REGENERATED CELLULOSE FILMS WITH HEAT SEALABILITY AND IMPROVED BARRIER PROPERTIES FOR SUSTAINABLE FOOD PACKAGING

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ABSTRACT

With the increasing demands of human life and production, non-degradable plastic packaging materials have caused serious disposal and pollution problems and promoted the interest and development of natural polymer-based packaging materials. Among them, cellulose is the most widely distributed and abundant natural polymer in nature and can be dissolved and regenerated to make transparent films. However, cellulose is not a thermoplastic, adhesives are usually needed to seal the cellulose films. In this study, regenerated cellulose (RC) films were endowed with heat sealability, as well as better mechanical and barrier properties for food preservation. Chapter 2 reviewed the recent research development of biodegradable food packaging materials derived from natural resources, focusing on the following three aspects: (1) sources and classification of biodegradable materials; (2) preparation methods of biodegradable packaging; and (3) performance and food-related applications of biodegradable packaging materials. Chapter 3 developed the heat sealable regenerated cellulose films by coating with zein and investigated the structure and mechanical and barrier properties of the composite cellulose/zein films. The composite films exhibited good heat-sealing and barrier properties with the addition of zein coating. Moreover, blueberries packed in heat-sealed cellulose/zein bags were protected from oxidation and spoilage for a storage period of 12 days, which was comparable to blueberries packed with Ziploc®. Chapter 4 aimed to further improve the water vapor barrier property of cellulose/zein composite films by the incorporation of sodium stearate. The results showed that the water vapor barrier property was significantly improved with the increased amount of sodium stearate. Especially, the composite film containing 20% sodium stearate had the lowest WVP value of $1.56 \pm 0.06 \times 10^{-7}$ g m^{-1} h^{-1} Pa^{-1}, although the tensile strength (31.77 ± 1.23 MPa) and strain ($1.52 \pm 0.22\%$) also decreased. All the sodium stearate-modified cellulose/zein composite films possessed good biodegradability and could prevent the weight loss of blueberries. Overall, this study demonstrates a facile method for manufacturing heat-sealable cellulose-based packaging materials with potential applications in sustainable food packaging.

RÉSUMÉ

Avec les exigences croissantes de la vie humaine et de la production humaine, les matériaux d'emballage en plastique non dégradable ont causé de graves problèmes d'élimination et de pollution et ont favorisé l'intérêt et le développement des matériaux d'emballage à base de polymères naturels. Parmi eux, la cellulose est le polymère naturel le plus répandu et le plus abondant dans la nature et peut être dissoute et régénérée pour fabriquer des films transparents. Cependant, la cellulose n'est pas un thermoplastique, des adhésifs sont généralement nécessaires pour sceller les films de cellulose. Dans cette étude, les films de cellulose régénérée (RC) ont été dotés d'une thermoscellabilité, ainsi que de meilleures propriétés mécaniques et barrières pour la conservation des aliments. Le chapitre 2 a passé en revue le développement récent de la recherche sur les matériaux d'emballage alimentaire biodégradables dérivés de polymères naturels, en se concentrant sur les trois aspects suivants : (1) les sources et la classification des matériaux biodégradables; (2) les méthodes de préparation des emballages biodégradables; et (3) les applications liées à la performance et à l'alimentation des matériaux d'emballage biodégradables. Le chapitre 3 a développé les films de cellulose régénérée thermosoudables par enduction de zéine et a étudié la structure et les propriétés mécaniques et barrières des films composites cellulose/zéine. Avec l'ajout d'un revêtement de zéine, les films composites présentaient de bonnes propriétés de thermoscellage et de barrière. De plus, les bleuets emballés dans des sacs de cellulose/zéine thermoscellés ont été protégées de l'oxydation et de la détérioration pendant une période de stockage de 12 jours, ce qui était comparable aux bleuets emballés dans du Ziploc®. Le chapitre 4 visait à améliorer encore la propriété barrière à la vapeur d'eau des films composites cellulose/zéine par l'incorporation de stéarate de sodium. Les résultats ont montré que la propriété de barrière à la vapeur d'eau était significativement améliorée avec l'augmentation de la quantité de stéarate de sodium, le film composite contenant 20% de stéarate de sodium avait la valeur PVW la plus faible de $1,56 \pm 0,06 \times 10^{-7}$ g m^{-1} h^{-1} Pa^{-1}, bien que la résistance à la traction (31,77 ± 1,23 MPa) et à la déformation (1,52 ± 0,22%) également réduit. Tous les films composites cellulose/zéine modifiés au stéarate de

sodium possédaient une bonne biodégradabilité et pouvaient empêcher la perte de poids des myrtilles. Dans l'ensemble, cette étude démontre une méthode facile pour fabriquer des matériaux d'emballage thermoscellables à base de cellulose avec des applications potentielles dans le domaine de l'emballage alimentaire durable.

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CONTRIBUTION OF AUTHORS

This thesis is edited in a manuscript format and consists of five chapters. Chapter 1 presents a general introduction that includes a brief overview of the recent relevant work in the literature and describes the research objectives presented in the thesis. Chapter 2 reviewed the research on the properties and applications of biodegradable food packaging derived from natural resources in the past five years. Chapter 3 developed the zein-coated RC films with heat sealability for food preservation. Chapter 4 examined the structure, mechanical properties, barrier properties, and biodegradability of sodium stearate-modified RC films, and finally, Chapter 5 presented the overall conclusion of the thesis, as well as some suggestions for future research.

The present author, Yaqi Chu, was responsible for the experimental work, data acquisition and analysis, and writing of the thesis. Dr. Yixiang Wang, the thesis supervisor, guided the research and revised the thesis prior to submission. Cassandra Popovich was responsible for reviewing and revising Chapter 3.

PUBLICATIONS

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LIST OF ABBREVIATIONS

ABTS:	2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid ammonium salt)
CNC:	Cellulose nanocrystal
CNF:	cellulose nanofiber
CO_2	Carbon dioxide
DPPH:	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric ion reducing antioxidant power
FT-IR	Fourier transform infrared spectroscopy
HCl:	Hydrogen chloride
H_2O	Water
H_2SO_4:	Sulfuric acid
NaOH:	Sodium hydroxide
O_2	Oxygen
OTR	Oxygen transmission rate
PLA:	Poly(lactic acid)
PPI:	Peanut protein isolate
PS:	Pea starch
PVA:	Polyvinyl alcohol
RC:	Regenerated cellulose
RH:	Relative humidity
SEM:	Scanning electron microscope
SiO_2:	Silicon dioxide

SPI:	Soybean protein isolate
SS:	Sodium stearate
TPTZ:	2,4,6-Tri (2-pyridyl)-s-triazine
UV	Ultraviolet
WVP	Water vapor permeability

CHAPTER 1. INTRODUCTION

The raw materials of synthetic plastics are taken from petroleum resources (Trivedi et al., 2016). The demand for these petroleum-based plastic products has steadily increased due to their good properties and wide range of applications. Petroleum-based plastics have contributed significantly to the economic growth of many industries such as packaging, electronics, and medical (Alshehrei, 2017). However, they are also causing serious environmental problems because of the short life span, rapid plastic consumption rate, and non-degradability. Petroleum-based plastic waste accounts for a significant portion of municipal waste. They can accumulate in the environment and remain for up to 2,000 years (DiGregorio, 2009). It is predicted that in 2050, approximately 12 billion metric tons of plastic waste will accumulate in the natural environment or in landfills (Geyer et al., 2017), which not only waste carbon feedstock, but also be harmful to animals and plants, as well as to humans (Dhanraj et al., 2022). The environmental issues caused by petroleum-based plastics are due to the lack of proper and environmentally friendly disposal methods. Some countries have banned the burning of plastics to avoid the persistent toxic pollutants produced by combustion, and the impact of carbon dioxide on global warming is unquestionable (Alshehrei, 2017). Moreover, petroleum-based plastics are more harmful to the marine system, where 100 million tons of plastics generate a cost of about $13 billion per year in lost ecosystem services (Naser et al., 2021). Therefore, there is an urgent need to find eco-friendly alternatives to petroleum-based plastics.

