

# **A DISSERTATION ON**

**Development and Characterization of Sodium Alginate and Lemon  
(*Citrus limon*) Waste-Based Biodegradable Film**

**SUBMITTED TO THE  
DEPARTMENT OF BIOENGINEERING  
FACULTY OF ENGINEERING & INFORMATION  
TECHNOLOGY  
INTEGRAL UNIVERSITY, LUCKNOW**



**IN PARTIAL FULFILMENT  
FOR THE  
DEGREE OF MASTER OF TECHNOLOGY  
IN FOOD TECHNOLOGY**

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I, **Alisha Ahmad**, a student of M.Tech Food Technology (II Year/ IV Semester), Integral University have completed my six months dissertation work entitled “**Development and Characterization of Sodium Alginate and Lemon (*Citrus limon*) Waste-Based Biodegradable Film**” successfully from **Integral University**, under the able guidance of **Dr. Khwaja Osama and Dr. Owais Yousuf**.

I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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Certificate that **Ms. Alisha Ahmad** (Enrollment Number 1700101932) has carried out the research work presented in this thesis entitled “**Development and Characterization of Sodium Alginate and Lemon (*Citrus limon*) Waste-Based Biodegradable Film**” for the award of **M. Tech Food Technology** from Integral University, Lucknow under our supervision. The thesis embodies results of original work and studies carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution. The dissertation was a compulsory part of **M. Tech Food Technology** degree.

We wish her good luck and bright future.

A handwritten signature in blue ink, appearing to read 'Owais'.

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## TO WHOM IT MAY CONCERN

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**DATE:**

**ALISHA AHMAD**

## LIST OF ABBREVIATIONS

<b>ANOVA</b>	Analysis of variance
<b>AOAC</b>	Association of Official Analytical Chemists
<b>ASTM</b>	American Society for Testing and Materials
<b>BPA</b>	Bisphenol A
<b>FAO</b>	Food and Agricultural Organization
<b>G</b>	$\alpha$ -1,4-L-guluronic acid
<b>HDPE</b>	High Density Polyethylene
<b>LDPE</b>	Low-Density Polyethylene
<b>LSD</b>	Least significant difference
<b>M</b>	$\beta$ -1,4-D-mannuronic acid
<b>MPa</b>	Megapascal
<b>MMT</b>	Million Metric Ton
<b>MSW</b>	Municipal Solid Waste
<b>PET</b>	Polyethylene terephthalate
<b>PLA</b>	Polylactic acid
<b>PP</b>	Polypropylene
<b>PVA</b>	Polyvinyl alcohol
<b>PVC</b>	polyvinyl chloride
<b>SPC</b>	Soy protein concentrate
<b>SPI</b>	Soy protein isolate
<b>SPSS</b>	Statistical Package for the Social Sciences
<b>T<sub>g</sub></b>	Glass transition temperature
<b>TGA</b>	Thermo gravimetric Analysis
<b>UNEP</b>	United Nations Environment Programme
<b>WVP</b>	Water vapor permeability



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## ABSTRACT

The aim of the study was to develop a biodegradable packaging film using sodium alginate and waste lemon peel. For this purpose, different concentration of both sodium alginate and lemon peel powder was taken in ratios Sodium alginate: lemon peel powder:: 0.5:1, 0.5:2, 0.5:3, 0.5:4, 1:1, 1:2, 1:3, 1:4, and 1.5:1, 1.5:2, 1.5:3, 1.5:4 to obtain 12 film specimens. The films were plasticized using glycerol (5% v/v) and crosslinked with a 2% CaCl<sub>2</sub> solution. The films were developed using the solution-casting method. Dried films obtained were visually homogenous, flexible, and without any cracks. The films were analyzed for various physical, mechanical, optical, and light barrier properties. The physicochemical properties of the film were thickness ( $0.07 \pm 0.01$  to  $0.27 \pm 0.01$  mm), grammage ( $246.72 \pm 0.35$  to  $445.78 \pm 0.23$  g/m<sup>2</sup>), moisture content ( $10.38 \pm 0.31\%$  to  $14.14 \pm 0.37\%$ ), ash content ( $3.23 \pm 0.05\%$  to  $5.13 \pm 0.04\%$ ), water solubility ( $64.14 \pm 0.07\%$  to  $81.91 \pm 0.23\%$ ), and water absorption ( $19.20 \pm 1.18\%$  to  $38.54 \pm 1.45\%$ ). The developed films displayed good mechanical properties with a maximum tensile strength of  $6.42 \pm 0.02$  MPa for sample SA1:LPP1 having sodium alginate: lemon peel ratio of 1:1. Elongation at break and burst strength ranged from  $4.67 \pm 0.12$  to  $15.27 \pm 0.12\%$  and  $0.23 \pm 0.01$  to  $0.66 \pm 0.02$  MPa, respectively. The highest retraction ratio of  $98.33 \pm 0.29\%$  was observed in sample SA1:LPP1. Lemon peel had a significant effect on the color values. An increase in its concentration resulted in an increase in the  $a^*$  and  $b^*$  values and decreased the  $L^*$  value. Lemon peel also improved the light barrier properties of the film with transparency ranging from  $3.56 \pm 0.47$  to  $7.03 \pm 0.09$ . The light transmittance decreased with an increase in lemon peel powder which was in accordance with obtained opacity. The films also showed 83.80 to 90.76% biodegradability in a soil burial test. Based on the results, the film sample SA1:LPP1, sodium alginate: lemon peel ratio of 1:1 had better properties as compared to other films. Therefore, the use of lemon peel can serve as a promising source for the development of biodegradable packaging.

**Keywords:** Lemon peel; Sodium alginate; biodegradable film; tensile strength; food packaging.

# CHAPTER 1

## INTRODUCTION

In recent decades, the rapid expansion of global consumerism has led to an alarming surge in plastic waste and environmental degradation. Plastic packaging, which constitutes a significant portion of this waste, poses a severe threat to the ecosystem, marine life, and human health (Kibria et al., 2023). Plastic packaging for food, while widely used and convenient, has several serious drawbacks that have far-reaching repercussions for the environment and human health (Jambeck et al., 2015; North & Halden, 2013). Certain food-packaging polymers, such as polycarbonate and polyvinyl chloride (PVC), include hazardous compounds like bisphenol A (BPA) and phthalates (Goodman & Peterson, 2014; J.-M. Liu et al., 2020). These chemicals may leach into the food, potentially causing developmental disorders, hormone disruption, and higher cancer risks (Caporossi & Papaleo, 2017; Giuliani et al., 2020; Hahladakis et al., 2018). The manufacturing of these plastics necessitates substantial quantities of fossil fuels which leads to resource depletion and a rise in greenhouse gas emissions. This exacerbates climate change and its related environmental issues. With the passage of time, plastic packaging breaks into small fragments known as microplastics, which contaminate the food if they come into contact, thereby entering the human body, and giving rise to serious health hazards (Revel et al., 2018). Moreover, the majority of food packaging is non-biodegradable, implying that it cannot naturally decompose into harmless elements. As a result, it remains in the environment, leading to persistent pollution and ecological damage. To address this pressing environmental crisis, there is an urgent need for sustainable and eco-friendly alternatives. Biodegradable packaging emerges as a solution that offers the potential to mitigate these adverse impacts of plastic waste, reduce carbon footprint, and foster a more sustainable future (A. Ahmad & Yousuf, 2021; Guillard et al., 2018; Noronha et al., 2014).

Sustainable packaging has witnessed a notable advancement in the past few years due to its safety, renewability, and biodegradability. Despite these favorable properties, the commercial utilization of biopolymer-based packaging films has been limited due to the high cost of commercial biopolymers (Taherimehr et al., 2021). Interestingly, discarded fruit processing by-products, usually considered as waste, contain valuable biopolymers such as

polysaccharides, proteins, and lipids, which serve as suitable matrices for developing food packaging films (Gupta et al., 2022). These by-products are rich in bioactive compounds such as polyphenols, pigments, alkaloids, and minerals which adds to the functionality of these films (Banerjee et al., 2017; Messinese et al., 2023). Therefore, by-products from fruit and juice processing offer a promising alternative to expensive biopolymers for the production of food packaging films.

In the realm of citrus fruits, global lemon production is approximately 20 million tons annually (FAO, 2021). The byproducts generated from the processing of lemons constitute around 50-60% of the original mass of the lemon (Balu et al., 2012; Satari & Karimi, 2018a). Improper disposal of these byproducts has led to a substantial environmental burden. Lemon waste contains a substantial amount of pectin (30-40%, dry basis), cellulose, hemicellulose, and lignin (Bátori et al., 2017). Unfortunately, these byproducts have not been fully utilized so far. Lemon peels, being a rich source of pectin has the potential to form biodegradable film due to their gelling ability. However, films based entirely on pectin exhibit poor mechanical strength (Chaichi et al., 2019). On the other hand, Sodium alginate, a polysaccharide obtained from marine brown algae is extensively used as a substrate for a biodegradable film on account of good mechanical properties (Frent et al., 2022). To address the limitations of single-component films, a novel approach is to combine two or more components via physical or chemical crosslinking to enhance their performance (Nur Hazirah et al., 2016). For instance, when pectin and sodium alginate, two polyanionic polysaccharides are combined in the presence of calcium ions, they can form an “egg-box” model. Both polysaccharides undergo chain-chain association and form synergistic mixed gels in the presence of  $\text{Ca}^{2+}$ . This combination proves beneficial in improving water resistance and enhancing synergistic physicochemical properties (Gohil, 2011).

Currently, there is no existing documentation on the direct utilization of lemon peel powder for developing biodegradable films. In this study, biodegradable films were developed using pectin and sodium alginate via crosslinking with calcium chloride. The films were plasticized using glycerol to increase their flexibility. Film forming solution was prepared by dispersing and homogenizing different compositions of the lemon peel and sodium alginate in distilled water. The films were formed using the solution casting method. Employing food

processing by-products for the production of packaging material presents a novel and impactful solution that has the potential to uplift the polymer and packaging industry. Furthermore, it can play a crucial role in mitigating environmental pollution.

### **1.1 Research Objectives**

1. To develop the biodegradable film using Sodium alginate and lemon waste.
2. To study the various physicochemical properties of the developed biodegradable film.
3. To study the effect of varying Sodium alginate and lemon waste compositions on biodegradable films.

## **CHAPTER 2**

### **REVIEW OF LITERATURE**

#### **2.1. Overview of plastic packaging**

Packaging plays a vital role in preserving and safeguarding food as it proceeds through the supply chain, thereby extending its shelf life. According to estimates, about 37% of packaging materials are composed of plastics, making them the most widely utilized packaging material (UNEP, 2022). Plastic packaging accounts for 26% of the total plastic market share. Food and beverage packaging accounts for approximately 60% of plastic packaging (Groh et al., 2019). The thermoplastics most commonly employed for food packaging include PP, PET, LDPE, HDPE, and nylon (Ncube et al., 2020). The use of plastic packaging has become dominant mainly because of its advantageous attributes like low production cost, mechanical strength, flexibility, and gas or water barrier properties (Dhall & Alam, 2020). Their widespread global use has led to significant environmental pollution. Nonetheless, the global recycling rate for plastic stands at a mere 3%. Improper disposal and insufficient recycling practices result in the persistence of plastics in the environment. The buildup of plastic materials poses a significant environmental hazard to the well-being of terrestrial and aquatic animals (Thompson et al., 2009; Thushari & Senevirathna, 2020). Over time, these plastics slowly degrade, breaking into microplastics that can readily enter the food chain, posing potential harm to life on Earth (Ncube et al., 2020). Additionally, the manufacturing processes involved in producing plastics generate substantial amounts of chemical pollutants. Municipal solid wastes (MSW) are becoming an increasingly concerning issue, primarily because of the challenges associated with disposing of plastic materials in landfills. Based on a projection, the amount of unmanaged plastic waste is expected to triple to a range of 155-265 million metric tons (MMT) per year if the widespread utilization of plastic persists at its current rate (Lebreton & Andrady, 2019).

In response to escalating concerns about health and the environment, there has been a notable surge in interest regarding the development of sustainable and biodegradable packaging to uphold the quality of fruits and vegetables (Chavan et al., 2023; Chisenga et al., 2020). The emergence of such biodegradable packaging presents a compelling alternative to curbing large volumes of waste generated from conventional plastic packaging materials. The

research and development in the realm of biodegradable packaging constitute a fascinating and distinctive area of exploration within the food packaging domain, holding immense potential both commercially and environmentally.

## **2.2. Biodegradable packaging**

Packaging materials based on polymers that are entirely or partially derived from renewable sources may solve the aforementioned problems (Imre et al., 2019). These sources include plants, animals, microorganisms, seafood, wood, and agricultural residues. Naturally, such materials are biodegradable and compostable. When utilized for packaging purposes, they can undergo degradation through the activity of living organisms after disposal (Peelman et al., 2013). Biodegradation is a fragmentation process triggered by heat, moisture, and microbial enzymes that converts complex molecular substances into smaller compounds (Ashok et al., 2016). As a result, they transform into CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>O, inorganic compounds, or biomass, effectively returning to nature in an environmentally friendly manner (Dhall & Alam, 2020; Flury & Narayan, 2021).

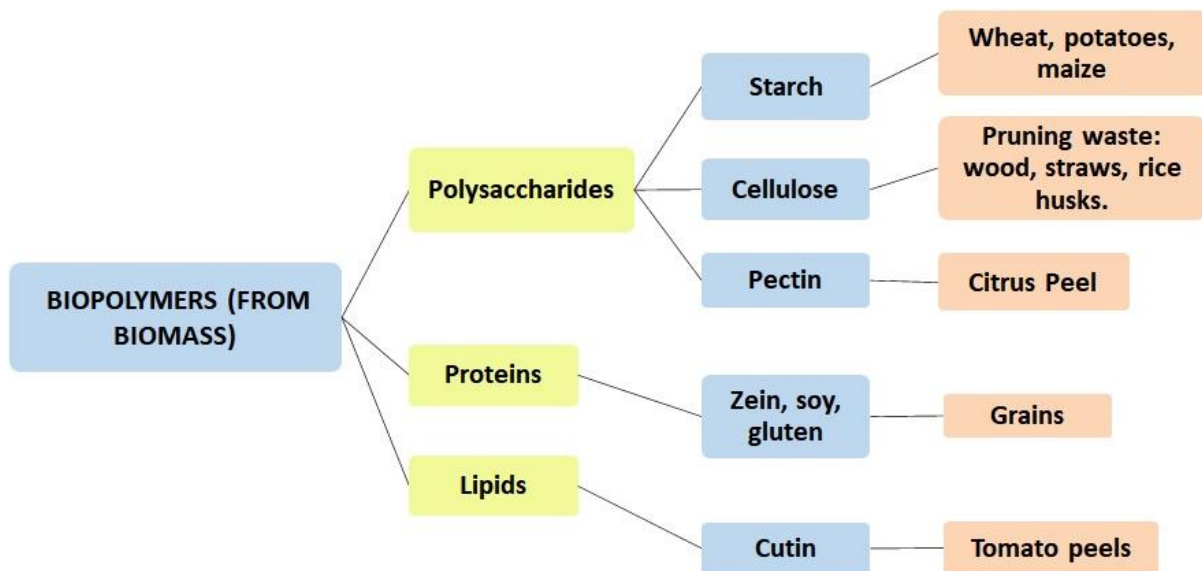
The market for biodegradable polymer-based packaging was assessed at \$4.65 billion in 2019, and it is projected to expand to \$9 billion by the year 2025 (Zhang et al., 2022). Biodegradable packaging derived from agricultural waste has also gained popularity as an eco-friendly and sustainable substitute for their non-biodegradable counterparts. Thus, biodegradable packaging holds significance not only in the context of reducing dependence on petroleum resources but also because they tend to biodegrade under normal environmental conditions. The success and growth of the biodegradable packaging industry rely on factors such as consumer preferences for environmentally friendly products, government policies promoting green packaging, and the utilization of renewable and biobased resources.

## **2.3. Biopolymers and their sources.**

Biodegradable polymers are large molecules with an elongated chain-like structure characterized by a high molecular weight. These polymers occur naturally in living organisms, either present in plants and animals or obtained from biomass. These polymers encompass naturally occurring substances like proteins, cellulose, starches, and other polysaccharides, with or without modifications, as well as chemically synthesized ones derived from natural

monomers like lactic acid. These materials are biodegradable, non-toxic, and sustainable, meaning they can easily break down in the environment through natural processes. Biopolymers offer two significant advantages over petroleum-based polymers: biodegradability and renewability. The general classification of biopolymers is provided below(Shaikh et al., 2021; Sid et al., 2021; Zhang et al., 2022):

1. Polymers originating from biomass (natural polymers) e.g. polysaccharides (starch, pectin, cellulose, alginate), proteins (whey, gluten), etc. as shown in Figure 2.1.
2. Polymers synthesized from renewable biobased monomers e.g. polylactic acid (PLA).
3. Polymers produced by microorganisms or bacteria e.g. polyhydroxy-alkanoates.



**Figure 2.1. Major classes of biopolymers obtained from biomass**

Biopolymers emulate the characteristics of conventional polymers and can be processed through various methods such as solution casting, melt-mixing, electrospinning, thermo-pressing, and extrusion (Yadav et al., 2018). During processing, polymers usually incorporate additives. Hence, it is essential to use biodegradable additives in conjunction with biodegradable packaging polymers to preserve their biodegradability (Havstad, 2020). Biobased polymers have not only replaced existing polymers in numerous applications but have also introduced novel combinations of properties (Murariu & Dubois, 2016). Particularly,

biobased materials hold significant value in three key areas of food-related applications: food packaging, food coating, and edible films (Fabra et al., 2014).

## **2.4. Films based on natural polymers**

Natural polymers and compounds derived from them hold significant importance in the food industry. The remarkable feature of these biopolymers lies in their diverse range, offering countless possibilities for structural modifications and applications. These polymers are sourced from plants, animals, and marine organisms. Examples are polysaccharides (starch, cellulose, pectin, alginate, chitosan), proteins (zein, casein, whey, and soy protein), and lipids. Biodegradable polymers possess several advantageous characteristics, including biodegradability, antioxidant, and antimicrobial properties, renewability, added nutritional value, low cost, widespread availability, and most importantly, they do not negatively impact the environment as opposed to conventional plastics (Sam et al., 2016). Studies related to the development of biodegradable films by utilization of these polymers are emerging.

