

casting method to produce edible films providing long shelf life that can be utilized as active package materials. In order to develop new edible active film applications with increased functionality and great sensory performance, research on this topic is therefore necessary. Along with cross-linking treatments like glutaraldehyde, acetic acid, gallic acid, sodium sulphate, graphen oxide additon or UV induced crosslinking on pea flour films, and their combinations, additional research should be conducted to determine the ideal protein/plasticizer ratio.

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APPENDICES

. STATISTICAL ANALYZES

Table A.1. Three-way ANOVA and Tukey's Comparison test for WVP values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	0,2523	0,2523	1,05	0,322
homogenization	1	0,3491	0,3491	1,46	0,247
onion s. powder extract	1	0,2446	0,2446	1,02	0,329
ph*homogenization	1	1,1082	1,1082	4,63	0,049
ph*onion s. powder extract	1	3,9064	3,9064	16,33	0,001
homogenization*onion s. powder extract	1	1,5890	1,5890	6,64	0,022
ph*homogenization*onion s. powder extract	1	6,8806	6,8806	28,77	0,000
Error	14	3,3484	0,2392		
Total	21	19,2596			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,489052	82,61%	73,92%	60,73%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

ph*homogenization	N	Mean	Grouping
8 hsh	6	3,71333	A
11 u	6	3,24000	A
11 hsh	4	3,04000	A
8 u	6	3,00167	A

Means that do not share a letter are significantly different.

Table A.2. Three-way ANOVA and Tukey's Comparison test for Solubility values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	38,21	38,214	0,96	0,341
homogenization	1	141,41	141,414	3,55	0,076
onion s. powder extract	1	10,58	10,578	0,27	0,613
ph*homogenization	1	7,35	7,347	0,18	0,673
ph*onion s. powder extract	1	363,51	363,509	9,12	0,007
homogenization*onion s. powder extract	1	19,21	19,215	0,48	0,496
ph*homogenization*onion s. powder extract	1	81,48	81,482	2,04	0,170
Error	18	717,70	39,872		
Total	25	1393,81			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6,31443	48,51%	28,48%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping
8-0,5-t	3	82,2967	A
11-1-t	4	80,4775	A
8-0,5-u	3	73,3667	A
11-1-u	3	72,8700	A
8-1-u	3	72,4167	A
8-1-t	4	70,7450	A
11-0,5-t	3	69,8200	A
11-0,5-u	3	65,8833	A

Means that do not share a letter are significantly different.

Table A.3. Three-way ANOVA and Tukey's Comparison test for Moisture Content values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	18,1106	18,1106	53,58	0,000
homogenization	1	3,8669	3,8669	11,44	0,004
onion s. powder extract	1	0,0038	0,0038	0,01	0,917
ph*homogenization	1	20,0121	20,0121	59,21	0,000
ph*onion s. powder extract	1	6,7195	6,7195	19,88	0,000
homogenization*onion s. powder extract	1	0,1881	0,1881	0,56	0,467
ph*homogenization*onion s. powder extract	1	6,5969	6,5969	19,52	0,000
Error	15	5,0701	0,3380		
Total	22	61,1971			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,581381	91,72%	87,85%	81,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample.	N	Mean	Grouping		
8-0,5-t	3	10,4633	A		
8-1-t	3	10,3167	A	B	
8-1-u	3	8,8700	A	B	
11-0,5-u	3	8,7533		B	
11-0,5-t	3	6,8000			C
11-1-u	3	6,7900			C
11-1-t	3	6,6333			C
8-0,5-u	2	6,4900			C

Table A.4. Three-way ANOVA and Tukey's Comparison test for TPC values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	1,443	1,443	0,55	0,474
homogenization	1	0,611	0,611	0,23	0,639
onion s. powder extract	1	156,500	156,500	59,26	0,000
ph*homogenization	1	171,746	171,746	65,03	0,000
ph*onion s. powder extract	1	0,010	0,010	0,00	0,953
homogenization*onion s. powder extract	1	12,468	12,468	4,72	0,051
ph*homogenization*onion s. powder extract	1	76,289	76,289	28,89	0,000
Error	12	31,693	2,641		
Total	19	485,791			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,62515	93,48%	89,67%	77,26%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

onion s. powder extract	N	Mean	Grouping	
1	10	33,9833	A	
0,5	10	28,2733		B

Means that do not share a letter are significantly different.

Table A.5. Three-way ANOVA and Tukey's Comparison test for Tensile Strength values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	0,01977	0,01977	1,68	0,215
homogenization	1	0,06730	0,06730	5,70	0,031
onion s. powder extract	1	0,31853	0,31853	26,99	0,000
ph*homogenization	1	0,01556	0,01556	1,32	0,269
ph*onion s. powder extract	1	0,06325	0,06325	5,36	0,035
homogenization*onion s. powder extract	1	0,02702	0,02702	2,29	0,151
ph*homogenization*onion s. powder extract	1	0,01977	0,01977	1,68	0,215
Error	15	0,17700	0,01180		
Total	22	0,71197			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,108628	75,14%	63,54%	44,01%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

ph*homogenization*onion s. powder extract	N	Mean	Grouping		
8 hsh 0,5	3	0,566667	A		
11 hsh 0,5	3	0,513333	A	B	
8 u 0,5	2	0,500000	A	B	C
11 hsh 1	3	0,253333		B	C
11 u 0,5	3	0,223333		B	C
11 u 1	3	0,220000		B	C
8 hsh 1	3	0,213333		B	C
8 u 1	3	0,166667			C

Means that do not share a letter are significantly different.