Cellulose is the most widely distributed natural polymer with attractive structure, renewability, and biodegradability for eco-friendly applications (Tu et al., 2021), for instance, bagasse cellulose nanofibers for food packaging (Ghaderi et al., 2014), biosynthesis of edible bacterial cellulose packaging (Yang et al., 2021), modified methyl cellulose composite films with antibacterial properties (Zhou & Wang, 2021), and so on. Cellulose can be dissolved in NaOH/urea aqueous solution and regenerated in a sulfuric acid bath to fabricate transparent cellulose films (Cai & Zhang, 2005). However, due to the lack of heat-sealing property, cellulose films usually need adhesives for food packaging applications. It has been reported

that zein has good heat-sealing capacity, making it an attractive biopolymer for food packaging (Tsai & Weng, 2019). Therefore, zein was selected to endow the RC films with heat sealability while maintaining the biodegradability of the composite films, and the structure and properties of the modified cellulose/zein composite films were investigated.

Apart from heat-sealing property, barrier properties are also essential for food packaging materials. The water vapor permeability of RC films is relatively high, so some studies have focused on improving the water vapor barrier property of RC films. For instance, Xu et al. (2019) added graphene oxide and nano-SiO_2 was combined by Reddy et al. (2018). Sodium stearate can be used as a water repellent in paper making process to ensure it is appropriate for writing and printing. It was reported that He et al. (2013) and Sobhana et al. (2017) have used sodium stearate to prepare highly hydrophobic cellulose films. In this study, sodium stearate was selected to modify the water vapor permeability of cellulose/zein composite films, and the resultant materials were characterized and tested for food preservation.

In general, the purpose of this study is to fabricate biodegradable materials with heat sealability and good mechanical and barrier properties from natural resources for sustainable food packaging. The objectives of the thesis include two aspects: (1) to study the heat-sealing property of the regenerated cellulose films coated with different amounts of zein; and (2) to study the barrier properties and food preservation effect of the sodium stearate modified cellulose/zein composite films.

CHAPTER 2. LITERATURE REVIEW

2.1 ABSTRACT

The need for alternative packaging materials and packaging forms is more urgent than ever due to the shortcomings of traditional food packaging materials in terms of their negative impacts on the environment and manufacturing requirements for non-renewable resources. In the packaging industry, the growing need for food safety and sustainability has led to research into ways to find bio-based polymers to replace synthetic plastics. Biodegradable films or coatings prepared from natural resources have become an important research direction in food packaging, and the raw materials can usually be classified as polysaccharides, proteins, and lipids. In this chapter, the classification of biodegradable packaging materials, preparation methods, properties and applications of biodegradable films and coatings are described.

Keywords: Food packaging, natural resources, biodegradable, films and coatings, applications

2.2 INTRODUCTION

In today's era of rapid food development, the packaging industry has grown tremendously in the past few decades. Consumer preference for ready-to-eat packaged foods has resulted in an increasing demand for the required packaging materials. To meet the current demand, the packaging industry is typically using petroleum-based, synthetic, non-biodegradable materials. The production process for these products is highly chemical-intensive and as a result, these products rank low in the green index (Agenda, 2016). Due to the non-degradable nature, they can cause environmental problems, such as leading to water, land, and air pollution. Based on these drawbacks, there is a turning trend towards biodegradable food packaging materials (Hoornweg & Bhada-Tata, 2012). Various techniques have been developed over the years to maintain the quality of food products, of which packaging is one of the most important methods. The use of biodegradable films and coatings as food packaging is a hot research topic, while improving the functionality and performance of these biodegradable films is one of the challenges facing the industry (Agenda, 2016; Nair & Laurencin, 2007).

The biopolymer materials can be degraded in the environment, which allows them to be disposed in soil after usage and can significantly reduce the need for landfills. By biological degradation, these materials produce only water, carbon dioxide, and inorganic compounds without any toxic residues (Siracusa et al., 2008). Due to these advantages, there is no doubt that biodegradable materials will receive more attention to replace synthetic plastics in food packaging applications.

Several bio-based materials, such as proteins, polysaccharides, and lipids, have been reported in the literature for the development of biodegradable packaging (Amin et al., 2021; Suderman et al., 2018). These novel systems have comparable mechanical, optical, and barrier properties to those synthetic packaging materials (Yuan et al., 2016). To further enhance the functionalities of biodegradable packaging materials, they were enriched with bioactive components, i.e., antimicrobials, antioxidants, and plasticizers (Nair & Laurencin, 2007). These packaging materials can be used to enhance the nutritional value and/or to extend the shelf life of packaged

food products. In general, biodegradable packaging materials can be categorized as films and coatings. Films are individually prepared layers that are casted and dried into suitable shapes as separate structures to wrap food (Riaz et al., 2020). Coatings, on the other hand, are thin layers of materials that are applied directly to the food products and act as a barrier between the product and the environment during transportation, processing, and storage. Coatings are generated by dipping the food product into a coating solution or spraying the coating solution directly onto the food surface (Sharma et al., 2019).

Considering the certain requirements of barrier performance, microbial stability, mechanical strength, production process, and cost, there are still some issues to overcome for the wide commercialization of biodegradable food packaging (Sedayu et al., 2019). This review aims to summarize the raw materials, production processes, properties, and applications of biodegradable food packaging derived from natural resources.

2.3 SOURCES OF BIODEGRADABLE PACKAGING

2.3.1 Polysaccharides

Polysaccharide films can be made from cellulose, starch, alginate, gums, and so on (Chiumarelli & Hubinger, 2012; Phan The et al., 2008), either by dissolution and regeneration or by thermal treatments (Mohamed et al., 2020).

Cellulose is the most abundant natural polymer and is widely used for many applications due to its biodegradability, excellent mechanical properties, renewability, low cost, and non-toxic and non-allergenic features (Jonoobi et al., 2015; Zhao et al., 2019). The films formed by cellulose and its derivatives are usually very efficient barriers to oxygen and aroma compounds. Cellulose extracted from durian rind (Zhao et al., 2019), methylcellulose (S. Li et al., 2020), carboxymethyl cellulose (Yaradoddi et al., 2020), and hydroxypropyl methylcellulose (Bigi et al., 2021) have already been reported to be used to prepare biodegradable food packaging.