### **2.4.1. Polysaccharides-based films**

Polysaccharides are gaining attention as a substitute for synthetic polymers. Starch, cellulose, pectin, alginates, chitin, and chitosan are some of the polysaccharides employed for this purpose. Polysaccharides are abundantly available biopolymers having a variety of functions. They consist of monosaccharide units joined together by glycoside linkages. Polysaccharides are selectively permeable to Carbon dioxide (CO<sub>2</sub>) and Oxygen (O<sub>2</sub>), thereby delaying the respiration and ripening process of fruits and vegetables by limiting the availability of O<sub>2</sub> (Cha & Chinnan, 2004). Starch is one of the most common polysaccharides present naturally in the roots of cassava, the pith of sago, the tuber of potato, and the seeds of corn, rice, and wheat. Also referred to as hydrocolloid biopolymer, it comprises two monomeric glucose units namely, amylose and amylopectin. Amylose consists of linear, helical chains linked with (1, 4)  $\alpha$ -D-glucopyranosyl units. Amylopectin has a branched chain formed by (1, 6)  $\alpha$ -D-glucopyranosyl linkages. Researchers have utilized starch-based biopolymers to develop biodegradable films. Corn starch and corn husk fiber have been employed to enhance film strength (Ibrahim et al., 2019). (Niu et al., 2021) developed a biodegradable film using potato starch, glycerol, and gelatin. The formulation was optimized via Response Surface Methodology and evaluated for physical properties namely, thickness,



water solubility (WS), tensile strength (TS) elongation at break (E %), and light barrier properties (Transmittance T %). The formulated film showed excellent properties with TS of 4.47 MPa, WS- 43.64%, E% 109.91%, and T% of 41.21% at 500 nm. Starch can be blended with other biopolymers or synthetic polymers in order to upgrade its properties. It allows for maintaining biodegradability and at the same time improves the mechanical strength. (Aini, 2010) observed that starch when blended with chitosan and gelatin increased moisture retention capacity. Arrowroot starch-based films with higher tensile strength and barrier properties, successfully extended the shelf life of cherry tomatoes for up to 10 days (Abdillah & Charles, 2021).

Cellulose, primarily composed of linear anhydroglucose polymers, stands as the most abundant natural polymer on the planet. Films developed using 75% cocoa pod husk cellulose and 25% sugarcane bagasse fibre demonstrated the lowest water vapor permeability and water absorption (Azmin et al., 2020). Cellulose nanocrystals are an emerging class of nanomaterials with potential applications. Cellulose nanofibre films crosslinked with  $\text{Ca}^{2+}$  ions exhibited 90% transmittance, high tensile strength (303MPa) as well as higher thermal stability and, water resistance (K. Lee et al., 2021). Chitin, extracted from shrimp cells and crab, is a linear copolymer with a  $\beta$ -1,4 linkage between N-acetyl glucosamine and N-glucosamine. It is the second most abundantly available biopolymer after cellulose. It exhibits properties such as biocompatibility, biodegradability, and film-forming ability, and can be easily blended with other polymers (Gzyra-Jagiela et al., 2019; M. Liu et al., 2012). The solubility of chitin is very low, so it is usually blended for packaging applications. The biopolymer films developed from chitin extracted from crab (*Portunus pelagicus*) shells had a tensile strength of 44.22 MPa as compared to commercially available plastic with only 18.90 MPa (Fernando et al., 2016). Chitosan is formed from partial N-deacetylation of chitin. Its compact and crystalline structure as well as strong hydrogen bonding makes it insoluble in water. Chitosan-based films generally have a smooth surface and display adhesiveness and cohesiveness (Hahn et al., 2019; D. Lee et al., 2013). Several studies on the use of chitosan for film formation have been reported (De Carli et al., 2022; Kumar et al., 2021; Riaz et al., 2020).

#### **2.4.2. Protein-based films**

Collagen is a protein found in animal connective tissue that is primarily made up of various polypeptides consisting of proline, hydroxyproline, glycine, and lysine. The glycine content influences the flexibility of the collagen chain. Natural collagen can be transformed into highly structured, three-dimensional scaffolds having high tensile strength and are innately biocompatible, biodegradable, and nontoxic when applied exogenously (Chattopadhyay & Raines, 2014). Collagen has unique mechanical, physicochemical, and biological properties and can be enzymatically degraded (Nair & Laurencin, 2007). Ahmad et al., (2016) investigated the potential of acid-solubilized collagen extracted from starry triggerfish skin to form a biodegradable film. The microstructure of the acid-solubilized films depicted a smooth and fine surface without any cracks. They also reported high tensile strength, elastic modulus, as well as increased glass transition temperature. Blend films based on collagen have also been developed to upgrade mechanical and barrier properties (Bhuimbar et al., 2019; Marangoni Júnior et al., 2021). The chemical degradation of collagen results in the formation of a high molecular weight polypeptide called gelatin (Nasrollahzadeh et al., 2021). Gelatin has exceptionally high water-holding capacity, gel-forming ability, and gas barrier properties and is used as starting material to produce biodegradable films for food packaging (Lu et al., 2022).

Soy protein, a residual product of the oil industry, presents an economical and eco-friendly option for crafting biofilms. It is accessible in the market as SPI (soy protein isolate, 90% protein) and SPC (soy protein concentrate, 70% protein). Unlike films made from other plant proteins, those based on SPI display greater clarity and flexibility. Nevertheless, soy protein films suffer from subpar barrier and mechanical attributes due to their hydrophilic properties (Shaikh et al., 2021). To address this limitation, these films are frequently combined with other biopolymers and plasticizers. SPI concentration at 8.65% along with 60% glycerol was found to be optimum for the development of soy protein-based edible film (Nandane & Jain, 2015). Wheat gluten, a protein constituent derived from wheat and produced as a byproduct of starch manufacturing, offers an inexpensive and feasible substitute for synthetic polymers. The consideration of wheat gluten stems from its captivating characteristics, such as its ability to form viscoelastic films, cross-link upon heating, limited water solubility, economical pricing, and widespread availability (Jansens et al., 2013; Lagrain et al., 2010;

Türe et al., 2012). Composite films developed using wheat gluten and lignocellulose are known to exhibit superior UV blocking, oxygen, and water vapor barrier properties (Chen et al., 2022).

### **2.4.3. Lipid-based films**

Lipids can be described as small, hydrophobic, and naturally occurring compounds, encompassing substances like fats, sterols, waxes, and fat-soluble vitamins. Lipid-based natural polymers find applications as edible biodegradable films and coatings (Saucedo-Pompa et al., 2009; Zafar et al., 2019). These wax polymers primarily consist of long-chain hydrocarbons and esters. While they are insoluble in water, they can dissolve in inorganic solvents. Hydrophobic substances (lipids) are commonly utilized as a barrier to impede water-vapor transfer, primarily due to their non-polar characteristics (Yousuf et al., 2022). One drawback of wax-based films in packaging applications is their temperature dependency. Researchers modified the hydroxyl groups of acetylated fatty acids to enable crosslinking between molecules, resulting in increased tensile strength (Ruiz et al., 2019).

### **2.5. Background of citrus fruit consumption and waste generation**

Citrus fruits are one of the major horticultural crops grown in the world. Globally, citrus fruits are now produced at an annual scale of around 15 million tons. Roughly three-quarters of these citrus fruits are consumed in their fresh state, while the remaining portion undergoes processing for food items like jam, jelly, juice, and essential oil (Mahawar et al., 2020). However, the consumption and processing of citrus fruits result in substantial waste generation, particularly peels, seeds, and pomace. Peels account for 60-65% of the total weight of citrus by-products, internal tissues about 30-35%, and seeds amount to 0-10% (Coman et al., 2020). These are either used as animal feed or disposed of directly into the environment. Citrus waste, if not treated properly, can cause environmental problems due to the high amount of organic matter and relatively low pH value (Angel Siles López et al., 2010). Improper disposal of these wastes can be harmful to ruminants and cause soil salinity. Consequently, the potential of citrus processing wastes remains largely untapped. Some of this waste is employed for the production of bioethanol, extraction of essential oils, pectin, dietary fiber, and flavoring agents or converted into dried pulp, molasses, purees, feed yeast, etc. They are a good source of flavonoids, ascorbic acid, essential oil, pectin, enzymes, natural antioxidants, and many other compounds (Satari & Karimi, 2018b; B. Singh et al., 2020; Suri et al., 2022). In this context, the

utilization of citrus processing wastes proves to be a valuable strategy for reclaiming these beneficial compounds and mitigating environmental pollution.

Recently, there has been a growing focus on the development of food packaging films using biopolymers and natural substances as a response to the environmental challenges posed by non-biodegradable conventional plastic packaging films (Yong & Liu, 2020, 2021). Citrus processing wastes have emerged as viable resources for developing biodegradable packaging films. Citrus by-products harbor various bioactive components like polyphenols and essential oil which can be integrated into biopolymer matrices to enhance the antioxidant and antimicrobial properties of packaging film (Han & Song, 2020; Jridi et al., 2019; Tongnuanchan et al., 2012). Moreover, citrus peels are abundant sources of pectin, which can serve as a foundational material for developing biodegradable food packaging films (Satari & Karimi, 2018b).

### **2.5.1. Pectin and its properties**

Pectin is a structural polysaccharide occurring in the primary cell walls of fruits and some vegetables, and is a good alternative to biodegradable materials (Mangiacapra et al., 2006). It consists of the methoxy esterified  $\alpha$ , D-1, 4-galacturonic acid units (Lizeth et al., 2018). Pectin is widely used for various packaging applications such as a thickening, gelling agent, emulsifier or texturizer, and as an edible coating on fresh and cut fruits or vegetables (Sucheta et al., 2019). Pectin has also been extracted from various waste biomass thereby contributing to waste management in agro and food processing industries. Pectin has the potential to form bio-based material for food packaging applications. Sources of pectin include by-products from juice processing of fruits like orange, mosambi, lime, apple, mango, and pomegranate. Perennial fruits like banana, papaya, pineapple, guava, etc. are also a rich source of pectin. By-products from these fruits are known for their film-forming ability. Brito et al. (2019) formed biodegradable films using both fruit (sweet orange, watermelon, passion fruit) and vegetable (zucchini, lettuce, spinach, mint, carrot yams, cucumber, and arugula) residue. The films obtained were homogeneous, malleable, and yellowish with high solubility. The formulation supplemented with pectin presented great technological and functional capability to produce biodegradable films. (Rodsamran & Sothornvit, 2019) reported that bioactive films from lime peel pectin can retard the oxidation of soybean oil during 30 days of storage. Sucheta

et al. (2019) found that a pectin-corn flour-based coating enhanced the shelf life of tomatoes by delaying respiration and reducing the weight loss and decay percentage. In a research conducted by Taghavi et al. (2020), bio-functional edible films based on gelatin were developed by incorporating orange peel powder (OPP) in different concentrations. The increase in OPP content resulted in films with enhanced thickness, moisture content, strength, and water vapor permeability. However, lower  $L^*$  and higher  $a^*$ ,  $b^*$ , and opacity values were observed in film incorporated with OPP. Borah et al. (2017) utilized sweet lime pomace as a pectin source and potato peel powder as a starch source to develop biopolymer film by ultrasound treatment. The films were analyzed for water vapor permeability ( $7.25 \times 10^{-9}$  g/Pa h m), water solubility ( $38.92 \pm 0.702\%$ ), moisture absorption ( $12.88 \pm 0.348\%$ ), tensile strength ( $242.01 \pm 3.074$  g) and elongation at break ( $7.61 \pm 0.824$  mm).

### **2.5.2. Lemon waste – as a biopolymer source**

Lemon (*Citrus lemon*) stands as one of the most plentiful crops globally, with an estimated production of approximately 20 million metric tons (FAO, 2021). During the processing of lemon juice, which constitutes the primary derived product, about 20-30% of the juice is obtained while around 50-60% constitutes the waste material, primarily composed of lemon peel (Di Vaio et al., 2010). Consequently, a significant quantity of lemon peel is generated on a global scale each year. Disposing of this waste presents challenges due to its minimal economic value and necessitates added costs. The presence of substantial levels of total solids, water, and an acidic pH range of 3-4 in the lemon waste can lead to significant environmental concerns if mishandled (Borah et al., 2017; Kowalska et al., 2017). To address this issue of waste reduction and reuse, numerous applications for lemon peel have been put into practice, including its use as an animal feed, fertilizer, pectin extraction, bioethanol production, and extraction of essential oils (Boluda-Aguilar & López-Gómez, 2013; Di Vaio et al., 2010; M'hiri et al., 2018).

Lemon peel contains considerable amounts of pectin (approximately, 30-40%, dry matter basis), cellulose, hemicellulose, lignin, starch, polyphenols, and flavonoids, alongside volatile substances like esters, alcohols, and terpenes (Bátori et al., 2017; Borah et al., 2017; Schmidt et al., 2015; N. Singh & Paudel, 2023). These constituents highlight both its technological attributes and nutritional qualities, underscoring the importance of recuperating and

repurposing lemon waste. (Karim et al., 2022) extracted pectin from lemon peel and utilized it for the fabrication of pectin-silica-based biodegradable film. The developed films presented excellent tensile strength (61.31 MPa), Young’s modulus (16.49 MPa), and toughness. In another study, biodegradable mulch films were developed using hydrolyzed lemon peel and low methoxy pectin in a ratio of 1:1, which demonstrated good mechanical properties (Merino & Athanassiou, 2022).

### 2.5.3. Utilization of citrus waste in packaging films

The utilization of citrus waste for the development of biodegradable packaging is an emerging area of study. So far, researchers have employed citrus by-products derived from various citrus fruits, including orange, lime, lemon, mosambi, and pomelo, for crafting active and biodegradable packaging films with enhanced functionality (**Table 2.1**). Due to the presence of a plethora of active components, citrus peel powder can serve as a filler within diverse polymer matrices like polyvinyl alcohol (Rathinavel & Saravanakumar, 2021), chitosan (Filho et al., 2020), chitosan/gelatin blend (Li et al., 2021), and starch (Chhatariya et al., 2022; Claudia Leites et al., 2021). Alternatively, the abundant presence of pectin and cellulose in citrus peel powder allows it to function as the primary material for the packaging film (Bátori et al., 2017). In such scenarios, other viscous polymers such as xanthan gum (Meydanju et al., 2022), and sodium alginate (H. Wu et al., 2019, 2020) are often introduced into the film formulation to act as adhesives. These films are generally prepared using casting techniques with glycerol as a plasticizer.

**Table 2.1. Application of citrus by-products in biodegradable packaging**

Citrus fruit	Film formulation	Findings	References
Orange	Orange peel powder (OPP), corn starch, glycerol, food-grade vinegar	Films with 40% OPP had the highest tensile strength (1.41 MPa), and lowest elongation at break (11.12%). Water absorption (145%) and solubility (37%) were highest in film with 50% OPP.	(Chhatariya et al., 2022)
Orange	Cassava starch, orange waste powder, glycerol	Incorporation of orange waste powder resulted in 3.7 MPa tensile strength, 89% elongation, and 21% water solubility.	(Claudia Leites et al., 2021)

		Films with orange waste powder had more opacity of $2.75 \text{ mm}^{-1}$ than control starch samples ( $0.51 \text{ mm}^{-1}$ ).	
Orange	Orange peel, chitosan, PVA, glycerol, acetic acid.	Orange peel increased the thickness ( $138.0 \text{ }\mu\text{m}$ ), WVP ( $0.478 \text{ g mm kPa}^{-1} \text{ h}^{-1} \text{ m}^{-2}$ ), and thermal stability (1.79 residual wt. % at $900 \text{ }^\circ\text{C}$ ). Improved light barrier property as indicated by low transparency value.	(Terzioğlu et al., 2021)
Orange	Gelatin, chitosan, orange peel essential oil, glycerol, tween 80	Orange peel essential oil reduced tensile strength, solubility, WVP, $L^*$ , and $a^*$ value. Increased elongation at break, opacity, and $\Delta E$ .	(Li et al., 2021)
Blood orange	Fish gelatin, blood orange peel pectin, glycerol	Equal concentration films had highest tensile strength (14 MPa), and glass transition temperature ( $79 \text{ }^\circ\text{C}$ ). Homogenous film structure High antioxidant and antibacterial properties (67% radical scavenging activity, 50.36% $\beta$ -carotene bleaching inhibition, 0.96 reducing power)	(Jridi et al., 2020)
Sweet lime	Potato peel starch, sweet lime pomace	The films exhibited water vapor permeability ( $7.25 \times 10^{-9} \text{ g/Pa h m}$ ), water solubility ( $38.92 \pm 0.702\%$ ), moisture absorption ( $12.88 \pm 0.348\%$ ), tensile strength ( $242.01 \pm 3.074 \text{ g}$ ) and elongation at break ( $7.61 \pm 0.824 \text{ mm}$ ).	(Borah et al., 2017)
Lemon	Lemon peel powder, xanthan gum, $\text{TiO}_2$ -Ag nanoparticles, glycerol	Increase in tensile strength, Young's modulus; decrease in solubility and water vapor permeability with the addition of xanthan gum and $\text{TiO}_2$ -Ag Improved antibacterial activity against <i>E.coli</i> and <i>S. aureus</i> .	(Meydanju et al., 2022)
Pomelo	Pomelo peel flour, Sodium alginate, glycerol	Compact microstructure and transparent films Improved tensile strength (23.55 MPa), elongation at break (17.68%), and WVP ( $2.08 \times 10^{-10} \text{ g/Pa s m}$ ).	(H. Wu et al., 2020)

Pomelo	White pomelo peel powder, casein, egg albumin, glycerol	Film with pectin: casein: albumin ratio 50:50:0 exhibited the highest tensile strength (5.73 MPa) and lowest WVP ( $0.52 \times 10^{-12}$ g/Pa h m). Addition of pectin into casein and albumin improved the thermal stability.	(Sood & Saini, 2022)
Pomelo	Pomelo peel flour, sodium alginate, glycerol, tea polyphenol (TP)	Optimal tensile strength (17.52 MPa) and elongation at break (19.46%). 10% tea polyphenol resulted in stronger intermolecular interaction Increased antioxidant activity.	(H. Wu et al., 2019)

## 2.6. Sodium Alginate

Alginates are naturally occurring linear, and anionic polysaccharides, obtained from marine sources. They are typically derived from the different genera of brown algae (*Laminaria digitata*, *Ascophyllum nodosum*, *Laminaria hyperborean*), where they are present in the form of sodium and calcium salts of alginic acid (Salem et al., 2016). These biopolymers find extensive applications in the food and pharmaceutical industries due to their bioavailability, biodegradability, biocompatibility, non-toxicity, and cost-effectiveness (Venkatesan et al., 2015). Its most valuable property is the ability to form robust gels or polymers with low solubility, primarily achieved through its interaction with polyvalent metal cations, especially  $\text{Ca}^{2+}$ . This has resulted in improved rigidity, cohesiveness, mechanical and barrier properties. During the gelation process of alginates, increasing the concentration of cations leads to the formation of a dense structure with reduced porosity and decreased water content (Alboofetileh et al., 2014). The composition of alginates makes them excellent filmogenic materials that can form strong films with a fibrous structure (Puscaselu et al., 2020).