Table A.6. Three-way ANOVA and Tukey's Comparison test for Elastic Modulus values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	88,695	88,695	8,91	0,008
homogenization	1	74,903	74,903	7,52	0,013
onion s. powder extract	1	16,637	16,637	1,67	0,212
ph*homogenization	1	0,750	0,750	0,08	0,787
ph*onion s. powder extract	1	86,937	86,937	8,73	0,008
homogenization*onion s. powder extract	1	27,794	27,794	2,79	0,112
ph*homogenization*onion s. powder extract	1	195,526	195,526	19,64	0,000
Error	18	179,208	9,956		
Total	25	656,791			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3,15532	72,71%	62,10%	41,28%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

ph*homogenization	N	Mean	Grouping	
8 hsh	7	20,0221	A	
8 u	6	16,9433	A	B
11 hsh	7	16,6417	A	B
11 u	6	12,8783		B

Means that do not share a letter are significantly different.

Table A.7. Three-way ANOVA and Tukey's Comparison test for Lightness (L*) values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	6,027	6,027	10,31	0,012
homogenization	1	686,178	686,178	1174,31	0,000
onion s. powder extract	1	15,249	15,249	26,10	0,001
ph*homogenization	1	7,812	7,812	13,37	0,006
ph*onion s. powder extract	1	24,157	24,157	41,34	0,000
homogenization*onion s. powder extract	1	122,656	122,656	209,91	0,000
ph*homogenization*onion s. powder extract	1	5,085	5,085	8,70	0,018
Error	8	4,675	0,584		
Total	15	871,839			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,764412	99,46%	98,99%	97,86%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample.	N	Mean	Grouping				
11-0,5-u	2	55,265	A				
8-0,5-u	2	51,510		B			
8-1-u	2	51,510		B			
11-1-u	2	48,095			C		
8-1-t	2	44,220				D	
11-1-t	2	40,265					E
8-0,5-t	2	35,400					F
11-0,5-t	2	34,105					F

Means that do not share a letter are significantly different.

Table A.8. Three-way ANOVA and Tukey's Comparison test for Redness (a*) values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	49,175	49,1752	10,68	0,01
homogenization	1	52,309	52,3091	11,36	0,01
onion s. powder extract	1	3,891	3,8908	0,84	0,38
ph*homogenization	1	0,166	0,1661	0,04	0,85
ph*onion s. powder extract	1	3,793	3,7928	0,82	0,39
homogenization*onion s. powder extract	1	0,023	0,0233	0,01	0,95
ph*homogenization*onion s. powder extract	1	0,636	0,6360	0,14	0,72
Error	8	36,840	4,6050		
Total	15	146,833			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2,14592	74,91%	52,96%	0,00%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

ph*homogenization	N	Mean	Grouping	
11 hsh	4	24,0500	A	
8 hsh	4	20,7475	A	B
11 u	4	20,6375	A	B
8 u	4	16,9275		B

Means that do not share a letter are significantly different.

Table A.9. Three-way ANOVA and Tukey's Comparison test for Yellowness (b*) values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	1120,41	1120,41	27,73	0,001
homogenization	1	338,84	338,84	8,38	0,020
onion s. powder extract	1	80,87	80,87	2,00	0,195
ph*homogenization	1	54,43	54,43	1,35	0,279
ph*onion s. powder extract	1	70,02	70,02	1,73	0,225
homogenization*onion s. powder extract	1	39,09	39,09	0,97	0,354
ph*homogenization*onion s. powder extract	1	62,21	62,21	1,54	0,250
Error	8	323,29	40,41		
Total	15	2089,15			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6,35698	84,53%	70,98%	38,10%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

ph*homogenization	N	Mean	Grouping	
8 hsh	4	48,0000	A	
8 u	4	42,4850	A	
11 hsh	4	34,9525	A	B
11 u	4	22,0600		B

Means that do not share a letter are significantly different.

Table A.10. Three-way ANOVA and Tukey's Comparison test for Total Color Change (ΔE*) values of film solutions of 8-0.5-hsh, 8-1-hsh, 11-1-hsh, 11-0.5-hsh, 11-0.5-u, 11-1-u, 8-0,5-u, 8-1-u.

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
ph	Fixed	2	11; 8
homogenization	Fixed	2	hsh; u
onion s. powder extract	Fixed	2	0,5; 1

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
ph	1	252,89	252,890	511,54	0,000
homogenization	1	40,74	40,736	82,40	0,000
onion s. powder extract	1	805,85	805,850	1630,06	0,000
ph*homogenization	1	0,11	0,114	0,23	0,644
ph*onion s. powder extract	1	6,09	6,089	12,32	0,008
homogenization*onion s. powder extract	1	1,52	1,519	3,07	0,118
ph*homogenization*onion s. powder extract	1	25,08	25,075	50,72	0,000
Error	8	3,95	0,494		
Total	15	1136,23			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,703114	99,65%	99,35%	98,61%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

sample	N	Mean	Grouping				
11-1-t	2	67,135	A				
11-1-u	2	60,655		B			
8-1-t	2	57,745			C		
8-1-u	2	56,610			C		
11-0,5-t	2	51,0550				D	
11-0,5-u	2	50,815				D	
8-0,5-t	2	44,205					E
8-0,5-u	2	39,295					F

Means that do not share a letter are significantly different.