Nanocellulose mainly includes cellulose nanocrystals (CNC), cellulose nanofibers (CNF), and

bacterial nanofibers. The main characteristics of nanocellulose are large surface area, large aspect ratio, excellent mechanical properties, and low thermal expansion. Nanocellulose has been applied in many different fields including food and biomedical industries in recent years. CNC and CNF have been excellent choices for improving the performance of biodegradable food packaging films (Mu et al., 2019). Most studies have shown that the addition of CNC and CNF to biodegradable food packaging films has a positive impact on their mechanical and barrier properties (Cazón & Vázquez, 2021; Lan et al., 2021). However, the high cost of applying CNC and CNF to packaging is currently one of the major challenges for their commercial applications.

Starch is also naturally abundant, cheap, non-toxic, renewable, and biocompatible, and has good film-forming property (Cheng et al., 2021). Unfortunately, it has a hydrophilic nature that makes the obtained materials sensitive to environmental humidity (López et al., 2011). For the preparation of food packaging materials, starch suffers from high viscosity, high brittleness, and poor water resistance. However, it can be chemically modified or blended with other film-forming compounds to enhance the performance of composite films and coatings. For example, hydroxypropylation of starch is a kind of etherification with propylene oxide in the presence of the alkaline catalyst, which lowers the gelatinization temperature and enthalpy, and increases the paste clarity, freeze-thaw stability and solubility in cold water (Lafargue et al., 2007). Native and acetylated corn starch-based biodegradable packaging films showed the heat-sealing property (López et al., 2011), and polylactic acid and starch were blended for preparing biodegradable packages (Muller et al., 2017).

Alginates are extracted from seaweeds and are linear anionic polysaccharides composed of 1,4-linked α-glucuronate (G) and β-D-mannuronate (M) residues (Park et al., 2021). Alginate coatings could extend the shelf life and improve food quality by increasing the water vapor barrier property, preserving flavor, and delaying fat oxidation (Mahcene et al., 2020). The water vapor barrier property of alginate films could be improved by the addition of calcium (Mohamed et al., 2020). When alginate reacts with calcium ions or multivalent metal ions, a

strongly insoluble or gelatinous structure can be produced. This calcium alginate gel has been used in rationalized foods such as crab sticks, onion rings, meat products, cocktail berries, and so on. (Gheorghita et al., 2020; Mahcene et al., 2020)

Pullulan is an extracellular polysaccharide secreted by the black yeast Aureobasidium pullulans. The structure of pullulan consists of linearly polymerized maltose units connected by α-1,6-glycosidic bonds, providing the structural flexibility specific to pullulan (Zhang et al., 2020). Pullulan is considered an ideal material for the preparation of food packaging films because of its non-mutagenic, non-toxic, odorless, and colorless features. Besides, pullulan is water soluble and has excellent film-forming property. The films produced by pullulan were uniform and transparent, and could offer high resistance to oil and grease and excellent mechanical and oxygen barrier properties (Priyadarshi et al., 2021).

Gums are hydrophobic or hydrophilic polysaccharides with high molecular weight that produce gels or viscous solutions in their specific solvents. Water-soluble gums, also known as hydrocolloids, have been widely used in food, biochemistry, cosmetics, and pharmaceutical industries due to their low cost, easy availability, and biocompatibility, as well as their chemical inertness and non-toxicity (Khezerlou et al., 2021). Among the more common gums are plant gums, such as Tragacanth gum, Ghatti gum, Persian gum, Cashew gum, and so on. The plant gum-based coatings incorporated with bioactive compounds for fruit preservation led to a selective modification of the gas permeability, resulting in an increased CO_2 concentration and a reduction in the amount of O_2 (Salehi, 2019). Plant gums also have the advantages of renewable, degradable, and edible, making them ideal polymeric substrates for the preparation of biodegradable films and coatings for waste reduction (Saha et al., 2017). Coatings prepared from plant gums have shown quite a few points such as delayed ripening, reduced respiration, reduced lipid oxidation and microbial growth, carrier of antioxidants, vitamins and antimicrobial compounds, and ultimate improvement of the shelf life of food products (Khezerlou et al., 2021).

Agar is a biopolymer derived from certain red algae. The chemical structure of agar is a mixture

of agar agglutinin (non-gelling portion) and agarose (gelling portion); the latter is a linear polysaccharide, while the former is slightly branched and sulfated (Mostafavi & Zaeim, 2020). Agar films have been reported to have a high retraction rate, which was mainly due to the dehydration of the agar gel during the drying process. They are transparent, heat-sealable, and biodegradable (Wongphan & Harnkarnsujarit, 2020). In addition, agar films are biologically inert and therefore can easily load different bioactive substances and cover the food surface. However, pure agar film is relatively fragile and has poor thermal stability and water vapor barrier property (Mostafavi & Zaeim, 2020).

In the development of biodegradable food packaging materials, carrageenan has become one of the most popular biopolymers for industrial production due to its wide source, complete biodegradability, short regeneration cycle, and easy to process (McKim et al., 2019). Carrageenan is available in two forms: natural and degraded, and is generally applied in its natural form as an additive. The thermal reversibility of carrageenan gel makes it easy to degrade the formed film, and the films are brittle and hard. Therefore, plasticizers are usually used to improve the mechanical properties of the carrageenan film (Simona et al., 2021), i.e. packaging films based on semi-refined kappa-carrageenan plasticized with glycerol and sorbitol (Farhan & Hani, 2017).

Chitosan is a product of chitin deacetylation and has many unique properties such as biodegradability, cell affinity, and biological effects. Especially, chitosan contains free amino groups, making it the only basic polysaccharide among natural polysaccharides (Yuan et al., 2016). The amino groups in the molecular structure of chitosan are more reactive than the acetylamino groups, so chitosan has excellent biological functions and can perform chemical modification reactions. The antibacterial and antifungal properties of chitosan make it a recognized food additive and be ideal for active packaging that comes into direct contact with food (Nguyen et al., 2021). Recently, essential oils were introduced into chitosan films to produce active packaging films with antibacterial and antioxidant properties, such as chitosan coating with clove oil to extend the shelf life of sausages (Lekjing, 2016), and chitosan-based

film containing Chinese chive root extract for food application (Riaz et al., 2020).

2.3.2 Proteins

Proteins are a class of nutrient-rich and renewable natural macromolecules that are widely used in biodegradable films. The mechanical and barrier properties of biodegradable films depend largely on the solubility of polymers in solution, the uniformity of polymer molecules, and the intermolecular interactions. Since proteins are relatively stable in solution and are easy to cross-link, protein films could have relatively good mechanical and barrier properties (Mohamed et al., 2020).

2.3.2.1 Animal Protein

Milk contains two major proteins: casein in the form of micelles and whey protein. Whey protein is neutral in flavor and texture and can be formulated to achieve a variety of barrier and mechanical properties, including controlling water migration, acting as an oxygen barrier, and having appropriate tensile strength and elasticity (Mohamed et al., 2020). Whey protein can produce films with good oxygen, aroma, and oil barrier properties at low to moderate relative humidity and sufficient mechanical properties to be durable when used as a coating or film for packaging applications, but their water vapor barrier property is poor (Cinelli et al., 2014).