### 2.6.1. Biosynthesis of alginates

The process of alginate extraction can be outlined in five stages. Initially, dried and crushed brown seaweeds are subjected to extraction using a mineral acid (e.g. HCl, 0.1M). This results in insoluble alginic acids, which can be easily separated from other unwanted glycans like sulfated fucoidans and laminarans through techniques like filtration or



centrifugation. Subsequently, the remaining insoluble residue is treated with an alkaline solution (using sodium carbonate, sodium hydroxide, or aluminum hydroxide, at a pH > 6.0) to transform insoluble alginic acid into sodium alginate. Following another separation step, the soluble sodium alginate is precipitated using either calcium chloride or cold alcohol. To achieve further purification, techniques such as acidification, the addition of Ca<sup>2+</sup> ions to form calcium alginate, or the introduction of ethanol as a dielectric stabilizer are employed (Fernando et al., 2020; Peteiro, 2018). To ensure successful alginate extraction, the biomass utilized must consist of a minimum of 83% dried matter with 17% moisture and should contain less than 3% sand (Hernández-Carmona et al., 2013). Another significant aspect of the extraction process is the size of the biomass. Reducing the size of the raw material facilitates the subsequent processing of algae (M. Silva et al., 2015). (Fertah et al., 2017) conducted an alginate extraction from *Laminaria digitata*, employing ground biomass (particle size 1-5 mm). They observed that using smaller particle sizes provided a larger surface area, leading to a more favorable extraction yield.

### **2.6.2. Structure of alginate**

Alginates consist of linear copolymers made up of repeating units of  $\alpha$ -1,4-L-guluronic acid (G) and  $\beta$ -1,4-D-mannuronic acid (M) (Agüero et al., 2017; Venkatesan et al., 2015). These repeating units maybe organized as blocks of G residues (GGGGGG) and M residues (MMMMMM), as well as alternating sequences of G and M residues (GMGMGM). The physical and mechanical properties of alginate are heavily influenced by the arrangement of these blocks in the biopolymer chain. An increased ratio of G blocks promotes the ease of gel formation, whereas the presence of MG blocks combined with a higher proportion of M blocks enhances the flexibility and elasticity of the alginate chain (Jiao et al., 2019; Ramos et al., 2018; Rhein-Knudsen et al., 2017). The molecular weight of alginates varies from 32 to 400 kDa, depending on their source and processing methods (Hu et al., 2021). Alginates are generally stiff because of the presence of inflexible six-membered sugar rings and limited rotation around the glycosidic linkage (Bogdanova et al., 2022). Additionally, electrostatic repulsion between charged groups on the polymer chain adds to the rigidity of the chains. The stiffness of the chains is influenced by factors like ionic strength and alginate composition, with increasing stiffness in the order of MG < MM < GG (Hecht & Srebnik, 2016).

### 2.6.3. Crosslinking of alginates

Alginate is widely recognized as a polyuronide and a natural ion exchanger (Kohn, 1975). Its charged state is advantageous for film formation. In the absence of any bivalent ions, alginate can only increase viscosity. However, it has ability to form gel by the addition of some bivalent cation through ion exchange (Doderio et al., 2019; Lu et al., 2006). The affinity of alginate towards cations follows the order  $\text{Ca}^{2+} < \text{Sr}^{2+} < \text{Ba}^{2+}$  (P. Lee & Rogers, 2012). The process of alginate gel formation is rather intricate. The hydrogel properties of alginate are highly influenced by the proportion and length of guluronic acid blocks (G-blocks), ability to bind cations, and type of gelling ions and conditions (Grant et al., 1973; Soazo et al., 2015; Tapia et al., 2008). The inclusion of  $\text{Ca}^{2+}$  ions, causes conformational alterations in alginate, leading to alignment of G-blocks and the creation of the “egg-box” model (Grant et al., 1973). This is because calcium ions binds between two chains and forms divalent salt bridges (Tapia et al., 2008).

According to (Costa et al., 2018), as the concentration of  $\text{CaCl}_2$  increases, the moisture content of the high G content film decreases, with  $\text{CaCl}_2$  ions predominantly reacting with G block in the chain sequentially. In terms of water solubility, the high G content film exhibits higher solubility due to its higher molecular weight and longer chain. While, with the increase in  $\text{CaCl}_2$  concentration a decrease in water solubility and water vapor permeability was observed, suggesting the presence of available G block in the matrix. Moreover, the tensile strength and young's modulus of the high G content film increased with a higher  $\text{CaCl}_2$  concentration. This improved mechanical property can be attributed to the reaction of  $\text{Ca}^{2+}$  ions with alginate. In another study, Liling et al. (2016) investigated the impact of different ionic crosslinking parameters on the characteristics of alginate mulching films. Crosslinking with  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ca}^{2+}$  resulted in an enhanced tensile strength and light transmission while reducing water vapor permeability (WVP). Among all the films,  $\text{Ca}^{2+}$  crosslinked films showed better properties. Moreover, WVP and light transmission decreased as the  $\text{Ca}^{2+}$  concentration increased, with optimal mechanical strength at 2% (w/v) calcium chloride.

## **2.7. Plasticizer**

Plasticizers are low-volatile molecules used to improve the flexibility and workability of biodegradable films. They work by interspersing themselves between polymer chains, disrupting the Hydrogen bonding, offering free space for polymers to move, and preventing them from coming closer, thereby softening the polymers by lowering the glass transition temperature  $T_g$ . Pores and cracks in the film could be also prevented by using plasticizers.

Glycerol, also known as 1,2,3- propanetriol is a polyol containing three hydroxyl groups. It is a widely used polymer for bio-polymer material. Glycerol has a strong ability to connect with polysaccharide matrix through Hydrogen bonding due to its multi-hydroxyl structure.(Thawien, 2008) in his study highlighted the effect of commonly used plasticizers type (sorbitol, glycerol, and polyethylene glycol) and concentration (20 to 60%) on blend film from rice starch-chitosan. The study reported that sorbitol plasticized films had the highest mechanical resistance but were most brittle. On the contrary, films plasticized with glycerol and polyethylene glycol exhibited flexible structure, with a low tensile strength. Increasing the plasticizer concentration resulted in higher solubility. The color of biodegradable film from rice starch-chitosan was more affected by the concentration of the plasticizer than by its type

## **2.8. Solution Casting Method**

Solution casting is the commonly used technique for biodegradable film formation. Thin films are prepared by this method at laboratory and pilot scales. In this method, the film-forming polymer solution is cast on a flat surface or mould, spread as a thin, uniform layer, and then dried. The thin film is obtained after solvent evaporation. It is based on the dispersion of macromolecules into a suitable solvent; this step is called solubilization, thus obtaining the film-forming suspension that is subjected to thermal gelatinization. The drying process provide sufficient time for solvent evaporation that makes a biodegradable film that adheres to the mould (Suhag et al., 2020). The main advantage of casting method is the ease of manufacturing without any requirement of specialized equipment at low cost. This method results in films with uniform thickness, low haze, and excellent optical properties (Suhag et al., 2020). Casting is used for the development of pectin-based films (I. S. V. da Silva et al., 2018). Pectin solutions (2–3 wt%) are mixed with the appropriate amount of plasticizer such as glycerol or

sorbitol to form film forming solution (Sganzerla et al., 2019). The solution is then cast and dried under controlled conditions to obtain thin films. (Gouveia et al., 2019) reported the successful production of pectin-based biodegradable films by thermo-compression molding. The pectin-glycerol films presented higher tensile strength values (Gouveia et al., 2019). Recently, pectin-based films using citrus waste have been developed using the solution casting method (Bátori et al., 2017). Other than the casting method, pressing and extrusion followed by blowing are other techniques used for the production of biodegradable films.

## CHAPTER 3

### MATERIALS AND METHODOLOGY

#### 3.1. Materials Required

##### Raw materials:

Lemon waste was collected from local juice vendors near Integral University, Lucknow, Uttar Pradesh. Sodium Alginate, Glycerol, and Calcium chloride were other materials used in study.

##### Other Materials:

Standard test sieves (As per ISS: 460-1962), beaker, petri plates, measuring cylinder, spatula, crucible, cuvette, glass rod, muslin cloth.

#### 3.2. Equipment Used:

##### 3.2.1. Electronic Weight Balance

High-precision electronic balance (WENSAR Model no.: PGB-6000) was used to weigh all the raw materials required for biodegradable film preparation and to measure the weight of various test samples. The maximum limit of the balance was 6000 grams. **Figure 3.1.** shows the electronic weight balance.



**Figure 3.1. Weighing balance**

### 3.2.2. Laboratory Grinder

A laboratory grinder (Crompton Greaves CG-DS51) shown in **Figure 3.2.** was used for grinding dried lemon peels to a fine powder.



**Figure 3.2. Laboratory grinder**

### 3.2.3. Tray Drier

A tray dryer is a conventional drying equipment with enclosed insulated chambers and trays placed on top of each other (**Figure 3.3**). It is based on the principle of convection drying which involves the removal of moisture from the material. The hot air is continuously circulated throughout the chamber by means of an electric heater or radiator coil. The heated air picks up moisture and discharge takes place through an outlet port.



**Figure 3.3. Tray drier**

### 3.2.4. Magnetic Stirrer

A magnetic stirrer (**Figure 3.4**) utilizes a rotating magnetic field that causes a stir bar placed inside a container, immersed in a liquid to spin. The rotating field is generated through a rotating magnet or a stationary electromagnet placed beneath the container. The agitation and rapid movement of the stir bar mix the solution thoroughly. The device is equipped with a speed controller and temperature adjustment knobs to control the rpm and heating, respectively.



**Figure 3.4. Magnetic stirrer**

### 3.2.5. Hot air Oven

Hot air oven (Science Tech) (**Figure 3.5**) was used for drying and to determine the moisture content of prepared biodegradable films. The hot air oven is made up of a double wall, with inner and outer chambers made of mild steel sheets. Mineral glass wool is used for insulation, in-between the two chamber walls. Door is insulated with brass hinges and a ball catch lock. Heating elements, made of Nichrome 80/20, are used at the bottom of the chamber.



**Figure 3.5. Hot air oven**

### 3.2.6. Muffle Furnace

A Muffle furnace (AMBASSADOR; Temperature- 1200°C max) was used to ignite the test samples in order to determine the ash content. It works on the principle of supplying heat to a chamber through induction or convection by a high-temperature heating coil within an insulated material. **Figure 3.6** shows the muffle furnace.



**Figure 3.6. Muffle furnace**

### 3.2.7. Digital Vernier Caliper

Vernier Caliper (Mitutoyo, Japan) as shown in **Figure 3.7** with a least count of 0.01 was used to determine the thickness of the various film samples.



**Figure 3.7. Vernier caliper**



### 3.2.8. Visible Spectrophotometer

Vis Spectrophotometer (LABMAN Model no. LMSP- UV1200) was used to study light transmission and film transparency properties. It is based on the principle of absorption of visible light by chemical compounds resulting in the production of distinct spectra. Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through a sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelengths.



**Figure 3.8. UV-vis spectrophotometer**

### 3.3. Preparation of Lemon peel powder

Procured lemon peels were sorted according to their color. Yellow peels were selected for the experiment and the remaining peels were discarded. Peels were washed with potable water to remove any dirt or adhering particles. After washing, peels were reduced to a smaller size and dried in a tray drier at 40 °C for 24 hours. Dried peels were pulverized to a fine powder using grinder. Post grinding, screening was done through a metal sieve (ISS: 460-1962) to obtain powdered particles of size <150 microns. The lemon peel powder was packed in an air tight packet and stored in a freezer at -18 °C prior to use.

### 3.4. Preparation of Calcium chloride solution

A 2% CaCl<sub>2</sub> solution was prepared by dissolving 2g Calcium chloride in 100mL distilled water and stored in a spray bottle.

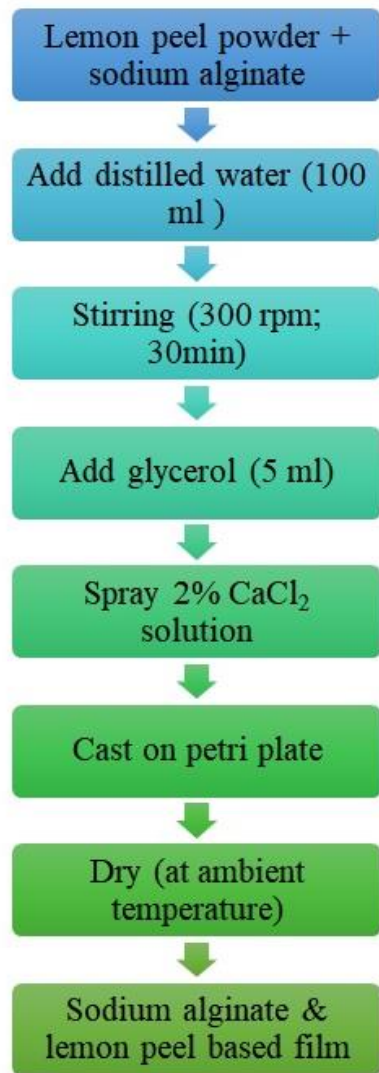
### 3.5. Formation of biodegradable film

The development of biodegradable films was done using sodium alginate and lemon peel. The film-forming variables were specified on the basis of preliminary experiments and review of the literature. Sodium alginate and lemon peel powder were taken in twelve different proportions of 0.5:1, 0.5:2, 0.5:3, 0.5:4, 1:1, 1:2, 1:3, 1:4, 1.5:1, 1.5:2, 1.5:3 and 1.5:4 (**Table 3.1**). The mixture was added to 100mL distilled water and stirred at 300 rpm for 30 minutes on a magnetic stirrer. Subsequently, 5mL glycerol was added as a plasticizer to the suspension and stirred for another 5 minutes. Films were prepared using the solution casting method. 30mL of this film-forming suspension was poured into a glass petri plate placed on a levelled flat surface to cast the film. After pouring, 2% CaCl<sub>2</sub> solution was sprayed onto the casted film surface as a crosslinking agent. The films were dried at room temperature for 48 hours. The flow chart for the formation of biodegradable film is represented in **Figure 3.9**. The resulting dried films were removed from the casting surface and kept in zippered bags at -18 °C for further analysis. **Plate 1-18** shows the various steps of film formation and their analysis.

### 3.6. Experiment design

**Table 3.1. Combinations of biodegradable film formulation**

<b>Film Sample</b>	<b>Sodium Alginate (g)</b>	<b>Lemon peel powder (g)</b>	<b>Glycerol (ml)</b>
<b>SA0.5:LPP1</b>	0.50	1.00	5.00
<b>SA0.5:LPP2</b>	0.50	2.00	5.00
<b>SA0.5:LPP3</b>	0.50	3.00	5.00
<b>SA0.5:LPP4</b>	0.50	4.00	5.00
<b>SA1:LPP1</b>	1.00	1.00	5.00
<b>SA1:LPP2</b>	1.00	2.00	5.00
<b>SA1:LPP3</b>	1.00	3.00	5.00
<b>SA1:LPP4</b>	1.00	4.00	5.00
<b>SA1.5:LPP1</b>	1.50	1.00	5.00
<b>SA1.5:LPP2</b>	1.50	2.00	5.00
<b>SA1.5:LPP3</b>	1.50	3.00	5.00
<b>SA1.5:LPP4</b>	1.50	4.00	5.00



**Figure 3.9.** Flowchart for the development of biodegradable film



**Figure 3.10.** Developed biodegradable films using sodium alginate and lemon peel.





**Plate 1. Lemon peels**



**Plate 2. Dried lemon peels**



**Plate 3. Grinding of lemon peels**



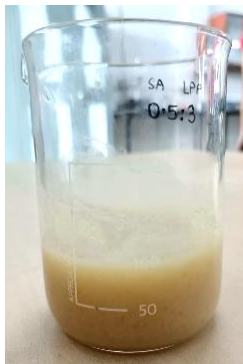
**Plate 4. Screening  
< 150 μm**



**Plate 5. Lemon peel powder**



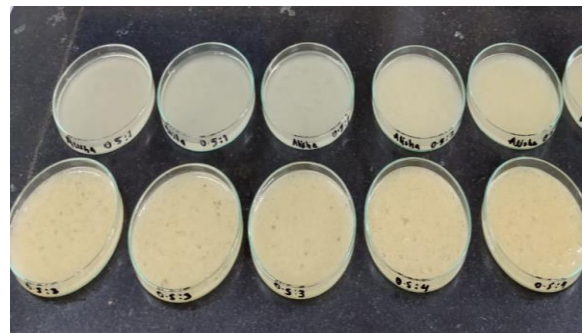
**Plate 6. Mixing of sodium alginate & lemon peel**



**Plate 7. Film forming solution**



**Plate 8. Film casting**



**Plate 9. Drying of film**



**Plate 10. Sodium alginate & lemon peel-based film**



**11 (a)**

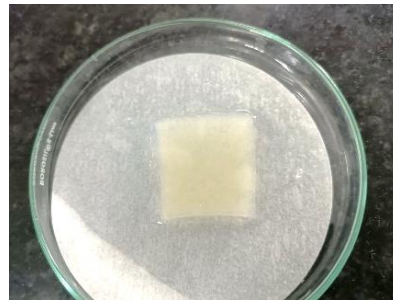


**11 (b)**

**Plate 11 (a) and (b).  
Water solubility test**



**12 (a)**



**12 (b)**

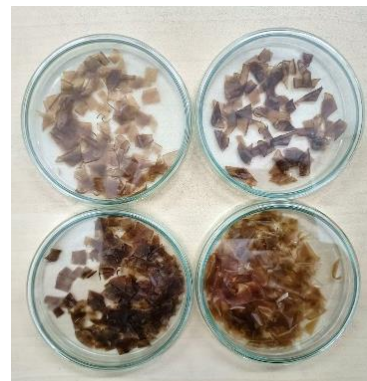


**12 (c)**

**Plate 12 (a), (b) & (c). Water absorption test**



**Plate 13. Thickness measurement**



**Plate 14. Moisture content determination**





15 (a)



15 (b)

Plate 15 (a) & (b). Determination of ash content



16 (a)



16 (b)



16 (c)

Plate 16 (a), (b) and (c). Biodegradability test

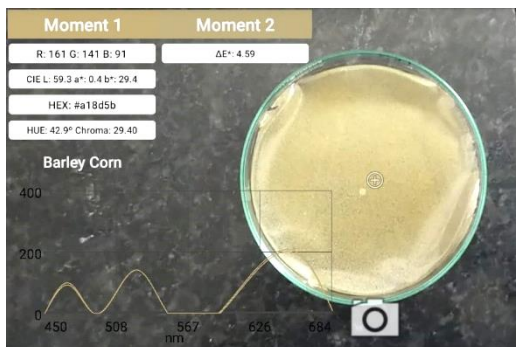


Plate 17. Color analysis



Plate 18. Burst strength measurement



### 3.7 Properties of film

#### 3.7.1. Thickness and Grammage

The thickness of the prepared film samples was measured using a digital vernier caliper having a sensitivity of 0.01 mm. Measurements were taken at three random positions of each film and the average thickness was calculated. The grammage of the film was determined using the mass-to-area ratio ( $\text{g}/\text{cm}^2$ ) following the guidelines of ISO 536:1995.

#### 3.7.2. Moisture Content

The moisture content of the film was determined using the oven-drying method. Films specimens were weighed initially and then kept in a hot air oven at 105 °C until constant weight was reached (AOAC, 2000). The films were weighed again and the resulting moisture content was calculated by the formula (1).

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100\% \quad (1)$$

Where  $W_1$  and  $W_2$  represent the initial and final weight of the film, respectively.

#### 3.7.3. Ash Content

Ash content of the film was determined according to (AOAC, 2000). 2 g of the film sample was accurately weighed and placed in a previously ignited and tarred silica crucible. The material was spread evenly and heated over a low Bunsen flame till fumes are no longer produced and then placed in a pre-heated muffle furnace to a temperature of 550°C for 4 hours. Crucibles were then removed carefully from the furnace. The samples were cooled in desiccators and weighed. Percentage total ash was calculated as per the following equation (2).

$$\% \text{ Ash} = \frac{\text{Weight of Ash}}{\text{Initial weight of sample}} \times 100\% \quad (2)$$

### 3.7.4. Tensile strength and Elongation at break

Tensile strength and elongation at break of the film were determined using a universal testing machine according to the method followed by (Li et al., 2021). The film was cut in a rectangular (2 cm × 5 cm) strip with a clamp distance of 40 mm. The film sample was stretched at a speed of 1mm/s until the film break. The tensile strength and elongation at break were calculated using the formula 3 & 4 respectively.

$$\text{Tensile strength (MPa)} = \frac{F_{max}}{S} \quad (3)$$

Where,  $F_{max}$  = maximum tensile force when the film break (N);  $S$  = cross-sectional area of the film (mm<sup>2</sup>).

$$\text{Elongation at Break (\%)} = \frac{L_f - L_0}{L_0} \quad (4)$$

Where,  $L_f$  = maximum length attained when the film broke (mm);  $L_0$  = initial length of film (mm).

### 3.7.5. Bursting strength

The burst strength of the film represents its ability to withstand progressively increasing pressure applied perpendicular to its surface, following specific predetermined conditions. The point at which the film fails, determining its burst strength, represents the pressure it can withstand and is essentially indicative of the energy absorption capacity of the film. Burst strength has been gauged using a burst strength tester, with a film specimen positioned between two angled rings within the machine. An inlet valve is opened to sustain air pressure. The sample is subjected to controlled air pressure, which is increased until the failure occurs. The pressure at the moment of failure is considered as the burst strength of the material.

### 3.7.6. Retraction ratio

The retraction ratio was calculated using a method described by (Phan The et al., 2009). It involved comparing the initial film thickness, which corresponds to the cast layer of the film-

forming solution, with the dry film thickness obtained after the film was dried. The retraction ratio was calculated using the following formula (5).

$$\text{Retraction ratio (\%)} = \frac{T_1 - T_2}{T_1} \times 100 \quad (5)$$

Where,  $T_1$  = thickness of cast layer;  $T_2$  = thickness of dried film

### 3.7.7. Water Solubility

The film's solubility in water was determined according to a method followed by (J. Wu et al., 2013). A 2 cm × 2 cm cross-sectional film area was cut and weighed. The film was immersed in a beaker filled with 50mL distilled water and kept at 25 °C for 1 hour. Next, the insoluble matter was separated and dried in a hot air oven at a temperature of 110 °C until a constant weight was attained. The final dry weight of the film was recorded. To determine the percentage of matter dissolved in water, the following equation (6) was used:

$$\text{Solubility (\%)} = \frac{\text{Initial dry weight} - \text{Final dry weight}}{\text{Initial dry weight}} \times 100\% \quad (6)$$

### 3.7.8. Water Absorption

The water absorption capacity was assessed through a water absorption test. For this test, a dried film sample measuring 2 cm × 2 cm was taken and placed in a beaker containing 50 ml of deionized water at room temperature for an hour. After the immersion period, the samples were removed from the beakers, excess water was wiped off, and their final weight was recorded (Sultan & Johari, 2017). The percentage (%) of water absorption for each sample was then calculated using equation (7):

$$\text{Water Absorption (\%)} = \frac{W_f - W_0}{W_0} \times 100\% \quad (7)$$

Where,  $W_0$  = dried sample weight

$W_f$  = weight of the sample after water absorption.

### 3.7.9. Color

The color of the developed film was evaluated by CIE Lab system using a digital colorimeter. The color measurements were expressed in terms of lightness and chromaticity parameters. The minimum and maximum value for lightness  $L^* = 0$  (black) and 100 (white), respectively. Chromaticity parameters:  $a^*$  signifies Green [-] to Red [+] and  $b^*$  indicated a trend from Blue [-] to Yellow [+].

### 3.8.9. Film Opacity and Transparency

Film opacity and transparency were calculated as per the method followed by (Meydanju et al., 2022). The films were cut into 4mm  $\times$  40 mm and placed at the inside transparent wall of the cuvette with distilled water as blank. Absorbance and transmittance was measured at 600 nm wavelengths using a UV-vis spectrophotometer. The opacity and transparency was calculated using the formula 8 & 9, respectively.

$$\text{Opacity} = \frac{Abs_{600}}{x} \quad (8)$$

$$\text{Transparency} = \frac{\log(\%T_{600})}{x} \quad (9)$$

Where,  $\%T_{600}$  = transmittance percent,  $Abs_{600}$  = absorbance at 600 nm and  $x$  = film thickness (mm)

### 3.8.11. Biodegradability

A small portion of the film was cut and weighed. The sample was placed in a muslin cloth and buried deep into the soil. The soil used was damp and loamy having some amount of moisture. After 15 days the film was observed and the results were recorded. The biodegradability was reported as percentage of change in weight of film.

### **3.8.12. Statistical Analysis**

Statistical significance was calculated by One-Way ANOVA using IBM® SPSS® Statistics for Windows Version: 20. LSD (least significant difference) was performed to obtain descriptive data and Duncan's multiple range test was used to determine significant differences among means at 95% confidence level. All the analyses were performed in three replicates and data is reported as mean  $\pm$  SD.

## CHAPTER 4

### RESULT AND DISCUSSION

Biodegradable films were prepared using Lemon (*Citrus limon*) peel powder and Sodium Alginate. Both the raw materials showed proficiency in film formation. Films were successfully developed using different proportions of Lemon peel powder and Sodium alginate and the effect of varying composition on film properties is discussed. All the films were analyzed for thickness, moisture content, ash content, water solubility, water absorption capacity, color, retraction ratio, folding endurance, and biodegradability.

#### 4.1. Thickness and Grammage

Film thickness is an important factor that influences the mechanical, optical, and barrier properties (Monjazez Marvdashti et al., 2017). The thickness of the films varied from  $0.07 \pm 0.01$  to  $0.27 \pm 0.01$  mm (Table 4.1). A slight increment in film thickness was observed in all sodium alginate films incorporated with 4% lemon peel powder than in other samples. Similar observations were made by (Terzioğlu & Parin, 2020), where lemon peel loading of 4 and 8 wt. % raised the thickness to 0.30 and 0.35 mm, respectively in polyvinyl alcohol and corn starch-based biocomposite films (Table 4.2). ANOVA results showed a significant difference ( $P < 0.05$ ) in the thickness of the film. Overall, an increase in film thickness was observed as the concentration of lemon peel powder increased as shown in Figure 4.1. This increase in film thickness resulting from the addition of lemon peel could be attributed to the increase in solid contents in the film. The thickness obtained was similar to those of pomelo peel flour films having a thickness in the range of 0.08 to 0.10 mm (H. Wu et al., 2020). Here, least thickness of  $0.07 \pm 0.01$  mm was observed in sample SA1:LPP1 having an equal concentration of sodium alginate and lemon peel powder, which was comparable to that obtained for sodium alginate-based film incorporated with guava leaf extract (Luo et al., 2019).

**Table 4.1. Thickness (mm) of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>THICKNESS (mm)</b>
SA0.5:LPP1	0.19 ± 0.01 <sup>cde</sup>
SA0.5:LPP2	0.20 ± 0.02 <sup>bcd</sup>
SA0.5:LPP3	0.22 ± 0.03 <sup>bc</sup>
SA0.5:LPP4	0.26 ± 0.01 <sup>a</sup>
SA1:LPP1	0.07 ± 0.01 <sup>h</sup>
SA1:LPP2	0.13 ± 0.03 <sup>g</sup>
SA1:LPP3	0.18 ± 0.01 <sup>def</sup>
SA1:LPP4	0.22 ± 0.01 <sup>bc</sup>
SA1.5:LPP1	0.22 ± 0.03 <sup>b</sup>
SA1.5:LPP2	0.16 ± 0.02 <sup>ef</sup>
SA1.5:LPP3	0.15 ± 0.01 <sup>fg</sup>
SA1.5:LPP4	0.27 ± 0.01 <sup>a</sup>

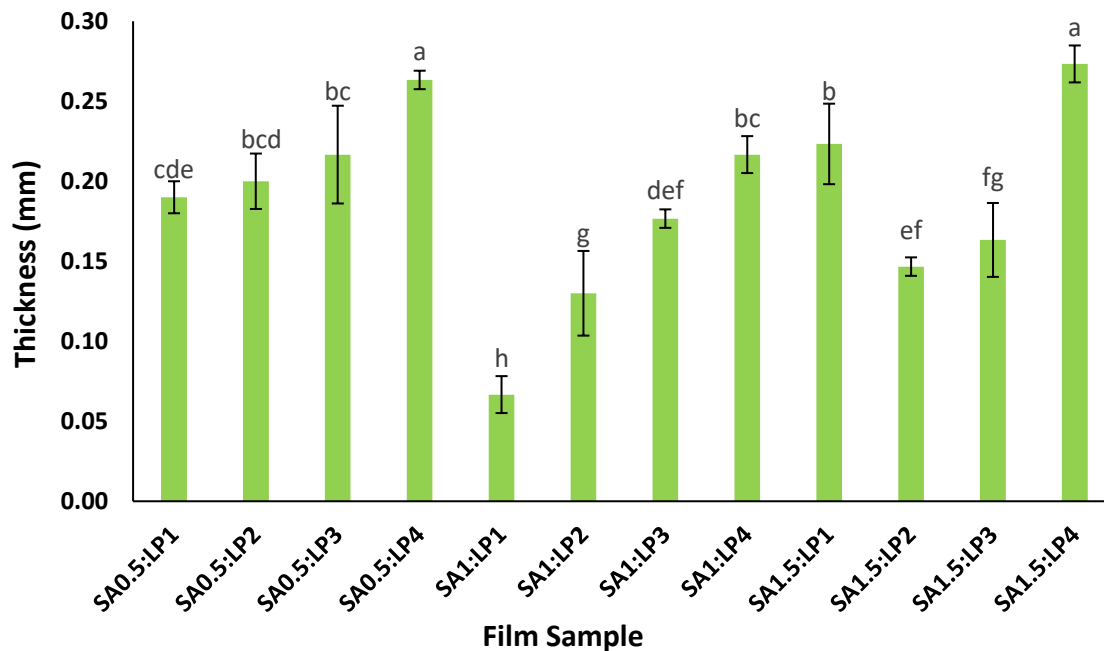
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.2. ANOV for thickness**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>(Combined)</b>			.110	11	.010	32.301	.000
<b>Between Groups</b>	<b>Linear</b>	<b>Contrast</b>	.001	1	.001	2.204	.151
	<b>Term</b>	<b>Deviation</b>	.109	10	.011	35.311	.000
	<b>Quadratic</b>	<b>Contrast</b>	.013	1	.013	43.244	.000
	<b>Term</b>	<b>Deviation</b>	.096	9	.011	34.430	.000
<b>Within Groups</b>			.007	24	.000		
<b>Total</b>			.117	35			



**Figure 4.1. Thickness of Lemon peel and Sodium alginate-based film**

The grammage value of the biodegradable films varied from  $246.72 \pm 0.35$  to  $445.78 \pm 0.23$  g/m<sup>2</sup>. It was lowest for sample SA0.5:LPP1 and highest for SA1.5:LPP4. The results were found to be significantly different ( $p < 0.05$ ). Table 4.3. and 4.4. show the grammage value and ANOVA results, respectively. The changes in film grammage can be elucidated by the disparities in thickness, structure, particularly, composition (Bilck et al., 2010). With the increase in the concentration of lemon peel powder, the grammage of the films also increased as evident from Figure. The dependency of grammage on film thickness is evident from the results of thickness. As can be seen that grammage is increasing with the increase in thickness. Therefore, it can be deduced that grammage is directly proportional to the thickness and composition of the film. The values obtained were higher than those reported by Luís et al. (2019) for carboxymethyl xylan film incorporated with licorice essential oil where the grammage of films ranged between 123.33 to 138.04 g/m<sup>2</sup>.



**Table 4.3. Grammage (g/m<sup>2</sup>) of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>GRAMMAGE (g/m<sup>2</sup>)</b>
<b>SA0.5:LPP1</b>	246.72 ± 0.35 <sup>l</sup>
<b>SA0.5:LPP2</b>	300.00 ± 1.00 <sup>j</sup>
<b>SA0.5:LPP3</b>	325.68 ± 0.96 <sup>h</sup>
<b>SA0.5:LPP4</b>	377.26 ± 0.46 <sup>f</sup>
<b>SA1:LPP1</b>	277.99 ± 0.94 <sup>k</sup>
<b>SA1:LPP2</b>	313.54 ± 1.21 <sup>i</sup>
<b>SA1:LPP3</b>	343.41 ± 0.75 <sup>g</sup>
<b>SA1:LPP4</b>	380.00 ± 1.00 <sup>e</sup>
<b>SA1.5:LPP1</b>	383.71 ± 0.98 <sup>d</sup>
<b>SA1.5:LPP2</b>	401.37 ± 0.74 <sup>c</sup>
<b>SA1.5:LPP3</b>	435.15 ± 0.86 <sup>b</sup>
<b>SA1.5:LPP4</b>	445.78 ± 0.23 <sup>a</sup>

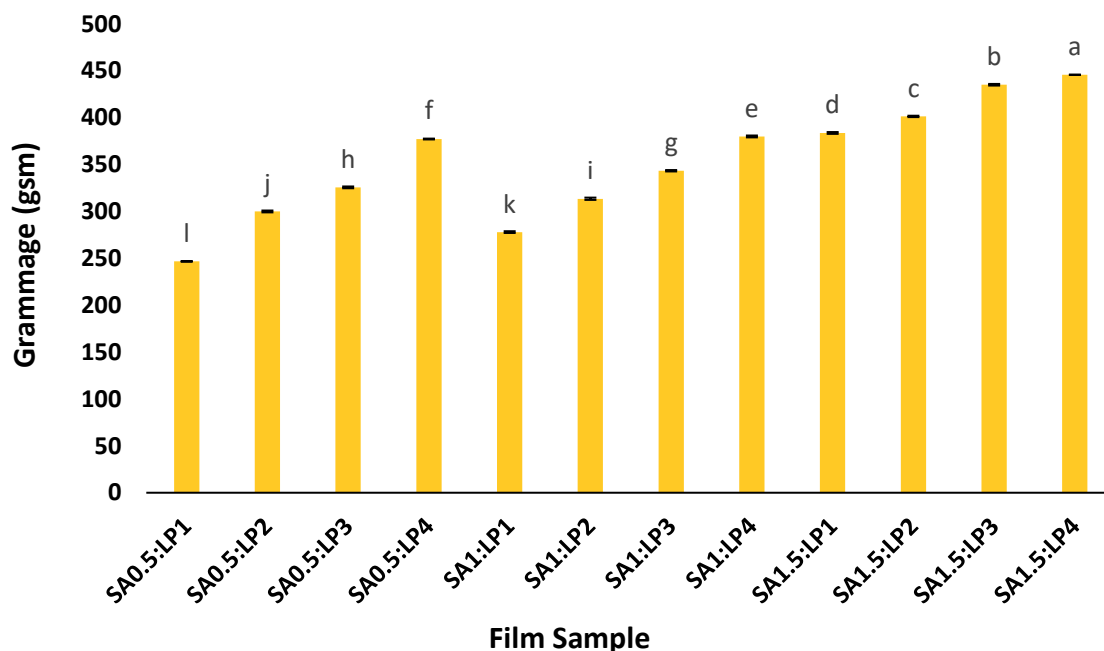
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different (P < 0.05).