Collagen can be obtained from animal processing co-products and wastes. It is a fibrous structural protein that is readily available, non-toxic, and non-hazardous, and provides an excellent basis for biological materials (Khodaei et al., 2021). Collagen films are arguably one of the most commercially successful biodegradable protein materials. They have good mechanical and oxygen barrier properties at low relative humidity, but their oxygen permeability increases rapidly with increasing relative humidity. The mechanical properties and thermal stability of the collagen films can be improved by using different cross-linking agents (Alizadeh & Behfar, 2013).

2.3.2.2 Plant Protein

Among various biopolymer materials, soy protein has attracted much attention because of its wide source, low-cost, environmental friendliness, and natural renewability. Soy protein has a wide range of promising applications in biotechnology and bioscience and has been used to produce biodegradable films. Soy protein was combined with nanocellulose (Yu et al., 2019) and diatomite/thymol complex (Lu et al., 2021) to form biodegradable packaging films with heat-sealing, antibacterial, and antioxidant properties.

Zein is the main storage protein of corn and is soluble only in organic solvents. It is considered as one of the ideal candidates among a variety of biopolymers because of its biodegradability, harmlessness, and excellent plasticity. In addition, zein has the characteristics of hydrophobicity, heat-seal ability, and GRAS grade, making it an attractive biopolymer for food packaging (Cui et al., 2020). Biodegradable zein composite films reinforced with chitosan nanoparticles and cinnamon essential oil were proven to have good mechanical and antimicrobial attributes (Vahedikia et al., 2019).

Peanut protein isolate (PPI) is a product prepared from defatted peanut flour by alkaline solubilization and acid deposition, and its protein content is higher than that of flour or peanut protein concentrate. PPI can also be used to prepare biodegradable films. For example, the combination of PPI and pea starch (PS) could take both the advantages such as good tensile stress, and is an attractive approach to developing biodegradable films (Sun et al., 2013).

Wheat gluten is a protein by-product of the starch preparation process and is an important raw material for biomaterial development. Currently, wheat gluten films are often prepared by dry (thermoplastic method) or wet (solution casting method) approaches (Mojumdar et al., 2011). Wheat gluten films can be used as food coatings or films for natural gluten-containing foods to slow down mass transfer such as water and oxygen. However, the water-sensitivity of wheat gluten films is one of the main problems when they are put into real applications. The mechanical and barrier properties of wheat gluten films are largely affected under wet conditions due to the adsorption of water and subsequent plasticization (Mohamed et al., 2020).

2.3.3 Lipids

Lipids are compounds derived from natural sources such as animals, insects, and plants. The diversity of lipid functional groups consists of phospholipids, monoglycerides, diglycerides, triglycerides, terpenes, cerebroside, fatty alcohols, and fatty acids. In recent years, the use of fats and oils in food preservation films and food cling films has been of increasing interest to the food industry. Lipids in coatings and films offer many functions; for example, they provide gloss, and reduce moisture loss, cost, and packaging complexity. Also, lipids are hydrophobic, so the films usually have excellent water vapor barrier property (Amin et al., 2021).

Shellac is a natural lipid found in the secretions of the purple gum worm, and has high safety and good film-forming, fast drying, and biodegradable properties. The outstanding moisture resistance and safety allow the shellac to be used as a coating material for food products (Ma et al., 2021). It could extend the shelf life of strawberries, apples, and citrus fruits by forming a semi-permeable barrier against carbon dioxide, oxygen, and moisture and creating a modified atmosphere (Phan The et al., 2008). Although shellac coating has many advantages, it also suffers from the reduced affinity with fruit peel and the lack of sufficient antibacterial and antioxidant capacities. Therefore, antioxidants and antimicrobial agents have been incorporated as functional components of shellac coatings (Y. Li et al., 2020; Ma et al., 2021).

Carnauba wax is a vegetable wax produced from the leaves of the Brazilian palm tree and is a good barrier against water transfer. It possesses high fatty ester and cinnamic acid diester contents and has the highest melting point and hardness among natural waxes. Carnauba wax has low solubility, and carnauba wax-based composite films could prolong the postharvest shelf-life of fruits and vegetables effectively (Devi et al., 2022).

Beeswax is usually combined with other substances to create coating materials to extend the shelf life of foods, such as strawberries (Velickova et al., 2013) and cherry tomatoes (Fagundes et al., 2015). Using beeswax as a hydrophobic coating could provide good water vapor resistance, minimize the weight loss, and allow full ripening of the fruit within 15 days in a

conditioned atmosphere (Oliveira et al., 2018). Besides, by heating the solvent mixture of wax, the wax particles in the emulsion could effectively increase the micro/submicron/nanostructure of the coating surface, thus enabling the super hydrophobicity of the coating (Du et al., 2021).

2.3.4 Use of Additives

In order to improve the flexibility and durability of biodegradable films and coatings, additives such as plasticizers are usually added to the matrix. In addition, additives with unique functions such as antimicrobial agents, antioxidants, and pigments can be added depending on the final applications of the biodegradable materials (Bilal et al., 2020). The type of coating solution and the method of application depend mainly on the surface properties of the food products, such as wettability, contact angle, surface tension, and so on.

2.3.4.1 Antimicrobials

An important emerging function of biodegradable coatings is to act as the carriers of antifungal and antimicrobial agents to improve the quality and shelf life of food products, and the carriers of nutrients to add nutritional value to final processed foods. The incorporation of natural or synthetic antimicrobial agents into various biodegradable packaging materials has become an effective alternative for controlling microbial growth (Janjarasskul & Krochta, 2010). The most commonly used antimicrobial agents are organic acids, Streptococcus lactis peptides, chitosan, lactoperoxidase systems, plant extracts, and essential oils (Fagundes et al., 2015; Simona et al., 2021).

2.3.4.2 Antioxidants

Antioxidants can be added to packaging materials to slow down the rate of oxidation reactions and improve the safety and quality of food products. These compounds can inhibit free radical activity through several different pathways, such as acting as scavengers of free radicals (i.e., glutathione), and chain-breaking antioxidants to neutralize intermediate peroxyl radicals (i.e., ascorbic acid). Thus, the use of antioxidants can protect food from color changes, taste and odor changes, reduced texture, and potential nutritional loss over time (Arnon-Rips &

Poverenov, 2016). Examples of naturally sourced antioxidants include plant extracts, essential oils, α-tocopherol, ascorbic and citric acids, and propolis, all of which are widely used individually or in combination (Rangaraj et al., 2021; Yuan et al., 2016). This type of active packaging effectively extends the shelf life of food products and contributes to the quality and safety of the package contents. Studies have shown that combining these natural compounds into product formulations or packaging materials not only inhibits fungal growth, but also enhances oxidative stability (Pavli et al., 2019).

2.3.4.3 Plasticizers

In most cases, plasticizers are essential components of biodegradable films and coatings, especially polysaccharides and proteins, because they become stiff due to the extensive interactions between polymer chains. To improve the thermal processability of polymers, plasticizers (low molecular weight agents) are added to the film-forming materials, exist between polymer molecules, and impede polymer-polymer interactions, leading to improved flexibility and processability (Suhag et al., 2020). Most plasticizers for natural polymers are extremely hydrophilic and hygroscopic and can absorb water molecules to form larger plasticizer-hydrodynamic complexes. Commonly used plasticizers include glycerin, propylene glycol, sorbitol, and polyethylene glycol (Farhan & Hani, 2017; Suderman et al., 2018).

2.4 PRODUCTION METHODS

2.4.1 Films

2.4.1.1 Casting

The casting method is the most commonly used method for film formation in the laboratory and industry. The method consists of three steps: (1) dissolve the raw materials in a suitable solvent, (2) cast the solution in a mold, and (3) dry the cast solution (Rhim et al., 2006). The biodegradable film formation begins with the selection of polymers or polymer blends. The selected ingredients are dissolved or dispersed in a suitable solvent. During the casting process,

the obtained solution is poured into predefined molds or Teflon-coated glass plates. The drying process provides sufficient time for the evaporation of the solvent with the help of various dryers, such as hot air ovens, tray dryers, microwave dryers, and vacuum dryers (Gupta et al., 2022). The main advantages of film casting are ease of manufacture, no need to use specialized equipment, and low cost (Fitch-Vargas et al., 2019). Wet casting produces better particle-particle interactions, resulting in more uniform particle packing and producing fewer defects. The effects of drying temperatures and drying methods on the production of biodegradable films have been studied (Tapia-Blácido et al., 2013). A fast drying process might have a negative impact on the physical and structural properties, for example, a high molding temperature might cause irreversible structural changes in the material (Suhag et al., 2020).

2.4.1.2 Extrusion

Extrusion is another method used to produce bio-polymer films and is one of the main polymer processing techniques currently used on a commercial scale. This method changes the structure of the material and improves the physicochemical properties of the extruded material (Fitch-Vargas et al., 2016). The extrusion process can be generally divided into three zones:(1) the feed zone; (2) the kneading zone; and (3) the heating zone in the last part/exit of the machine (Suhag et al., 2020). This method operates best with minimal water or solvent content; therefore, it is also referred to as a dry method. However, plasticizers such as polyethylene glycol or sorbitol, are required to increase the thermal processability (Fitch-Vargas et al., 2019).

The main advantages of the extrusion film formation method are the short processing time and low energy consumption compared to the casting method (Fitch-Vargas et al., 2016). It is a high-performance, low-cost, and efficient process for commercial production in the food sector. In addition, other advantages of this technology include no need for solvents, easy handling of high-viscosity polymers, a wide range of processing conditions, and better control of feed residence time and degree of mixing (Liu et al., 2009). The extrusion film formation method is more efficient in controlling the mechanical properties of the film and can produce a variety of forms that are not available with the solvent casting method (Fitch-Vargas et al., 2016).

However, the extrusion method has the problem that only temperature and low moisture-resistant raw material blends can be processed, which limits the use of certain polymers (Suhag et al., 2020). In addition, the higher initial cost of specialized equipment and higher maintenance cost affect the use of this process.

Table 2.1 Recently developed biodegradable packaging films for food preservation.

Natural Polymers	Production Methods	Applications	References
Corn starch	Extrusion	Mango	(Sothornvit & Rodsamran, 2008)
Sodium alginate	Extrusion-casting	Ham Slices	(Pavli et al., 2019)
Starch	Extrusion-casting	Tomato	(Fitch-Vargas et al., 2019)
Agar-sodium	Casting	Cheese	(Suhag et al., 2020)
SPI, diatomite	Casting	Blueberry	(Lu et al., 2021)
Pullulan, Chitosan	Casting	Strawberry	(Du et al., 2021)

2.4.2 Coating

2.4.2.1 Dipping

Dipping is the most common method of coating food products, which is dipping the food sample into the coating solution. This method consists of three steps: (1) immersion and dwell; (2) deposition; and (3) solvent evaporation (Gupta et al., 2022). In the first step, the substrate is dipped into the coating emulsion/solution at a constant rate and the dweller ensures that there is sufficient solution to wet the substrate and that there is complete interaction between the substrate and the coating matrix (Valdés et al., 2017). This deposition process is used to form a thin layer of the coating material on the food surface, and the excess surface liquid is drained and removed. During the evaporation process, the solvent and excess liquid are evaporated from the food surface by heating and drying (Atieno et al., 2019). The thickness of the coating

film depends on the density of the coating material, viscosity of the coating solution, surface tension, and surface pull-back rate (Gupta et al., 2022; Suhag et al., 2020).

The dipping method is widely used for the coating of fresh products. This method is simple and cost-effective (Atieno et al., 2019), while the disadvantage is the dipping may dilute the outer layer and reduce the functionality; i.e., after dipping, the natural wax coating of fruits and vegetables might fall off (Lin & Zhao, 2007). The impregnation method facilitates the complete coating of food surfaces. It ensures a good uniformity of coating on the rough and complex food surfaces. However, the impregnation method also has problems, including dilution of the coating and accumulation of waste or dirt.

2.4.2.2 Spraying

The spraying method increases the surface of the liquid by forming droplets and distributing them over the food surface through a set of nozzles. Three types of spraying techniques have been applied to process coatings. (1) Air spray atomization: a high-velocity air stream surrounds a fluid flowing at low velocity from inside a tube, and the fluid-air friction accelerates and interrupts the flow of the liquid fluid, causing atomization. This spraying method is cost-effective because air is used for fine spraying of micro-droplets (Valdés et al., 2017). (2) Air-assisted airless atomization: air-assisted airless guns first partially atomize the fluid with a special fluid nozzle tip similar to a standard airless nozzle. They finish the atomization with a small amount of compressed air from the face of the air nozzle, resulting in a fine atomized spray pattern very similar to a compressed air system (Lin & Zhao, 2007). Air-assisted airless atomization solves many of the problems associated with using high viscosity and high solid content coatings, as well as the problems associated with heating, providing high production levels and high quality finishes (Gupta et al., 2022). (3) Pressure atomization: the coating is applied to the food using pressure. As air is not used in this technique, the technology is also known as airless atomization. Small size nozzles are used for high-pressure energy to provide surface tension and high-viscosity coating solutions for application to food products. The pressure is kept below 3.5 bars during the process to avoid damage to the film-forming

system (Suhag et al., 2020).

Table 2.2 Recently developed biodegradable coatings for food preservation.