Subset for alpha = 0.05

**Table 4.4. ANOVA for grammage**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>Between Groups</b>	<b>(Combined)</b>		126230.076	11	11475.461	16187.955	.000
	<b>Linear</b>	<b>Contrast</b>	97139.475	1	97139.475	137030.607	.000
	<b>Term</b>	<b>Deviation</b>	29090.602	10	2909.060	4103.690	.000
	<b>Quadratic</b>	<b>Contrast</b>	1190.873	1	1190.873	1679.915	.000
	<b>Term</b>	<b>Deviation</b>	27899.729	9	3099.970	4372.998	.000
<b>Within Groups</b>			17.013	24	.709		
<b>Total</b>			126247.090	35			



**Figure 4.2. Grammage ( $\text{g/m}^2$ ) of Lemon peel and Sodium alginate-based film**

#### 4.2. Moisture Content

The moisture content value serves as an indicative parameter associated with the overall volume of empty spaces filled by water molecules within the microstructural network of the film. Moisture content influences the processibility, shelf-life, usability, and quality of a product. The moisture content of lemon peel and sodium alginate-based film ranged from  $10.38 \pm 0.31\%$  to  $14.14 \pm 0.37\%$ . The values of moisture content are presented in Table 4.5. Statistical analysis showed a significant difference ( $P < 0.05$ ) in the moisture content values of the developed film as shown in Table 4.6. Neat sodium alginate-based films are reported to have 66.92% moisture content (Luo et al., 2019). The results thus reveal that the addition of lemon peel powder significantly reduced the moisture content of the films as evident from Figure 4.3. It was observed that the moisture content of the films decreased with an increase in the concentration of both lemon peel powder and sodium alginate. This reduction in moisture content could be clarified by the fact that the interactions between lemon peel powder and sodium alginate reduce the accessibility of hydroxyl groups, consequently restricting the water and composite matrix interactions via hydrogen bonding (Ciannamea et al., 2016; Wu et al., 2019). By contrast, the moisture content of the developed lemon peel and sodium alginate film

was lower than pomelo peel flour with 19.72% (Wu et al., 2020), and sweet potato starch/lemon waste pectin incorporated with TiO<sub>2</sub> nanoparticles-based film with 23.12% moisture content (Dash et al., 2019). However, the results obtained were in line with gelatin-orange peel powder films with a moisture content of 10.72 to 12.67% (Taghavi et al., 2020), and mosambi/sago based film with a moisture content of 10 to 14.67% (Ahmad et al., 2022)

**Table 4.5. Moisture content (%) of Lemon peel and Sodium alginate-based film**

FILM SAMPLE	MOISTURE CONTENT (%)
SA0.5:LPP1	13.22 ± 0.31 <sup>bcd</sup>
SA0.5:LPP2	12.34 ± 0.24 <sup>ef</sup>
SA0.5:LPP3	11.24 ± 0.33 <sup>gh</sup>
SA0.5:LPP4	10.38 ± 0.31 <sup>i</sup>
SA1:LPP1	13.21 ± 0.35 <sup>bcd</sup>
SA1:LPP2	12.50 ± 0.85 <sup>de</sup>
SA1:LPP3	11.75 ± 0.75 <sup>fg</sup>
SA1:LPP4	10.82 ± 0.06 <sup>hi</sup>
SA1.5:LPP1	14.14 ± 0.37 <sup>a</sup>
SA1.5:LPP2	13.96 ± 0.19 <sup>ab</sup>
SA1.5:LPP3	13.42 ± 0.37 <sup>abc</sup>
SA1.5:LPP4	12.81 ± 0.31 <sup>cde</sup>

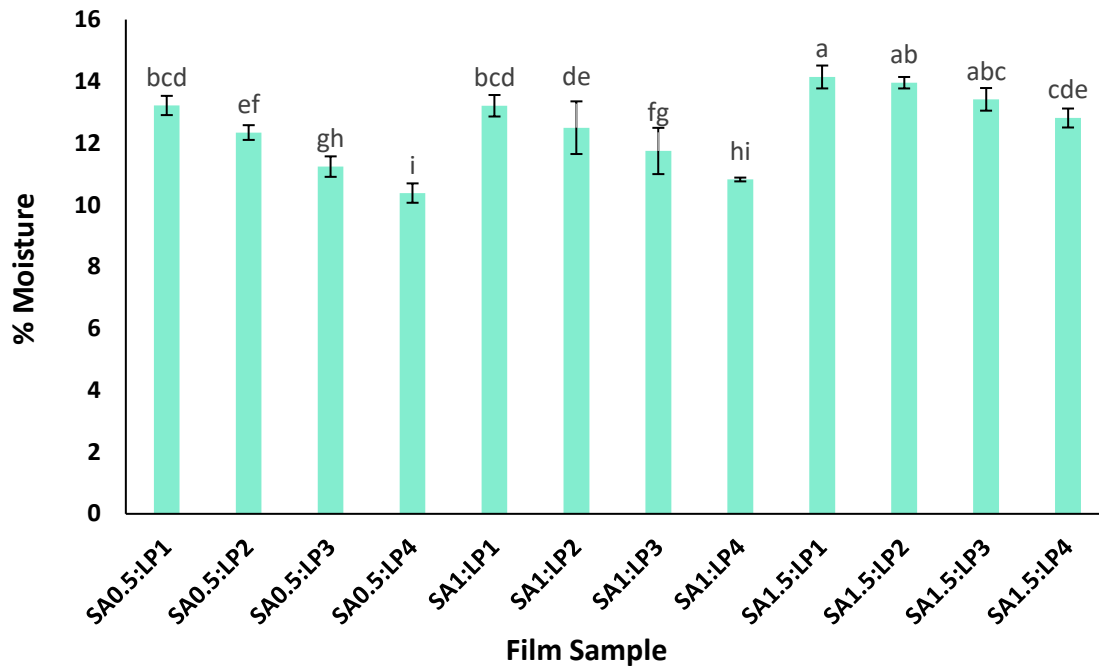
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different (P < 0.05).

Subset for alpha = 0.05

**Table 4.6. ANOVA for moisture content**

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		48.720	11	4.429	24.457	.000
	Linear	Contrast	6.452	1	6.452	35.627	.000
	Term	Deviation	42.268	10	4.227	23.340	.000
	Quadratic	Contrast	4.793	1	4.793	26.467	.000
	Term	Deviation	37.475	9	4.164	22.993	.000
Within Groups			4.346	24	.181		
Total			53.066	35			



**Figure 4.3. Moisture content (%) of Lemon peel and Sodium alginate-based film**

### 4.3. Ash Content

Ash content quantifies the complete mineral content within a specimen, signifying the inorganic remains left behind after the elimination of water and organic substances through combustion. The residue encompasses minerals like calcium and potassium as well as other hazardous elements (Schwalfenberg et al., 2013). The ash content of the films was determined as shown in Table 4.7. It ranged from  $3.23 \pm 0.05\%$  to  $5.13 \pm 0.04\%$ . Statistical analysis showed a significant difference ( $P < 0.05$ ) in the ash content of the films (Table 4.8). Ash content was greatly influenced by both sodium alginate and lemon peel powder in the formulation (Figure 4.4). Film sample SA1.5:LPP4, with a maximum concentration of both sodium alginate and lemon peel, showed the highest ash content of  $5.13 \pm 0.04\%$ .

**Table 4.7. Ash content (%) of Lemon peel and Sodium alginate-based film**

FILM SAMPLE	ASH CONTENT (%)
SA0.5:LPP1	3.23 ± 0.05 <sup>h</sup>
SA0.5:LPP2	3.42 ± 0.05 <sup>g</sup>
SA0.5:LPP3	4.01 ± 0.05 <sup>e</sup>
SA0.5:LPP4	4.22 ± 0.10 <sup>d</sup>
SA1:LPP1	3.85 ± 0.06 <sup>f</sup>
SA1:LPP2	4.04 ± 0.10 <sup>e</sup>
SA1:LPP3	4.18 ± 0.05 <sup>d</sup>
SA1:LPP4	4.65 ± 0.09 <sup>c</sup>
SA1.5:LPP1	4.26 ± 0.09 <sup>d</sup>
SA1.5:LPP2	4.59 ± 0.02 <sup>c</sup>
SA1.5:LPP3	4.95 ± 0.06 <sup>b</sup>
SA1.5:LPP4	5.13 ± 0.04 <sup>a</sup>

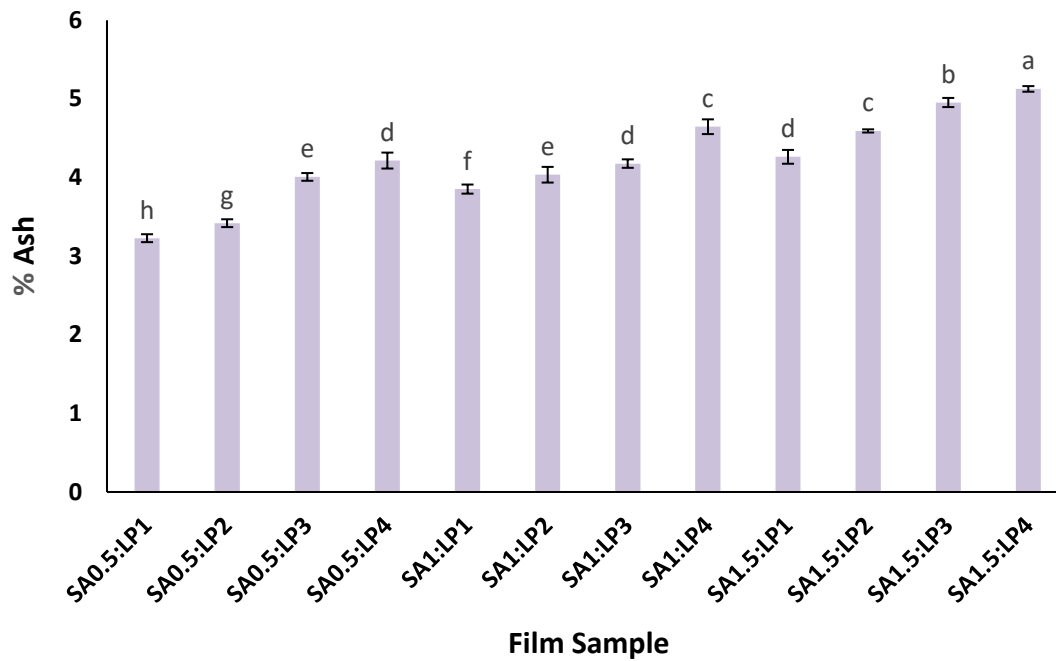
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.8. ANOVA for Ash content**

			Sum of Squares	df	Mean Square	F	Sig.
<b>(Combined)</b>			10.568	11	.961	207.725	.000
<b>Between Groups</b>	<b>Linear Term</b>	<b>Contrast</b>	9.055	1	9.055	1957.923	.000
		<b>Deviation</b>	1.513	10	.151	32.706	.000
	<b>Quadratic Term</b>	<b>Contrast</b>	.000	1	.000	.018	.895
		<b>Deviation</b>	1.513	9	.168	36.338	.000
<b>Within Groups</b>			.111	24	.005		
<b>Total</b>			10.679	35			



**Figure 4.4. Ash content (%) of Lemon peel and Sodium alginate-based film**

#### **4.4. Tensile strength and Elongation at break**

Tensile strength is defined as the maximum stress experienced by a film in a tensile test (C. Liu et al., 2021). The tensile strength of the lemon peel and sodium alginate-based film ranged from  $0.79 \pm 0.03$  to  $6.42 \pm 0.02$  MPa (Table 4.9). ANOVA results showed a significant difference ( $P < 0.5$ ) in the tensile strength of the films (Table 4.10). Maximum tensile strength of  $6.42 \pm 0.02$  MPa was observed in sample SA1:LPP1 with sodium alginate: lemon peel ratio of 1:1 (Figure 4.5). This could be due to higher molecular interaction between sodium alginate and lemon peel, resulting in higher tensile strength (Henning et al., 2022). The tensile strength obtained was higher than corn starch film loaded with orange peel (Chhatariya et al., 2022). The results were comparable to that of pomelo peel pectin/casein/albumen blend films with a tensile strength of 1.85 to 5.73 MPa (Sood & Saini, 2022). It was observed that tensile strength was more in films with a lower concentration of lemon peel powder. A low concentration of citrus peel is known to increase the tensile strength. This is because of the interfacial adhesiveness between the peel powder and film matrix (Yun & Liu, 2022). Whereas, an increase

in peel powder caused a significant reduction in tensile strength due to the formation of agglomerated particles (Rathinavel & Saravanakumar, 2021; Taghavi et al., 2020).

**Table 4.9. Tensile strength (MPa) of Lemon peel and Sodium alginate-based film**

FILM SAMPLE	TENSILE STRENGTH (MPa)
SA0.5:LPP1	1.05 ± 0.01 <sup>k</sup>
SA0.5:LPP2	1.73 ± 0.03 <sup>h</sup>
SA0.5:LPP3	1.38 ± 0.03 <sup>j</sup>
SA0.5:LPP4	0.79 ± 0.03 <sup>l</sup>
SA1:LPP1	6.42 ± 0.02 <sup>a</sup>
SA1:LPP2	3.08 ± 0.01 <sup>d</sup>
SA1:LPP3	2.75 ± 0.07 <sup>e</sup>
SA1:LPP4	2.49 ± 0.02 <sup>f</sup>
SA1.5:LPP1	3.83 ± 0.06 <sup>b</sup>
SA1.5:LPP2	3.71 ± 0.03 <sup>c</sup>
SA1.5:LPP3	2.05 ± 0.06 <sup>g</sup>
SA1.5:LPP4	1.66 ± 0.02 <sup>i</sup>

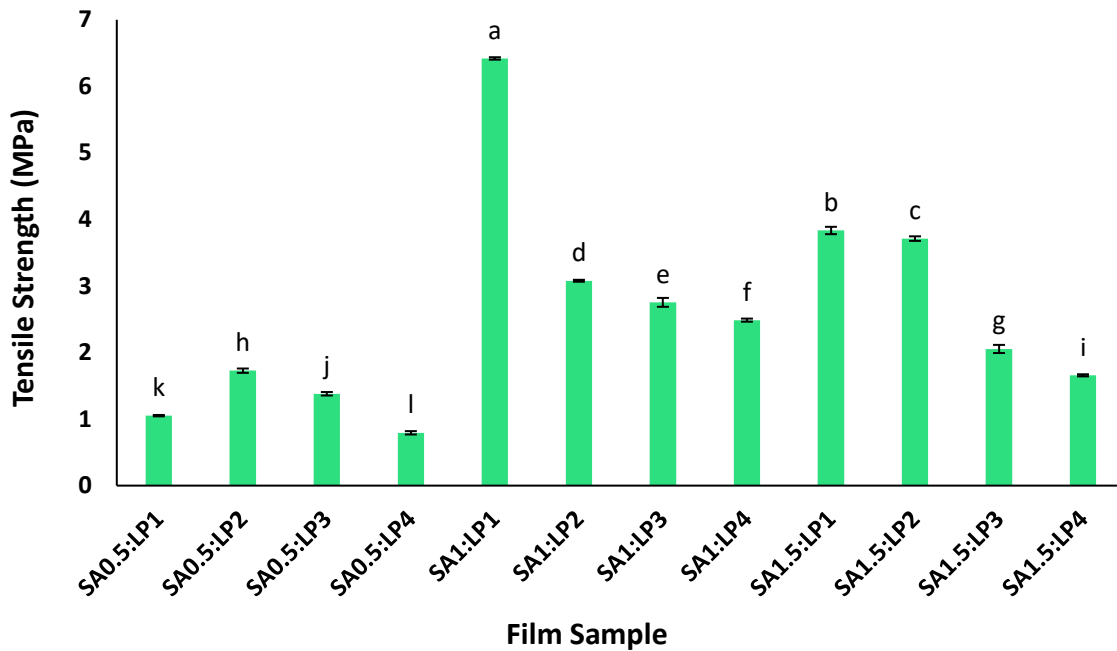
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.10. ANOVA for tensile strength**

			Sum of Squares	df	Mean Square	F	Sig.
<b>Between Groups</b>	<b>(Combined)</b>		80.112	11	7.283	5373.861	.000
	<b>Linear</b>	<b>Contrast</b>	4.412	1	4.412	3255.754	.000
	<b>Term</b>	<b>Deviation</b>	75.700	10	7.570	5585.672	.000
	<b>Quadratic</b>	<b>Contrast</b>	21.366	1	21.366	15765.709	.000
	<b>Term</b>	<b>Deviation</b>	54.333	9	6.037	4454.557	.000
<b>Within Groups</b>			.033	24	.001		
<b>Total</b>			80.145	35			



**Figure 4.5. Tensile strength (MPa) of Lemon peel and Sodium alginate-based film**

The percentage elongation is significantly influenced by the tensile strength. As tensile strength increases, the percentage elongation of films declines as evident from the Figure 4.6. The increase in tensile strength corresponds to an increase in polymeric interactions within the film matrix, leading to the formation of a more compact film structure. The increased compactness of the film matrix reduces its ability to stretch, consequently resulting in a decrease in the percentage of elongation. The percentage elongation of the films varied from  $4.67 \pm 0.12$  to  $15.27 \pm 0.12\%$  (Table 4.11). Statistical analysis showed a significant difference ( $P < 0.05$ ) in the elongation values (Table 4.22). Lower elongation % was observed in sample SA1:LPP1 and SA0.5:LPP2 as compared to other films due to more compact film structure. Similar observations were made by (Sood & Saini, 2022) for pomelo peel pectin/casein/albumen films where elongation at break decreased with an increase in tensile strength.



**Table 4.11. Elongation at break (%) of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>ELONGATION AT BREAK (%)</b>
SA0.5:LPP1	11.27 ± 0.12 <sup>d</sup>
SA0.5:LPP2	7.40 ± 0.10 <sup>h</sup>
SA0.5:LPP3	9.60 ± 0.20 <sup>g</sup>
SA0.5:LPP4	10.87 ± 0.31 <sup>e</sup>
SA1:LPP1	4.67 ± 0.12 <sup>i</sup>
SA1:LPP2	7.60 ± 0.20 <sup>h</sup>
SA1:LPP3	11.53 ± 0.42 <sup>d</sup>
SA1:LPP4	12.00 ± 0.20 <sup>c</sup>
SA1.5:LPP1	10.00 ± 0.20 <sup>f</sup>
SA1.5:LPP2	10.60 ± 0.20 <sup>e</sup>
SA1.5:LPP3	14.07 ± 0.12 <sup>b</sup>
SA1.5:LPP4	15.27 ± 0.12 <sup>a</sup>

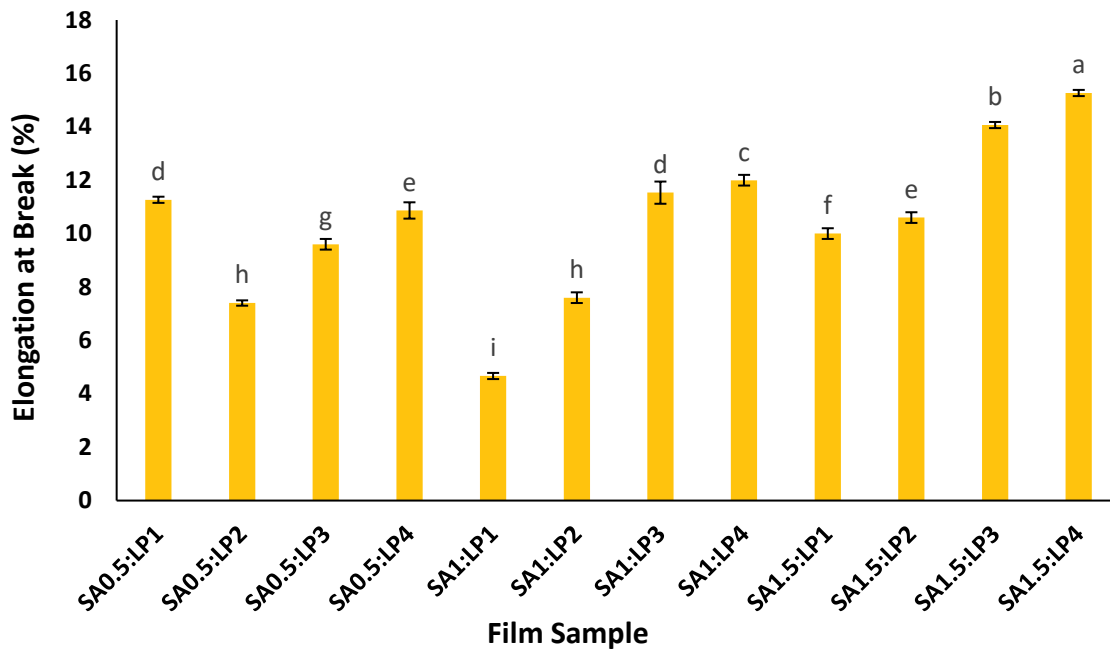
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.12. ANOVA for Elongation at break**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>Between Groups</b>	<b>(Combined)</b>		277.479	11	25.225	571.140	.000
	<b>Linear</b>	<b>Contrast</b>	92.217	1	92.217	2087.938	.000
	<b>Term</b>	<b>Deviation</b>	185.262	10	18.526	419.460	.000
	<b>Quadratic</b>	<b>Contrast</b>	64.570	1	64.570	1461.963	.000
	<b>Term</b>	<b>Deviation</b>	120.692	9	13.410	303.627	.000
<b>Within Groups</b>			1.060	24	.044		
<b>Total</b>			278.539	35			



**Figure 4.6. Elongation at break (%) of Lemon peel and Sodium alginate-based film**

#### 4.5. Bursting Strength

The burst strength of the developed lemon peel and sodium alginate films ranged from  $0.23 \pm 0.01$  to  $0.66 \pm 0.02$  MPa (Table 4.13). ANOVA results showed a significant difference ( $P < 0.05$ ) in the burst strength of films (Table 4.14). Samples SA0.5:LPP4, SA1:LPP4, and SA1.5:LPP4 with sodium alginate: lemon peel ratio 0.5:4, 1:4, and 1.5:4 had a higher burst strength of  $0.65 \pm 0.01$ ,  $0.66 \pm 0.02$ , and  $0.65 \pm 0.0$  MPa, respectively as compared to other films. The results are in accordance with the thickness of the film as the burst strength is more for films with a higher thickness. From Figure 4.7., it is evident that burst strength is increasing with an increase in peel powder, which eventually caused an increase in film thickness. The bursting strength obtained was much higher than rice bran reinforced LDPE films having 0.031 to 0.060 MPa burst strength (Dabash et al., 2022).

**Table 4.13. Bursting strength (MPa) of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>BURSTING STRENGTH (MPa)</b>
SA0.5:LPP1	0.27 ± 0.01 <sup>f</sup>
SA0.5:LPP2	0.27 ± 0.01 <sup>f</sup>
SA0.5:LPP3	0.52 ± 0.01 <sup>c</sup>
SA0.5:LPP4	0.65 ± 0.01 <sup>a</sup>
SA1:LPP1	0.23 ± 0.01 <sup>g</sup>
SA1:LPP2	0.31 ± 0.01 <sup>e</sup>
SA1:LPP3	0.63 ± 0.02 <sup>b</sup>
SA1:LPP4	0.66 ± 0.02 <sup>a</sup>
SA1.5:LPP1	0.30 ± 0.00 <sup>e</sup>
SA1.5:LPP2	0.28 ± 0.01 <sup>f</sup>
SA1.5:LPP3	0.37 ± 0.01 <sup>d</sup>
SA1.5:LPP4	0.65 ± 0.01 <sup>a</sup>

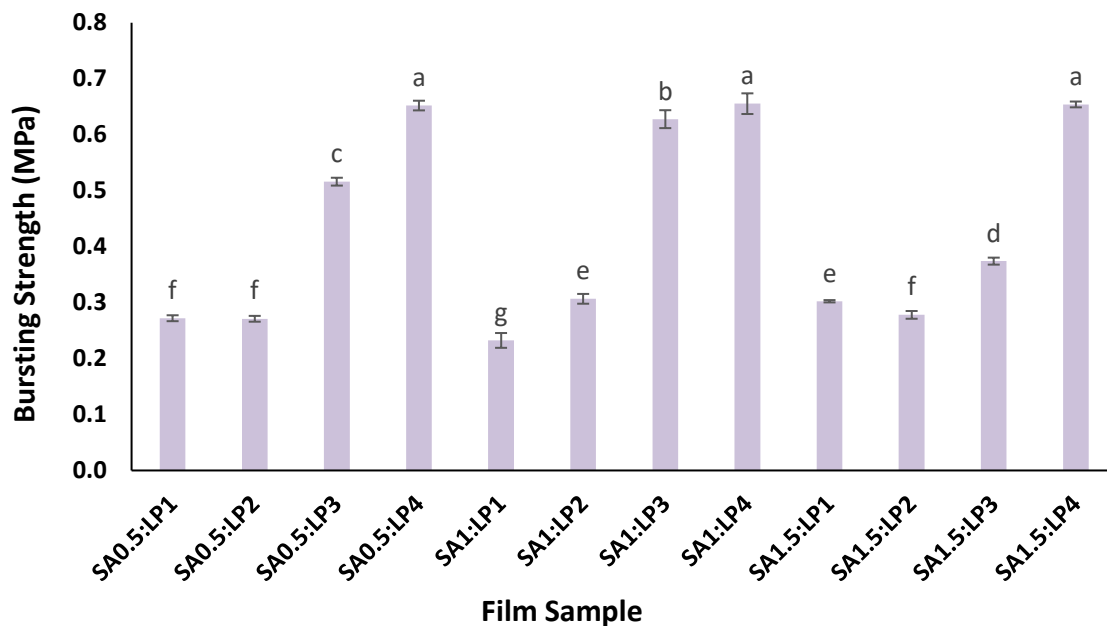
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different (P < 0.05).

Subset for alpha = 0.05

**Table 4.14. ANOVA for bursting strength**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>Between Groups</b>	<b>(Combined)</b>		1.039	11	.094	850.389	.000
	<b>Linear</b>	<b>Contrast</b>	.057	1	.057	510.928	.000
	<b>Term</b>	<b>Deviation</b>	.983	10	.098	884.335	.000
	<b>Quadratic</b>	<b>Contrast</b>	.011	1	.011	99.601	.000
	<b>Term</b>	<b>Deviation</b>	.972	9	.108	971.527	.000
<b>Within Groups</b>			.003	24	.000		
<b>Total</b>			1.042	35			



**Figure 4.7. Bursting strength (MPa) of Lemon peel and Sodium alginate-based film**

#### 4.6. Retraction ratio

The retraction ratio is an essential parameter for producing the film at an industrial level. The retraction ratios of the films are represented in Table 4.15. It is linked to the film's dry matter composition and contraction during drying (Phan et al., 2009). The ratio ranged from  $90.43 \pm 0.35\%$  to  $98.33 \pm 0.29\%$ . ANOVA results showed a significant difference ( $P < 0.05$ ) in the retraction ratio of the films (Table 4.16). Sample SA1:LPP1, having an equal concentration of sodium alginate and lemon peel powder retracted the most.  $98.33 \pm 0.29\%$  retraction ratio was observed in this film which indicates the highest interaction of hydrocolloids on drying (Puscaselu & Gheorghe, 2019). The least shrinkage was observed in sample SA1.5:LPP4, with only  $90.43 \pm 0.35\%$  contraction. It was found that the retraction ratio decreased with an increase in the concentration of lemon peel powder. The values obtained were slightly higher than those obtained by (Prasetyo et al., 2017), where a 91.45 to 93.55% retraction ratio was reported for glycerol plasticized sugar palm starch films.

**Table 4.15. Retraction ratio (%) of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>RETRACTION RATIO (%)</b>
SA0.5:LPP1	95.25 ± 0.25 <sup>cd</sup>
SA0.5:LPP2	95.00 ± 0.43 <sup>de</sup>
SA0.5:LPP3	94.32 ± 0.40 <sup>f</sup>
SA0.5:LPP4	92.02 ± 0.03 <sup>g</sup>
SA1:LPP1	98.33 ± 0.29 <sup>a</sup>
SA1:LPP2	96.70 ± 0.30 <sup>b</sup>
SA1:LPP3	95.58 ± 0.14 <sup>c</sup>
SA1:LPP4	94.68 ± 0.12 <sup>ef</sup>
SA1.5:LPP1	94.92 ± 0.38 <sup>de</sup>
SA1.5:LPP2	94.58 ± 0.38 <sup>ef</sup>
SA1.5:LPP3	92.23 ± 0.24 <sup>g</sup>
SA1.5:LPP4	90.43 ± 0.35 <sup>h</sup>

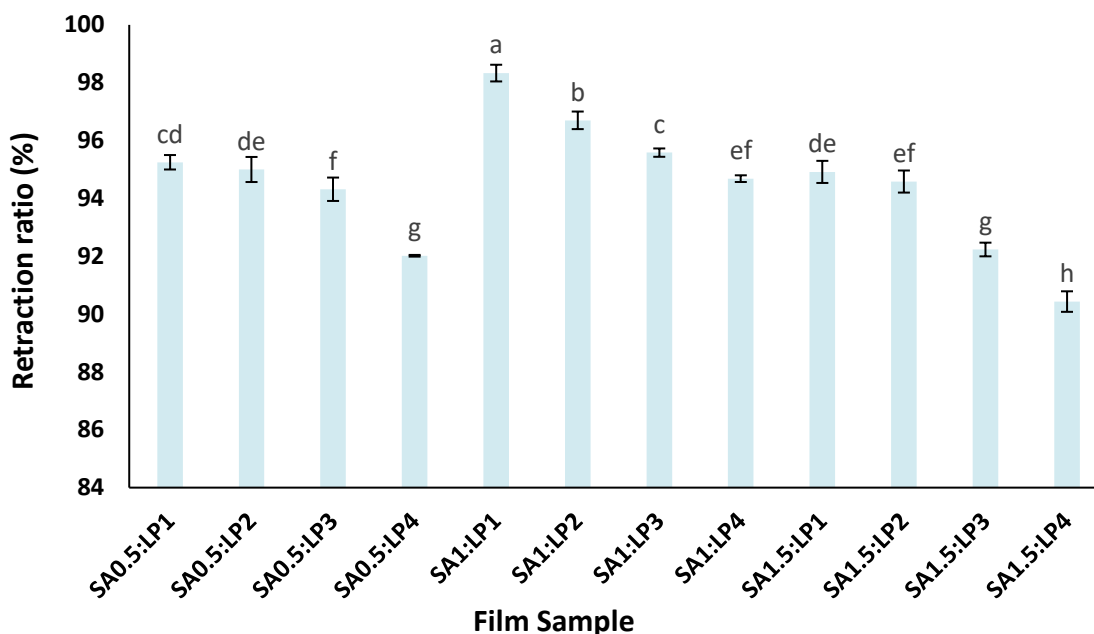
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

#### 4.16. ANOVA for retraction ratio

			<b>Sum of</b>	<b>df</b>	<b>Mean</b>	<b>F</b>	<b>Sig.</b>
			<b>Squares</b>		<b>Square</b>		
	<b>(Combined)</b>		148.832	11	13.530	147.938	.000
<b>Between</b>	<b>Linear</b>	<b>Contrast</b>	28.398	1	28.398	310.499	.000
	<b>Term</b>	<b>Deviation</b>	120.434	10	12.043	131.682	.000
	<b>Quadratic</b>	<b>Contrast</b>	44.677	1	44.677	488.496	.000
	<b>Term</b>	<b>Deviation</b>	75.757	9	8.417	92.036	.000
<b>Within Groups</b>			2.195	24	.091		
<b>Total</b>			151.027	35			



**Figure 4.8. Retraction ratio (%) of Lemon peel and Sodium alginate-based film**

#### 4.7. Water Solubility

Water solubility is crucial for assessing the water resistance of packaging films. It serves as a standard indicator for evaluating the water resistance and biodegradability of films. Additionally, films exhibiting high water solubility have the capacity to effectively release antimicrobial agents (Abdollahi et al., 2012). The water solubility of lemon peel and sodium alginate-based film varied from  $64.14 \pm 0.07\%$  to  $81.91 \pm 0.23\%$  (Table 4.17, Figure 4.9). The solubility is largely affected by the chemical composition of the biopolymer utilized for film formation, as well as its interaction with water molecules. The developed films exhibited high solubility due to the hydrophilicity of both sodium alginates as well as lemon peel powder. ANOVA results showed a significant difference ( $P < 0.05$ ) in the water solubility of the films (Table 4.18). Least solubility of  $64.14 \pm 0.07\%$  was observed in sample SA1:LPP1. The solubility of the films increased with an increase in lemon peel powder. Similar observations were made by Terzioğlu et al. (2021), where the solubility increased from 78.2% to 83.1% on the incorporation of orange peel into chitosan/PVA composite films.

**Table 4.17. Water Solubility (%) of Lemon peel and Sodium alginate-based film**

FILM SAMPLE	WATER SOLUBILITY (%)
SA0.5:LPP1	75.93 ± 0.35 <sup>d</sup>
SA0.5:LPP2	78.29 ± 0.22 <sup>c</sup>
SA0.5:LPP3	79.93 ± 0.30 <sup>b</sup>
SA0.5:LPP4	81.91 ± 0.23 <sup>a</sup>
SA1:LPP1	64.14 ± 0.07 <sup>j</sup>
SA1:LPP2	70.32 ± 0.32 <sup>h</sup>
SA1:LPP3	71.08 ± 0.22 <sup>g</sup>
SA1:LPP4	73.61 ± 0.36 <sup>e</sup>
SA1.5:LPP1	69.24 ± 0.43 <sup>i</sup>
SA1.5:LPP2	70.70 ± 0.43 <sup>gh</sup>
SA1.5:LPP3	72.27 ± 0.14 <sup>f</sup>
SA1.5:LPP4	78.12 ± 0.19 <sup>c</sup>

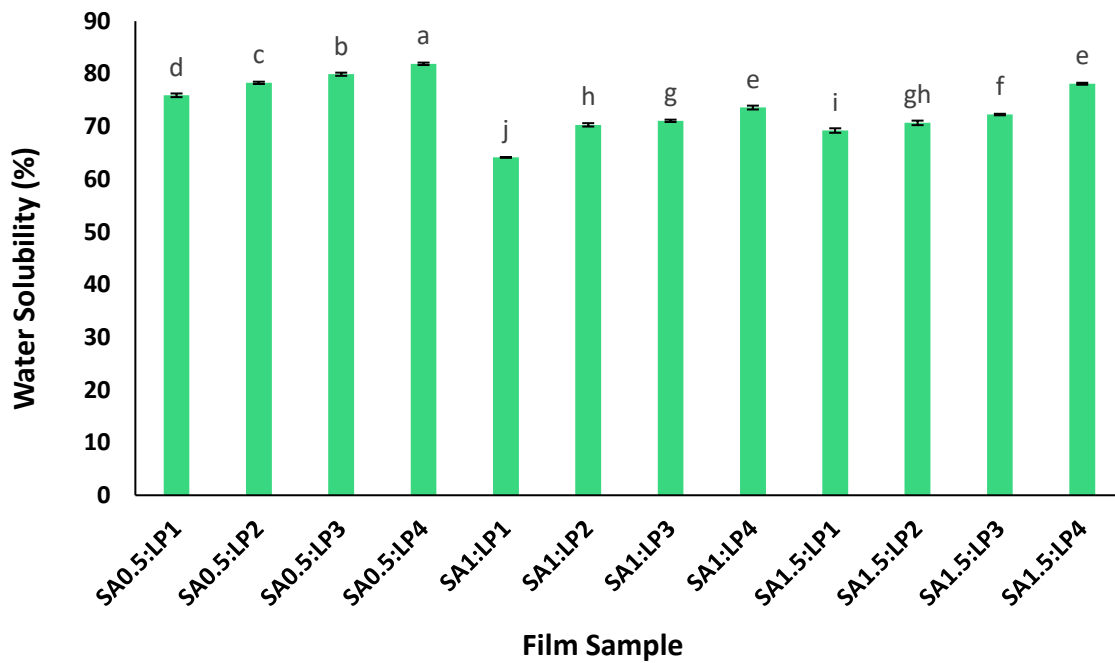
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different (P < 0.05).

Subset for alpha = 0.05

**Table 4.18. ANOVA for solubility**

			Sum of	df	Mean	F	Sig.
			Squares		Square		
	(Combined)		877.410	11	79.765	933.427	.000
Between Groups	Linear	Contrast	87.155	1	87.155	1019.911	.000
	Term	Deviation	790.255	10	79.026	924.779	.000
	Quadratic	Contrast	191.063	1	191.063	2235.874	.000
	Term	Deviation	599.192	9	66.577	779.101	.000
Within Groups			2.051	24	.085		
Total			879.461	35			



**Figure 4.9. Water Solubility (%) of Lemon peel and Sodium alginate-based film**

#### 4.8. Water Absorption

Water absorption of the lemon peel and sodium alginate-based film ranged from  $19.20 \pm 1.18\%$  to  $38.54 \pm 1.45\%$  (Table 4.19). ANOVA showed a significant difference ( $P < 0.05$ ) in the water absorption of films (Table 4.20). Water absorption was more for films with a lower concentration of lemon peel powder. The films should possess minimal water absorption capacity, which is essential for packaging foods with high moisture content. Water absorption of the film significantly decreased with an increase in peel powder content as depicted in Figure 4.10. Similar deductions were made in a previous study, where an increase in mosambi peel resulted in lower water absorption value of mosambi peel and sago based film (Ahmad et al., 2022). It is also evident from the results that increase in sodium alginate also lead to an increased water absorption. As can be seen, film sample SA1.5:LPP1, with a highest sodium alginate concentration has a absorption of  $38.54 \pm 1.45\%$  which is much greater than those of sample SA0.5:LPP1 and SA1:LPP1 with a water absorption of  $27.30 \pm 0.88$  and  $33.60 \pm 0.92\%$ , respectively.