Natural Polymers	Production Methods	Applications	References
Whey protein	Dipping	Apple	(Chiumarelli & Hubinger, 2012)
Alginate, chitosan	Spraying	Bell pepper	(Poverenov et al., 2014)
Cassava	Spraying, dipping	Cassava	(Atieno et al., 2019)
Decolorized Hsian-tsao leaf gum	Spraying	Pork slices	(Umaraw et al., 2020)
Shellac, tannic acid	Dipping	Mango	(Ma et al., 2021)
Gellan gum	Dipping	Strawberries	(Du et al., 2021)

2.5 FOOD PRESERVATION APPLICATIONS

2.5.1 Mechanical Protection

Food packaging needs to have the ability to delay product spoilage, improve product shelf life, and maintain food quality and safety; that is, food packaging should be able to protect food in three ways: physical, chemical, and biological (Pooja Saklani et al., 2019). Physical protection usually involves avoiding mechanical damage to the food, including cushioning against shock and vibration during transport, which requires biodegradable packaging films to be mechanically strong. Tensile strength and elongation at break are usually measured by tensile test to indicate the mechanical properties of films (Vahedikia et al., 2019). Chemical protection is the reduction of composition changes induced by the environment. These environmental effects are from O_2 (Oinonen et al., 2016) and CO_2, the increase or decrease of moisture (Phan The et al., 2008), and such as UV (Li et al., 2021). Biological protection is the avoidance of microbial influences on the food product, such as spoilage and pathogenic infestation (Lu et

al., 2021), thus preventing the occurrence of diseases.

So far, few biodegradable packaging films have been industrially produced and commercially applied. One of the important reasons is the insufficient mechanical strength of biodegradable films. The common synthetic plastic films can reach ten times higher tensile strength than the biodegradable films, while the elongation at break is also tens of times higher. Various strategies have been applied to improve the mechanical properties of biodegradable films. For example, compared to the individual films, the blend of pectin and branched starch produced a biodegradable film with an increased strength of 25.5 ± 0.6 MPa and well-maintained flexibility (3.1 ± 0.3%) and stiffness (Priyadarshi et al., 2021). Biodegradable films made of Qodume Shirazi (AHSG)/PVA showed a tensile strength of 23.3 MPa and a tensile strain of 53.3% (Marvdashti et al., 2017), while sage seed gum/chitosan films had a similar strength of 25.84 MPa but less elongation of 7.64% (Davoodi et al., 2020). Soy protein-based packaging materials were incorporated with cellulose nanofibers and cedar pine needle extracts, which obviously increased the tensile strength of the composites and endowed the strong antioxidative and antibacterial capacities against foodborne pathogens (*Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Listeria monocytogenes*) (Yu et al., 2019). Zein films were prepared by incorporating cinnamon essential oil and chitosan nanoparticles to provide an antibacterial effect and enhanced mechanical strength from 0.95 MPa (control sample) to 2.15 MPa (Vahedikia et al., 2019). Tragacanth/hydroxypropyl methylcellulose/beeswax films with silver nanoparticles had a strength of 30.1 MPa and an elongation of 26.2% (Bahrami et al., 2019). Other methods such as heat treatment of the raw materials and the addition of nanofillers have also been reported to improve the performance of biodegradable packaging films (Mohamed et al., 2020).

2.5.2 Barrier Functions

Many food packaging materials are required to have barrier properties, including the ability to block the passage of gases, liquids, and other permeable materials through the packaging

materials. Especially, high barrier properties are usually essential to slow down food product deterioration and extend shelf life (Marvdashti et al., 2017).

Moisture resistance is the most studied property and is usually labeled by the water permeability coefficient. Due to the hydrophilic nature of natural polymer-based materials, moisture resistance is relatively poor in most cases, and different humidity environments can affect the mechanical properties of biodegradable films (Zibaei et al., 2021). The incorporation of oils and fats can be invoked to improve the moisture barrier properties of films, but they may also reduce the mechanical properties. The biodegradable bilayers consisting of agar and ethanol-cast shellac exhibited very low water vapor permeability (1.03×10^{-11} g m^{-1} s^{-1} Pa^{-1}), high contact angle value (≈92°), and low water adsorption rate (26×10^{-3} μL s^{-1}) (Phan The et al., 2008). Biodegradable films with good moisture barrier property were also prepared from starch and their maleated counterpart with poly (butylene adipate-co-terephthalate). Compared with the control films, the modified films showed an obvious improvement of 86.8% in moisture resistance, which was up to 10.33×10^{-13} kg·m/(m^2·s·Pa) (Chang et al., 2021). The biodegradable films prepared from pectin and branched starch showed the improved water vapor barrier property ($1.16 \pm 0.16 \times 10^{-9}$ g·mm·m^{-2} s^{-1} Pa^{-1}) as well as good oil resistance and mechanical properties (Priyadarshi et al., 2021).

The specific gas in the package directly affects the quality of the packaged food. Protein films usually have better gas barrier properties than the films prepared with grease and polysaccharides (Jeya Jeevahan et al., 2020). According to Cinelli et al. (2014), the modification of PLA films with whey protein significantly improved the oxygen barrier property while ensuring their biodegradability. New wood films were developed from the chemical thermomechanical pulp inside streams. The microfibrillated cellulose and galactoglucomannan-lignin network was cross-linked with laccase, and the films showed good mechanical strength, thermal stability and possessed a very low oxygen permeability (0.69 ± 0.01 cm^3 μm m^{-2} d^{-1} kPa^{-1}), much lower than that of pure cellulose film (Oinonen et al., 2016). The biodegradable films prepared by Chang et al. (2021) also showed a significant

improvement in oxygen barrier with a 65.6%-74.3% reduction in oxygen permeability, making them an attractive alternative to synthetic plastics for food packaging applications. Besides, lipids such as terpene resin, shellac, and wood resin have been proven to have excellent barrier properties for water vapor and oxygen. Usually, wax films are substantially more resistant to moisture migration than other lipid or non-lipid biodegradable films. The lactic serum is a good barrier for CO_2, and banana flour could form films that showed excellent oxygen barrier property. Using xanthan gum as a coating could form a protective barrier on the surface of acerola to prevent oxygen penetration and delay the ripening process of acerola (Mohamed et al., 2020).

Oil resistance is expressed by the oil permeability coefficient. Natural polymer films such as pectin and branched starch have good oil resistance (with oil absorption value of 6.45 ± 0.51 $g \cdot m^{-2}$) (Priyadarshi et al., 2021). The bioactive films of chitosan and white turmeric were prepared by solvent casting technique and exhibited good tensile properties, water resistance, and oil resistance, and the oil absorption of the blended films was reduced by 46% compared to the original films (Hiremani et al., 2021).

High transparency is beneficial for displaying food products, while UV-blocking property is important for protecting foods against photo-oxidation and degradation (Nguyen et al., 2021). According to Li et al. (2021), the soy protein films exhibited limited UV transmission, although they had a high visible light transmission of over 85.0%. By combining with amino-containing hyperbranched polysiloxanes, the composite films had improved UV shielding properties and blocked 100% of UV-C and UV-B spectra and 88.4% of UV-A spectrum, while maintaining a high visible transmittance of more than 80%. Similarly, the chitosan films were modified using *Piper betle* Linn oil and the composite films achieved a visible light transmission of 70% and blocked almost all the lights in the UV-C and UV-B range (Nguyen et al., 2021). Alyssum holocarpum seed gum films modified with PVA exhibited excellent UV-blocking properties along with improved mechanical and oxygen barrier properties (Marvdashti et al., 2017).