**Table 4.19 Water Absorption (%) of Lemon peel and Sodium alginate-based film**

FILM SAMPLE	WATER ABSORPTION (%)
SA0.5:LPP1	27.30 ± 0.88 <sup>e</sup>
SA0.5:LPP2	25.08 ± 1.07 <sup>g</sup>
SA0.5:LPP3	21.33 ± 1.29 <sup>g</sup>
SA0.5:LPP4	19.20 ± 1.18 <sup>h</sup>
SA1:LPP1	33.60 ± 0.92 <sup>c</sup>
SA1:LPP2	31.26 ± 0.80 <sup>d</sup>
SA1:LPP3	27.97 ± 0.89 <sup>e</sup>
SA1:LPP4	26.29 ± 0.73 <sup>f</sup>
SA1.5:LPP1	38.54 ± 1.45 <sup>a</sup>
SA1.5:LPP2	35.79 ± 0.69 <sup>b</sup>
SA1.5:LPP3	33.44 ± 0.67 <sup>c</sup>
SA1.5:LPP4	29.94 ± 1.12 <sup>d</sup>

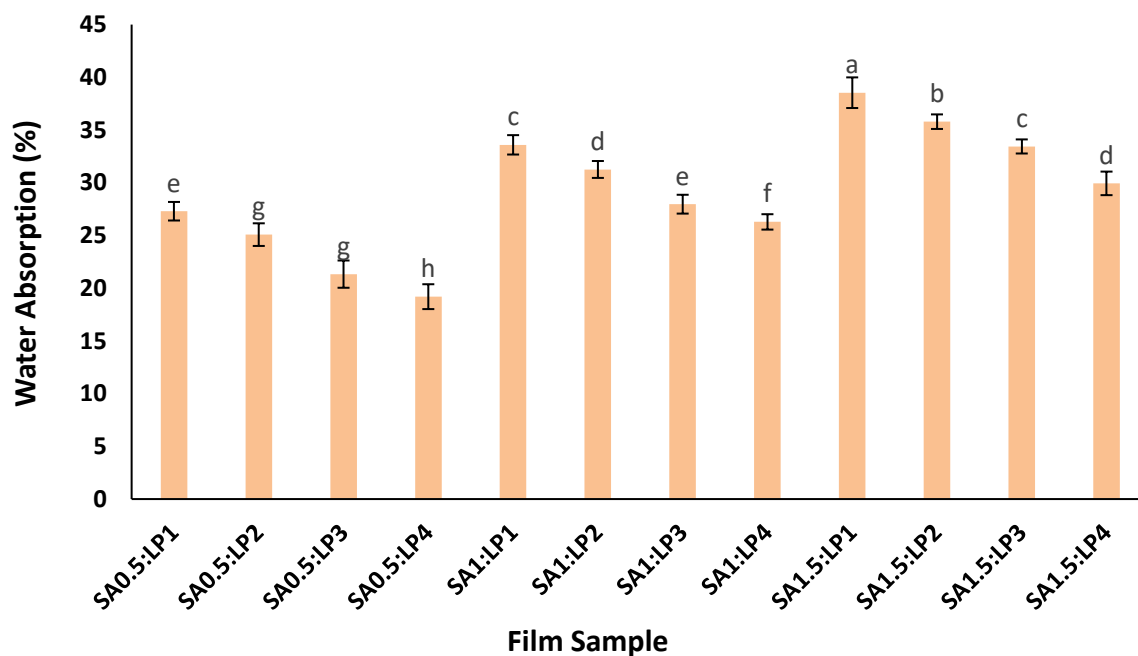
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.20. ANOVA for water absorption**

			Sum of	df	Mean	F	Sig.
			Squares		Square		
	(Combined)		1096.029	11	99.639	98.920	.000
Between Groups	Linear	Contrast	402.493	1	402.493	399.588	.000
	Term	Deviation	693.536	10	69.354	68.853	.000
	Quadratic	Contrast	3.758	1	3.758	3.731	.065
	Term	Deviation	689.778	9	76.642	76.089	.000
Within Groups			24.174	24	1.007		
Total			1120.203	35			



**Figure 4.10. Water Absorption (%) of Lemon peel and Sodium alginate-based film**

## 4.9. Color

Color characteristics hold significance for the visual aspect of the film, as they directly impact the attractiveness of the product and its acceptance by consumers. The  $L^*$ ,  $a^*$ , and  $b^*$  values are represented in Table 4.21, 4.22, and 4.23, respectively. Both Sodium alginate and lemon peel powder had a significant effect on color values which is discussed further.

### 4.9.1. $L^*$ value

The  $L^*$  value indicates the intensity of color i.e. lightness which varies from  $L=100$  for perfect white to  $L=0$  for black. The lightness value of the sodium alginate and lemon peel-based film varied from  $60.60 \pm 0.62$  to  $73.43 \pm 0.85$ . ANOVA results showed that  $L^*$  value of the films were significantly different ( $P < 0.05$ ) from each other (Table 4.22). Sample SA0.5:LPP1 with minimum sodium alginate and lemon peel concentration showed the highest lightness value of  $73.43 \pm 0.85$ , while sample SA1.5:LPP had the least  $L^*$  value of  $60.60 \pm$

0.62. A high concentration of lemon peel powder promoted lower  $L^*$  value (Figure 4.11). This may be attributed to the presence of solid residue in peel powder.

**Table 4.21.  $L^*$  value of Lemon peel and Sodium alginate-based film**

FILM SAMPLE	$L^*$ VALUE
SA0.5:LPP1	73.43 ± 0.85 <sup>a</sup>
SA0.5:LPP2	67.07 ± 0.61 <sup>d</sup>
SA0.5:LPP3	66.83 ± 1.12 <sup>d</sup>
SA0.5:LPP4	66.13 ± 0.84 <sup>d</sup>
SA1:LPP1	70.80 ± 0.44 <sup>b</sup>
SA1:LPP2	67.30 ± 0.40 <sup>d</sup>
SA1:LPP3	64.10 ± 0.20 <sup>e</sup>
SA1:LPP4	63.43 ± 1.33 <sup>e</sup>
SA1.5:LPP1	69.27 ± 0.47 <sup>c</sup>
SA1.5:LPP2	66.00 ± 0.66 <sup>d</sup>
SA1.5:LPP3	63.73 ± 0.40 <sup>e</sup>
SA1.5:LPP4	60.60 ± 0.62 <sup>f</sup>

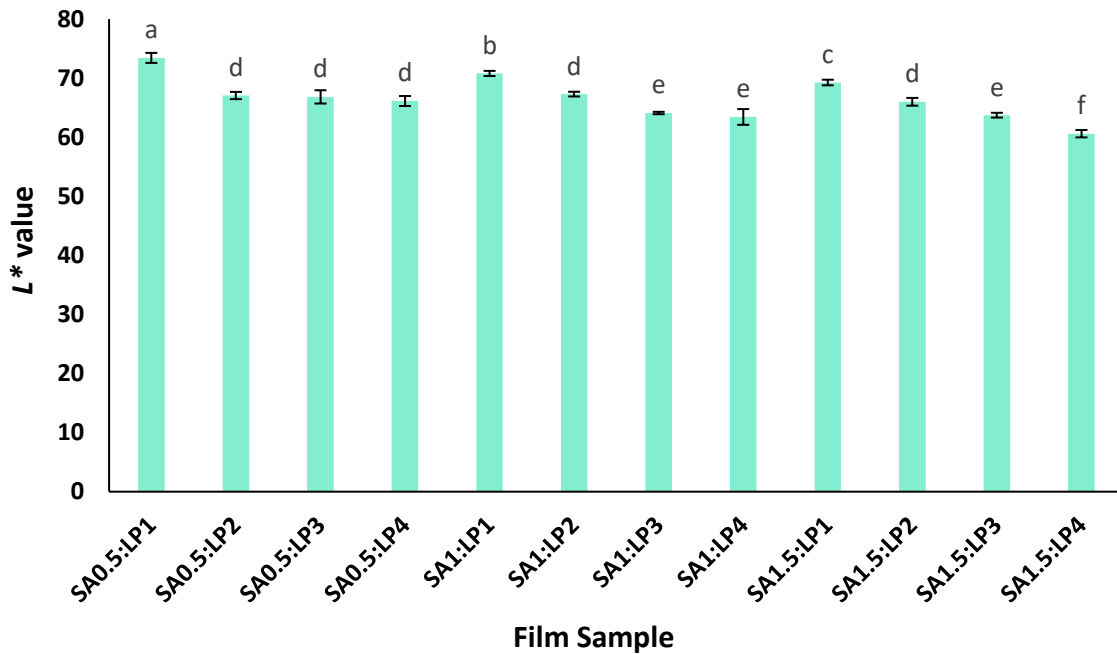
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.22. ANOVA for  $L^*$  value**

			Sum of Squares	df	Mean Square	F	Sig.
<b>Between Groups</b>	<b>(Combined)</b>		399.781	11	36.344	67.791	.000
	<b>Linear</b>	<b>Contrast</b>	182.685	1	182.685	340.760	.000
	<b>Term</b>	<b>Deviation</b>	217.096	10	21.710	40.494	.000
	<b>Quadratic</b>	<b>Contrast</b>	.089	1	.089	.166	.687
	<b>Term</b>	<b>Deviation</b>	217.006	9	24.112	44.975	.000
<b>Within Groups</b>			12.867	24	.536		
<b>Total</b>			412.647	35			



**Figure 4.11.  $L^*$  value of Lemon peel and Sodium alginate-based film**

#### 4.9.2. $a^*$ value

Chromaticity parameter  $a^*$  represents green–red color opponents with values ranging from -128 to +127. The ‘- ve’  $a^*$  value indicates a trend towards green and ‘+ ve’ towards red. The  $a^*$  value of the films ranged from  $-0.67 \pm 0.74$  to  $2.57 \pm 0.57$ .  $a^*$  value of the films were highly influenced by the concentration of lemon peel powder in the formulation (Figure 4.12). High concentration of lemon peel powder indicated a trend towards redness of the film. ANOVA showed a significant difference ( $p < 0.05$ ) in the  $a^*$  value of the film (Table 4.24). Sample SA0.5:LPP4, having sodium alginate and lemon peel in the concentration 0.5 and 4 respectively, was darkest among all with an  $a^*$  value of  $2.57 \pm 0.57$ . Sodium alginate also has a considerable effect on the  $a^*$  value. Films formulated with 0.5 and 1% sodium alginate were more towards red while those formulated with 1.5% sodium alginate indicated a trend towards green with values varying from  $-0.67 \pm 0.74$  to  $-1.90 \pm 0.36$ . Overall,  $a^*$  values of the film increased with increase in peel powder and decreased with an increase in sodium alginate. The results were similar to that of pectin/sodium alginate/xanthan gum based film (Yang et al., 2021).

**Table 4.23.  $a^*$  value of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b><math>a^*</math> VALUE</b>
<b>SA0.5:LPP1</b>	$1.10 \pm 0.56^b$
<b>SA0.5:LPP2</b>	$1.67 \pm 0.32^{ab}$
<b>SA0.5:LPP3</b>	$1.70 \pm 0.36^{ab}$
<b>SA0.5:LPP4</b>	$2.57 \pm 0.57^a$
<b>SA1:LPP1</b>	$1.07 \pm 0.55^b$
<b>SA1:LPP2</b>	$1.57 \pm 0.51^b$
<b>SA1:LPP3</b>	$1.63 \pm 0.57^{ab}$
<b>SA1:LPP4</b>	$1.83 \pm 0.15^{ab}$
<b>SA1.5:LPP1</b>	$-1.90 \pm 0.36^d$
<b>SA1.5:LPP2</b>	$-1.33 \pm 0.42^{cd}$
<b>SA1.5:LPP3</b>	$-1.03 \pm 0.71^{cd}$
<b>SA1.5:LPP4</b>	$-0.67 \pm 0.74^c$

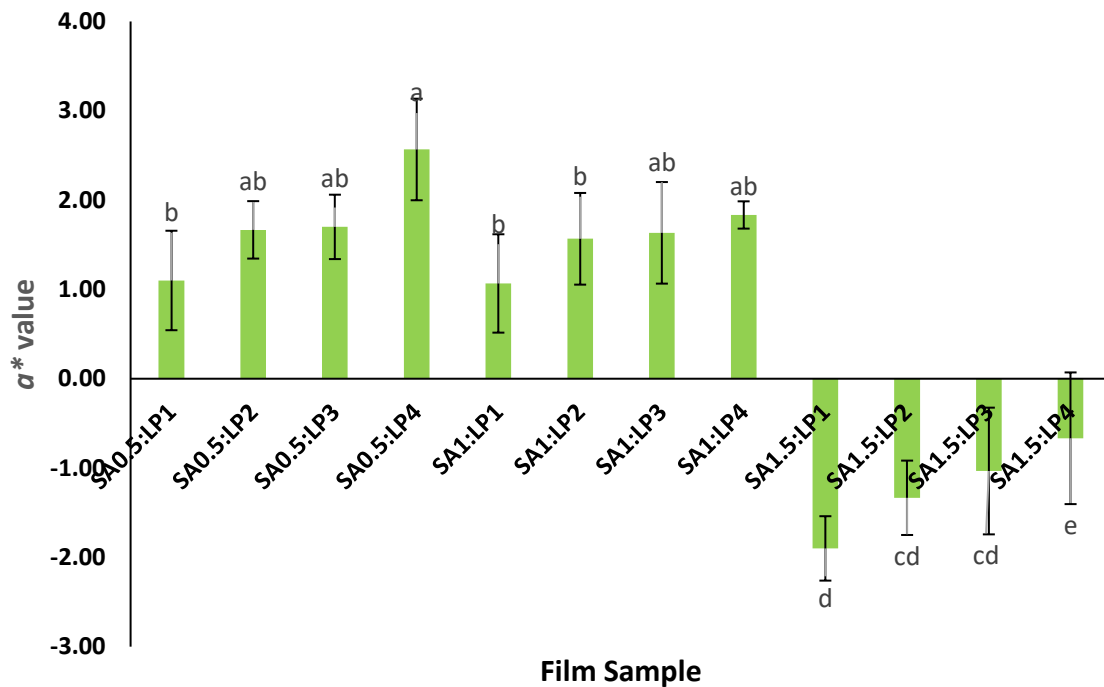
Values are expressed as means  $\pm$  standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.24. ANOVA for  $a^*$  value**

			<b>Sum of</b>	<b>df</b>	<b>Mean</b>	<b>F</b>	<b>Sig.</b>
			<b>Squares</b>		<b>Square</b>		
	<b>(Combined)</b>		73.150	11	6.650	25.495	.000
<b>Between</b>	<b>Linear</b>	<b>Contrast</b>	37.834	1	37.834	145.050	.000
	<b>Term</b>	<b>Deviation</b>	35.316	10	3.532	13.540	.000
	<b>Quadratic</b>	<b>Contrast</b>	6.999	1	6.999	26.832	.000
	<b>Term</b>	<b>Deviation</b>	28.317	9	3.146	12.063	.000
<b>Within Groups</b>			6.260	24	.261		
<b>Total</b>			79.410	35			



**Figure 4.12.  $a^*$  value of Lemon peel and Sodium alginate-based film**

### 4.9.3. $b^*$ value

The  $b^*$  value indicates yellow and blue opponents of a color, where  $b^*$  (+) and  $b^*$  (-) represents yellow and blue color, respectively and range from -128 to + 127.  $b^*$  value of the lemon peel and sodium alginate-based film ranged from  $10.20 \pm 0.46$  to  $30.07 \pm 0.76$ . Statistical analysis showed a significant difference ( $P < 0.05$ ) in the  $b^*$  values of the film (Table 4.26). As evident from figure 4.13, the yellowness of the film increased with an increase in lemon peel concentration. Sample SA0.5:LPP1, with the least concentration of 0.5% and 1% sodium alginate and lemon peel, respectively had lowest  $b^*$  value of  $10.20 \pm 0.46$  while those with maximum concentration of both was more yellow with a  $b^*$  value of  $30.07 \pm 0.76$ . The results were similar to those obtained by (Taghavi et al., 2020) for gelatin based films enriched with orange peel powder.

**Table 4.25. *b*\* value of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b><i>b</i>* VALUE</b>
SA0.5:LPP1	10.20 ± 0.46 <sup>h</sup>
SA0.5:LPP2	17.57 ± 0.57 <sup>e</sup>
SA0.5:LPP3	19.23 ± 0.35 <sup>d</sup>
SA0.5:LPP4	26.20 ± 1.15 <sup>b</sup>
SA1:LPP1	12.20 ± 1.64 <sup>g</sup>
SA1:LPP2	14.53 ± 0.78 <sup>f</sup>
SA1:LPP3	26.93 ± 0.49 <sup>b</sup>
SA1:LPP4	28.67 ± 0.60 <sup>a</sup>
SA1.5:LPP1	16.17 ± 0.86 <sup>e</sup>
SA1.5:LPP2	23.50 ± 1.04 <sup>c</sup>
SA1.5:LPP3	26.17 ± 1.20 <sup>b</sup>
SA1.5:LPP4	30.07 ± 0.76 <sup>a</sup>

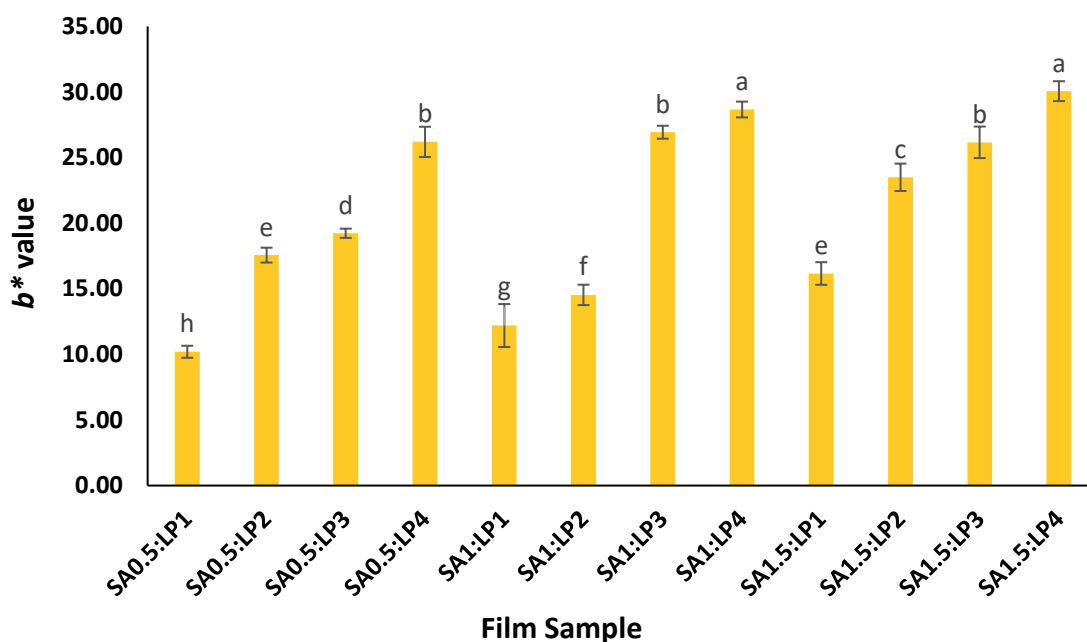
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.26. ANOVA for *b*\* value**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>Between Groups</b>	<b>(Combined)</b>		1530.936	11	139.176	171.704	.000
	<b>Linear</b>	<b>Contrast</b>	597.174	1	597.174	736.747	.000
	<b>Term</b>	<b>Deviation</b>	933.762	10	93.376	115.200	.000
	<b>Quadratic</b>	<b>Contrast</b>	.009	1	.009	.011	.917
	<b>Term</b>	<b>Deviation</b>	933.753	9	103.750	127.999	.000
<b>Within Groups</b>			19.453	24	.811		
<b>Total</b>			1550.390	35			



**Figure 4.13. *b\** value of Lemon peel and Sodium alginate-based film**

#### **4.10. Film Opacity and Transparency**

Color and UV-vis light barrier properties are significant characteristics that serve as an indicator of packaging efficacy in safeguarding food and beverages, acting as a protective barrier against the harmful effects of light-induced food degradation (Hamdi et al., 2019; Kwon et al., 2018). The UV-visible light barrier attributes of the films were assessed by measuring the absorbance and transmittance of the sample. Film opacity and transparency values of the film are shown in Tables 4.27 and 4.29, respectively. The absorbance value of the films increased with the increase in lemon peel powder resulting in an increase in opacity. As a result, less light could pass through the film incorporated with a higher concentration of lemon peel. ANOVA showed a significant difference ( $P < 0.05$ ) in the opacity of the film (Table 4.28). The opacity of the lemon peel and sodium alginate based films ranged from  $4.70 \pm 0.12$  to  $7.14 \pm 0.37$ . No significant difference was observed in sample SA0.5:LPP1, SA1:LPP1, SA1.5:LPP1, with varying sodium alginate concentrations of 0.5, 1, and 1.5, respectively along with constant 1% lemon peel concentrations in all the three samples. As demonstrated in Figure 4.14, the opacity of the films increased with an increase in lemon peel powder concentration.



Similar trend was observed by (Terzioğlu & Parın, 2020), where increase in lemon peel in PVA/starch film resulted in higher opacity lower and transparency of films.

**Table 4.27. Opacity of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>OPACITY</b>
<b>SA0.5:LPP1</b>	4.75 ± 0.09 <sup>f</sup>
<b>SA0.5:LPP2</b>	5.34 ± 0.19 <sup>de</sup>
<b>SA0.5:LPP3</b>	5.51 ± 0.34 <sup>cd</sup>
<b>SA0.5:LPP4</b>	5.97 ± 0.15 <sup>b</sup>
<b>SA1:LPP1</b>	4.70 ± 0.12 <sup>f</sup>
<b>SA1:LPP2</b>	4.85 ± 0.10 <sup>f</sup>
<b>SA1:LPP3</b>	4.99 ± 0.11 <sup>ef</sup>
<b>SA1:LPP4</b>	5.84 ± 0.30 <sup>bc</sup>
<b>SA1.5:LPP1</b>	4.70 ± 0.33 <sup>f</sup>
<b>SA1.5:LPP2</b>	6.17 ± 0.27 <sup>b</sup>
<b>SA1.5:LPP3</b>	6.91 ± 0.11 <sup>a</sup>
<b>SA1.5:LPP4</b>	7.14 ± 0.37 <sup>a</sup>

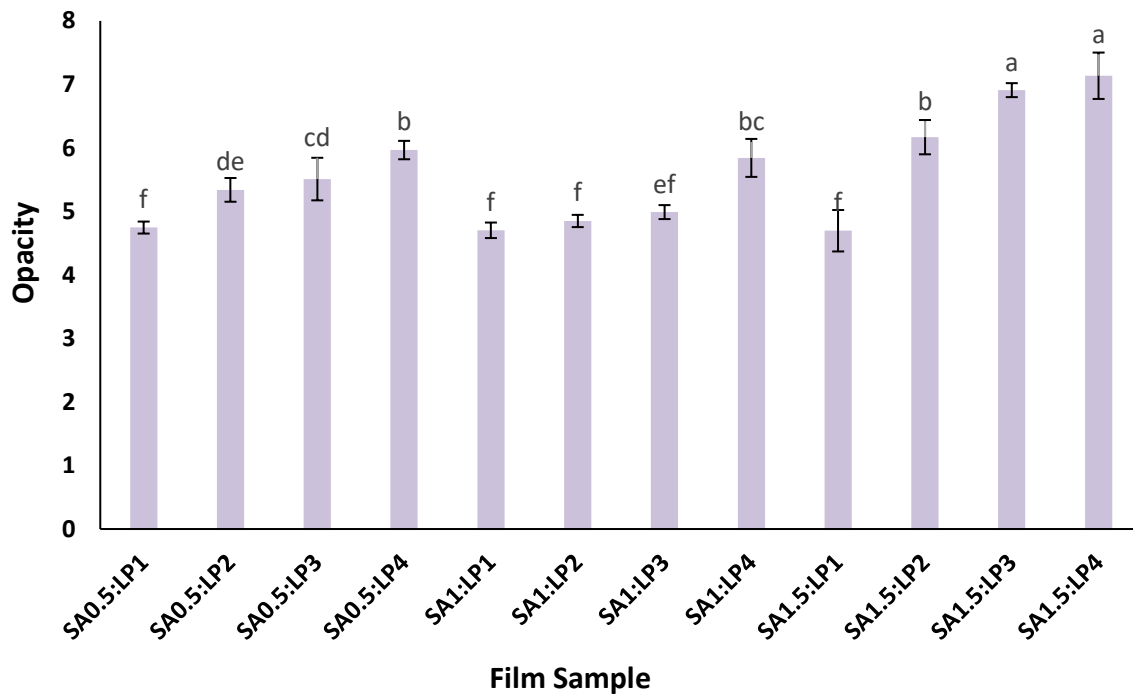
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different (P < 0.05).

Subset for alpha = 0.05

**Table 4.28. ANOVA for opacity**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>(Combined)</b>			23.835	11	2.167	41.431	.000
<b>Between Groups</b>	<b>Linear</b>	<b>Contrast</b>	9.362	1	9.362	179.010	.000
	<b>Term</b>	<b>Deviation</b>	14.473	10	1.447	27.673	.000
	<b>Quadratic</b>	<b>Contrast</b>	4.894	1	4.894	93.574	.000
	<b>Term</b>	<b>Deviation</b>	9.579	9	1.064	20.351	.000
<b>Within Groups</b>			1.255	24	.052		
<b>Total</b>			25.090	35			



**Figure 4.14. Opacity of Lemon peel and Sodium alginate-based film**

The transparency of the lemon peel and sodium alginate based-film ranged from  $3.56 \pm 0.47$  to  $7.03 \pm 0.09$ . Statistical analysis showed a significant difference ( $P < 0.05$ ) in the transparency of films (Table 4.30). The higher transparency value of lemon peel and sodium alginate films in the visible range (600 nm), significantly decreased with an increase in lemon peel in the formulation (Figure 4.15), which were in agreement with the film opaqueness. The films showed good light barrier properties. Since, citrus peels are rich in polyphenols, they highly contribute to the light barrier capacity of the films (Yun & Liu, 2022). Therefore, they have potential to be used as a packaging for photo-sensitive foods. The results obtained were in accordance with the findings of (Terzioğlu & Parm, 2020). They reported transparency in the range of 5.55 to 6.71 for lemon peel incorporated PVA/starch films.

**Table 4.29. Transparency of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>TRANSPARENCY</b>
SA0.5:LPP1	7.03 ± 0.09 <sup>a</sup>
SA0.5:LPP2	4.81 ± 0.56 <sup>d</sup>
SA0.5:LPP3	3.83 ± 0.28 <sup>ef</sup>
SA0.5:LPP4	3.57 ± 0.44 <sup>f</sup>
SA1:LPP1	6.85 ± 0.11 <sup>ab</sup>
SA1:LPP2	5.72 ± 0.21 <sup>c</sup>
SA1:LPP3	5.46 ± 0.07 <sup>c</sup>
SA1:LPP4	4.31 ± 0.26 <sup>e</sup>
SA1.5:LPP1	6.76 ± 0.14 <sup>ab</sup>
SA1.5:LPP2	6.53 ± 0.12 <sup>ab</sup>
SA1.5:LPP3	6.40 ± 0.18 <sup>b</sup>
SA1.5:LPP4	3.56 ± 0.47 <sup>f</sup>

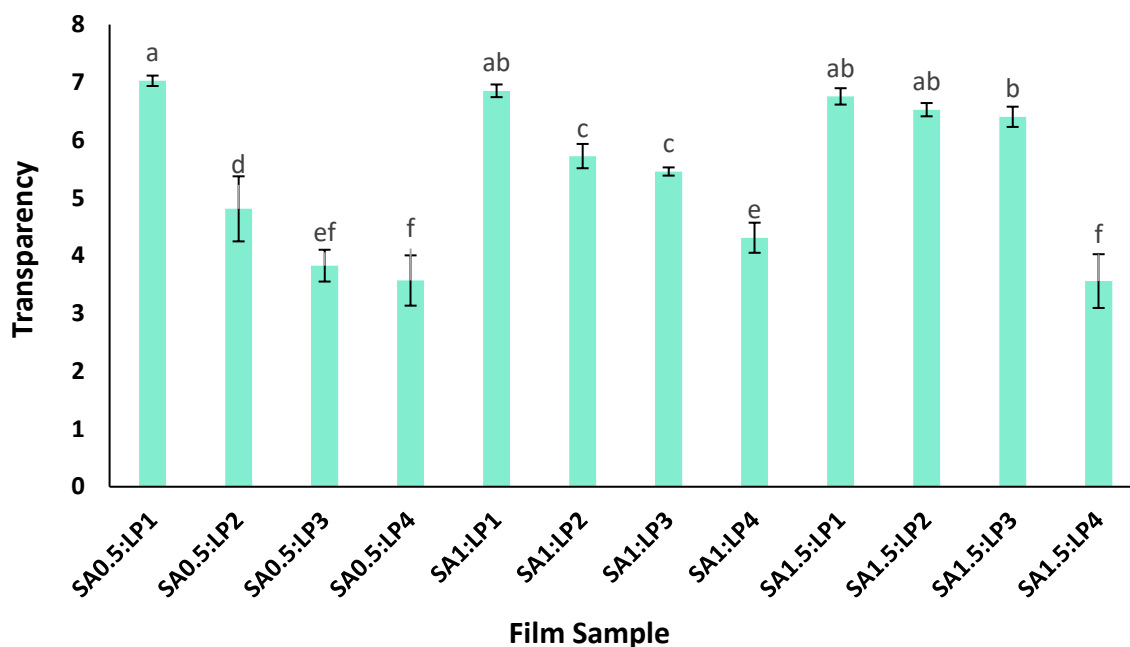
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different ( $P < 0.05$ ).

Subset for alpha = 0.05

**Table 4.30. ANOVA for transparency**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>Between Groups</b>	<b>(Combined)</b>		59.186	11	5.381	64.575	.000
	<b>Linear</b>	<b>Contrast</b>	.052	1	.052	.622	.438
	<b>Term</b>	<b>Deviation</b>	59.134	10	5.913	70.970	.000
	<b>Quadratic</b>	<b>Contrast</b>	.078	1	.078	.932	.344
	<b>Term</b>	<b>Deviation</b>	59.056	9	6.562	78.752	.000
<b>Within Groups</b>			2.000	24	.083		
<b>Total</b>			61.186	35			



**Figure 4.15. Transparency of Lemon peel and Sodium alginate-based film**

#### 4.11. Biodegradability

The visual appearance of lemon peel powder and sago-based film after burial for a definite period can be visualized in Plate 16(c). The developed film buried for 15 days in loamy soil showed significant changes. As per ASTM 15448-2 guidelines, materials are classified as biodegradable when a minimum of 90% organic carbon is transformed into CO<sub>2</sub> in a duration of six months. The development of cracks and color changes suggest the occurrence of disintegration. The biodegradability of the film ranged from  $83.80 \pm 0.36\%$  to  $90.76 \pm 0.21\%$  (Table 4.31, Figure 4.16). The biodegradability was relatively higher than the biodegradability of the starch-pectin blend films (10-15% kept for 7 days) as reported by (Jeyasubramanian & Balachander, 2016). ANOVA results showed a significant difference ( $P < 0.05$ ) in the degradability of films (Table 4.32). Highest biodegradability of  $90.76 \pm 0.21\%$  was observed in sample SA1.5:LPP3 and SA1.5:LPP4.

**Table 4.31. Biodegradability (%) of Lemon peel and Sodium alginate-based film**

<b>FILM SAMPLE</b>	<b>BIODEGRADABILITY (%)</b>
SA0.5:LPP1	83.80 ± 0.36 <sup>d</sup>
SA0.5:LPP2	84.72 ± 0.33 <sup>d</sup>
SA0.5:LPP3	84.63 ± 0.45 <sup>d</sup>
SA0.5:LPP4	84.80 ± 0.72 <sup>d</sup>
SA1:LPP1	84.90 ± 1.30 <sup>d</sup>
SA1:LPP2	86.67 ± 0.83 <sup>c</sup>
SA1:LPP3	86.67 ± 0.83 <sup>c</sup>
SA1:LPP4	86.67 ± 0.83 <sup>c</sup>
SA1.5:LPP1	89.37 ± 0.35 <sup>b</sup>
SA1.5:LPP2	88.94 ± 0.42 <sup>b</sup>
SA1.5:LPP3	90.76 ± 0.21 <sup>a</sup>
SA1.5:LPP4	90.76 ± 0.21 <sup>a</sup>

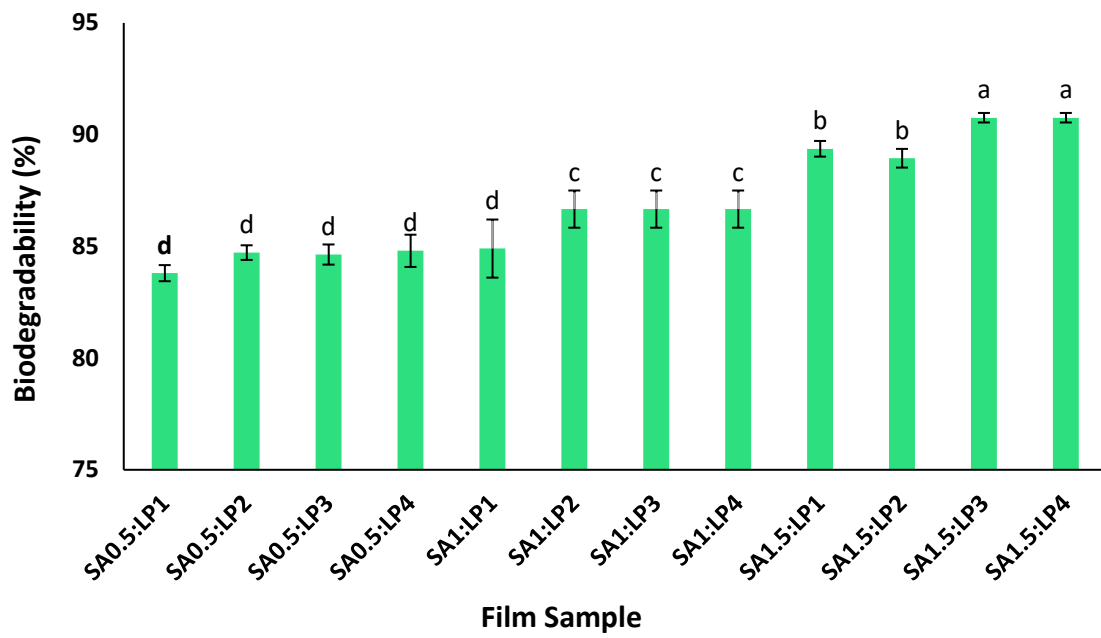
Values are expressed as means ± standard deviation.

Means in columns followed by different letters are significantly different (P < 0.05).

Subset for alpha = 0.05

**Table 4.32 ANOVA for Biodegradability**

			<b>Sum of Squares</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>(Combined)</b>			204.239	11	18.567	43.519	.000
<b>Between Groups</b>	<b>Linear</b>	<b>Contrast</b>	187.659	1	187.659	439.845	.000
	<b>Term</b>	<b>Deviation</b>	16.580	10	1.658	3.886	.003
	<b>Quadratic</b>	<b>Contrast</b>	6.249	1	6.249	14.646	.001
	<b>Term</b>	<b>Deviation</b>	10.332	9	1.148	2.691	.026
<b>Within Groups</b>			10.240	24	.427		
<b>Total</b>			214.478	35			



**Figure 4.16. Biodegradability (%) of Lemon peel and Sodium alginate-based film**

## CHAPTER 5

### SUMMARY AND CONCLUSION

Sodium alginate and lemon peel-based biodegradable packaging film was successfully developed. All the twelve films with the following ratios of Sodium alginate: lemon peel powder:: 0.5:1, 0.5:2, 0.5:3, 0.5:4, 1:1, 1:2, 1:3, 1:4, and 1.5:1, 1.5:2, 1.5:3, 1.5:4 were found to have acceptable appearance and properties to be categorized as a biodegradable film. The use of glycerol had a plasticizing effect on the film resulting in the formation of flexible films with no sign of brittleness. Varying concentrations of sodium alginate and lemon peel powder in the formulation had a significant effect on film properties. The developed films were assessed for their physical, mechanical, optical, light barrier, and biodegradability properties. An increase in film thickness and grammage was observed as the concentration of lemon peel powder increased. Thickness was slightly greater in all sodium alginate films incorporated with 4% lemon peel powder. While the least thickness was reported in SA1:LPP1 having an equal ratio of sodium alginate and lemon peel powder. Grammage is highly proportional to the film thickness as the least value was observed in sample SA1:LPP1. Comparison with the other published work established that the addition of lemon peel to sodium alginate significantly improved the moisture content of the films and the sample having equal concentration depicted the lowest moisture resulting from restricted interaction between the film matrix and water. Both sodium alginate and lemon peel contributed to the ash content.

Tensile strength characterized by the maximum tension the film can withstand was found to improve. The elongation percentage was in line with the results of tensile strength as they presented a lower elongation percentage for films with higher tensile strength. The least elongation percentage was observed in SA1:LPP1 which reportedly had the highest tensile strength. Burst strength is largely dependent on thickness. It was more for films with a higher concentration of peel powder and hence thickness. The developed films exhibited high solubility due to the hydrophilicity of both sodium alginates as well as lemon peel powder. While the water absorption was reduced with an increase in lemon peel in the formulation. Sample SA1:LPP1 had the highest retraction ratio, signifying a higher mechanical strength. Varied concentrations of lemon peel and sodium alginate had a significant effect on color

values. Sample SA0.5:LPP1 with minimum sodium alginate and lemon peel concentration showed the highest lightness values. An increase in peel powder resulted in a concurrent increase in darkness due to the presence of residual particles in peel powder. Higher concentrations of peel powder resulted in an increase of both  $a^*$  and  $b^*$  values. However,  $a^*$  values further decreased and showed a trend toward greenness with the increase in the concentration of sodium alginate. The absorbance of film at visible wavelength increased with an increase in peel powder, marked by a significant increase in the opacity of the film. Conversely, light transmission decreased with an increase in peel powder. Thereby, films possessed excellent light barrier properties. Moreover, the films also showed 90.76% degradability within 15 days.

The results obtained indicate that film sample SA1:LPP1 having sodium alginate and lemon peel powder in the ratio of 1:1 had better tensile strength, elongation percentage, water solubility, and water absorption. It also displayed the least thickness, grammage, and highest retraction ratio and other parameters within the acceptable range. The findings suggest that lemon peel, a by-product of the juice processing industry has the potential to be crafted into a biodegradable packaging film. Valorization of this zero-cost by-product can reduce the load on petroleum resources which are non-renewable and on the verge of depletion. Employing sustainable and biodegradable films for packaging could contribute to mitigating the drawbacks associated with plastic packaging and addressing the issue posed by the disposal of substantial amounts of lemon waste.



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