2.5.3 Functional Additive Carriers

For most foods, microbial overgrowth is the main cause of food spoilage, and the differences in processing or packaging sanitation can affect the microbial residue in the finished food (Park et al., 2021). In later storage and transportation, if the temperature, humidity, and gas volume are suitable, microorganisms will still grow and multiply, accelerating food deterioration. Chitosan films supplemented with *Sonneratia caseolaris (L.)* Engl. leaf extract had significant inhibitory activity against Gram-negative bacteria (Nguyen et al., 2020). Essential oils contain terpenoids, terpenoids, and other aromatic compounds with important antimicrobial activity. Randazzo et al. (2016) added citrus essential oils extracted from lemon, mandarin, and orange to methylcellulose or chitosan films. The chitosan films were more effective for the incorporation of essential oils, which showed antimicrobial activity and maintained tensile and water vapor barrier properties. Similarly, sodium alginate films incorporated with oregano essential oil exhibited excellent antibacterial ability against *Listeria monocytogenesw*, and were effective in extending the shelf life and maintaining the organoleptic properties of food products (Pavli et al., 2019).

There have been several recent studies on antioxidant films. Clove oil, a natural essential oil with antibacterial and antioxidant activity, was blended with chitosan to prepare a coating that could effectively extend the shelf life of frozen cooked pork sausages. The product was tested for thiobarbituric acid reactive substances and peroxide value, and the results were significantly better than those of uncoated sausages. It was noted that rosemary and peppermint extracts, oregano oil, and thyme oil also had antioxidant activity (Lekjing, 2016). Nguyen et al. (2021) assessed the antioxidant activity of the packaging films by the half maximum inhibitory concentration (IC_{50}) for the king orange and confirmed the antioxidant properties of the essential oil-modified chitosan films.

The addition of plasticizers to biodegradable films can change their mechanical and barrier properties (Pooja Saklani et al., 2019). There are some commonly used food-grade plasticizers such as glycerol, sorbitol, mannitol, sucrose, and polyethylene glycol. Farhan and Hani (2017) prepared and characterized kappa-carrageenan films plasticized with glycerol and sorbitol. The

films containing plasticizers had higher tensile strength and elongation at break than the non-plasticized films. The plasticized films were more transparent and had significantly increased seal strength while having reduced oil permeability. The films plasticized with sorbitol were more effective in blocking oxygen than those plasticized with glycerol. The chitosan film modified with *Piper betle* Linn oil also incorporated glycerol as a plasticizer, which greatly improved the tensile strain. The king oranges were selected as fruit samples for testing, and the oranges wrapped in the prepared films showed a good appearance and extended shelf life (Nguyen et al., 2021).

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CONNECTING STATEMENT I

Chapter 2 reviewed the recent development of biodegradable food packaging derived from natural resources. This review summarized the raw materials, preparation methods, properties, and potential applications in food industry of biodegradable films and coatings. **Chapter 3** developed the heat sealable regenerated cellulose films by coating with zein and investigated the structure and mechanical and barrier properties of the composite cellulose/zein films.

CHAPTER 3. HEAT SEALABLE REGENERATED CELLULOSE FILMS ENABLED BY ZEIN COATING FOR SUSTAINABLE FOOD PACKAGING

3.1 ABSTRACT

The development of green packaging materials is requested due to the growing concerns about plastic waste. As the most abundant natural polymer on earth, cellulose can be dissolved and regenerated to make transparent films. However, cellulose is not a thermoplastic, so cellulose films are usually sealed by an adhesive for packaging applications. Herein, this study aimed to endow regenerated cellulose (RC) films with heat sealability by coating a layer of zein, and the mechanical and barrier properties of the composite cellulose/zein films were characterized. The results revealed that, with a thin zein coating, all the composite films were able to be sealed by a tabletop impulse sealer without the need for high temperature or high pressure and showed larger tensile strain and better water vapor and oxygen barrier properties compared to the RC films. Moreover, it was worth noting that the blueberries packed in the heat-sealed cellulose/zein bags were protected from oxidation and spoilage during a 12-day storage period and were comparable to the ones packed by Ziploc®. Thus, this work demonstrates a facile way to fabricate cellulose-based packaging materials that are heat sealable and have potential applications in sustainable food packaging.

Keywords: regenerated cellulose, zein, heat sealable, barrier properties, food packaging

3.2 INTRODUCTION

Currently, much attention has been paid to the non-biodegradable plastic waste, which is a major cause of environmental issues and has resulted in widespread concerns [1]. Therefore, the attempts to develop eco-friendly materials have been facilitated [2]. Biodegradable materials are mainly from three sources: proteins (soy protein, whey protein, zein, etc.) [3, 4], polysaccharides (cellulose, starch, chitosan, alginate, etc.) [2, 5], and lipids such as waxes [6]. These natural resources are renewable, low-cost, and non-toxic, and have potential applications in many areas [7]. Food packaging is one of the major sources of plastic waste, so a lot of studies focused on the development of biodegradable packaging. For example, the combination of diatomite and thymol was introduced into soybean protein isolate to prepare heat-sealable films for blueberry preservation [8]. Biodegradable packaging materials with antimicrobial property was fabricated by electrospinning zein/acetic acid solutions [9], while cinnamaldehyde was loaded with carboxymethyl cellulose as a coating to improve the mechanical performance, antibacterial property, and oxidation resistance of the low-density polyethylene films for the preservation of tilapia [10].

Cellulose is the most abundant polysaccharide on earth, accounting for more than 50% of plant carbon content [11]. With the advantages of low cost, renewability, biodegradability, and good mechanical properties, cellulose and its derivatives have been actively used for developing food packaging in recent years [12-14]. Cellulose can be dissolved in a suitable solvent and regenerated into biodegradable films [2, 15, 16], but their applications in food packaging are limited by poor flexibility and the lack of heat-sealing property. To overcome these drawbacks, a plasticizer such as glycerol is usually added to improve the flexibility, but it may further reduce the water vapor barrier capacity of the cellulose films. Huang et al. reported the heat-sealable cellulose film enabled by a confined solvent. The heat-sealing temperature was 78°C with the addition of plasticizers; however, this method was only effective when the content of residual solvents was in the range of 20%-35% [17]. Xu et al. suggested to reduce the degree of polymerization of cellulose and modulate the degree of substitution of cinnamate to prepare

cellulose cinnamate with both thermal processability and biodegradability, which could be applied as a coating for heat-sealing packaging [18]. Considering the versatility of natural polymers, herein, we hypothesized that the addition of a natural polymer coating could improve the performance of regenerated cellulose (RC) films while maintaining the biodegradability. Zein is a non-water-soluble fraction in corn and is largely available as the by-product of the starch processing. It has good film-forming capacity, and the film is heat sealable, making it an attractive biopolymer for food packaging [19, 20]. Therefore, zein was selected to modify the properties of RC films. There have been a few studies on the composite materials from cellulose/cellulose derivatives and zein. For instance, the incorporation of zein together with 1,2,3,4-butane tetracarboxylic acid as a crosslinker could largely enhance the toughness and water vapor and oxygen barrier properties of the pristine cellulose nanocrystal film for flexible packaging applications [19], while cellulose acetate was employed to improve the poor mechanical and wetting properties of electrospun zein scaffolds for tissue engineering and other biomedical devices [21]. Wei et al. assembled the zein-cellulose nanocrystals core-shell microparticles through electrostatic and hydrophobic interactions, in which the higher cellulose contents slowed down the release of the loaded curcumin in gastrointestinal tract and decreased its bioaccessibility [22]. However, as far as we know, RC films combined with zein coating for heat sealable food packaging applications has never been reported.

To develop biodegradable and heat sealable food packaging materials, for the first time, RC films were prepared using NaOH/urea aqueous system and modified with glycerol and zein coating to improve the flexibility and heat-sealing property. The structure and properties of the modified RC films were characterized, and the biodegradable bags were easily fabricated with a tabletop heat-sealer and tested for the preservation of fresh blueberries.

3.3 MATERIALS AND METHOD

3.3.1 Materials

The bleached kraft pulp of pine was used to prepare RC films and was kindly supplied by FPInnovations (Pointe-Claire, QC, Canada). Sulfuric acid (95.0%-98.0%) and hydrochloric acid (37%) were purchased from Millipore-Sigma (Oakville, ON, Canada). Ethyl alcohol anhydrous was purchased from Greenfield Global (Brampton, ON, Canada). Sodium hydroxide (≥97.0%), urea (≥99.6%), glycerol (≥99.5%), 2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95%), 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid ammonium salt) (ABTS, ≥98.0%), potassium persulfate (≥99%), glacial acetic acid (≥99.7%), sodium acetate (≥99%), 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ, ≥99%), ferric chloride hexahydrate (≥98%), and iron(II) sulfate heptahydrate (≥99.5%) were purchased from Fisher Scientific (Ottawa, ON, Canada). Zein F4400C-FG was purchased from FloZein Products (Ashburnham, MA, USA). Fresh blueberries were purchased from a local supermarket in Montreal and were harvested in Piedmont (Piedmont, NC, USA).

3.3.2 Cellulose/Zein Composite Films Preparation

Desired amount of pine pulp was immersed in 30% w/w H_2SO_4 solution and stirred at 200 rpm and 25°C for 48 hours to decrease the molecular weight. After that, the pulp was washed with deionized water and dried in the oven at 100°C for 24 hours [23]. The hydrolyzed pulp (4% w/w) was dissolved in a pre-cooled aqueous solution containing 7% w/w of NaOH and 12% w/w of urea by stirring at 2000 rpm for 8 minutes [24]. The solution was degassed and the insoluble fraction was precipitated by centrifuging at 1000 rpm and 25°C for 5 minutes. After that, the solution was poured onto a flat plate and coagulated in a H_2SO_4 aqueous bath (5% w/w) for 5 minutes at 25°C [23]. The wet RC films were immersed in 20% w/w and 30% w/w glycerol aqueous solutions for 24 hours and were coded as RC-20G and RC-30G, respectively, and then compressed with a hot press machine (Carver 3895, Wabash, IN, USA) for 10 minutes at 90°C and 0.4 MPa, followed by another 10 minutes at 120°C and 0.6 MPa. The coating solutions were prepared by dissolving zein (15% w/w, 20% w/w, and 25% w/w) and glycerol (20% w/w of the dry weight of zein) in 80% ethanol aqueous solutions. The dried RC films were coated on one side and marked as RC-20G-15Z, RC-20G-20Z, RC-20G-25Z, RC-30G-

15Z, RC-30G-20Z, and RC-30G-25Z, respectively, based on the different glycerol and zein contents.

3.3.3 Characterization

3.3.3.1 Cellulose/Zein Composite Film Structure

The structure of the modified RC films was characterized by FT-IR spectrometer (Cary 630, Agilent Technology, Santa Clara, CA, USA), and the spectra were recorded as the average of 72 scans with a 2 cm^{-1} resolution. The modified RC films were coated with gold-platinum (4nm) by a low vacuum coater (Leica EM ACE200, Vaughan, ON, Canada), and their surfaces and cross sections were observed by a scanning electron microscope (Hitachi TM1000, Schaumburg, IL, USA) with an acceleration voltage of 4 kV.

3.3.3.2 Cellulose/Zein Composite Film Properties

Before the tests, the modified RC films were conditioned at 43% RH and 25°C for 48 hours. The mechanical properties of the modified RC films were tested following ASTM D-882 with a texture analyzer (ADMET MTEST Quattro eXpert 7601, Norwood, MA, USA) at 25°C and a crosshead speed of 5 mm/min. The determination of heat-sealing property was carried out following the method of Su, Wang [4] with minor modifications. The modified RC films were cut into strips (50 mm × 10 mm), and two strips were positioned overlapped and heat sealed by KF-300HC 8-inch impulse sealer with cutter (St-Laurent, QC, Canada). A tensile test was conducted at a crosshead speed of 5 mm/min and 25°C.

The water vapor permeability (WVP) of the modified RC films was tested following the ASTM E96-92 standard [25]. The modified RC films were placed on the top of the glass vials that held anhydrous calcium chloride, and then the vials were kept in a desiccator with a 100% RH. The weight of the vials was periodically monitored, and the WVP values (g m^{-1} h^{-1} Pa^{-1}) of the films were calculated by Equation (3.1):

$$WVP = \frac{\Delta m \times k}{A \times \Delta T \times \Delta P} \tag{3.1}$$

where Δm is the variation of the vial weight (g), k is the thickness of the modified RC films (m), A is the area of the modified RC film (m^2), ΔT stands for the time, and ΔP represents the difference of partial pressure between the two sides of the modified RC films (Pa).

The oxygen transmission rate (OTR) ($cc/m^2 \cdot day$) of the modified RC films was analyzed by an oxygen permeability tester (AMETEK MOCON OX-TRAN 2/22, Brooklyn Park, MN, USA) at 25℃ and 0% RH.

3.3.4 Blueberry preservation

The preservation effect of the modified RC films on blueberries was assessed. Fresh blueberries were sealed in either RC-20G-20Z bags or commercial storage bags (Ziploc®). The blueberries without any package were used as the control. All the samples were stored at 25℃ and 50% RH for 12 days and the changes in appearance and antioxidant activity were monitored.

The antioxidant activity was analyzed on days 0, 3, 6, 9, and 12, respectively. The extraction was based on the method of Lang et al. [26] with minor modifications. The blueberries were pulped and added to ethanol (1:10 w/v) and treated in an ultrasonic bath (Emerson, St. Louis, MO, USA) at 40℃ for 60 minutes. The extract was centrifuged at 7830 rpm for 30 minutes, and the supernatant was separated to measure the antioxidant activity.

The DPPH radical scavenging activity was tested using the method described by Tang et al. [27] with some modifications. The sample (100 μL) was mixed with 0.1 mM DPPH (100 μL) in ethanol and stored in dark at 25℃ for 30 minutes. The absorbance was recorded by Synergy HTX Multimode Reader (BioTek, Santa Clara, CA, USA) at 517 nm, and the DPPH radical scavenging rate was calculated using Equation (3.2):

$$DPPH\ \ radical\ \ scavenging\ \ activity(\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100 \qquad (3.2)$$

where A_0 is the absorbance of DPPH in ethanol at 517 nm, and A_1 is the absorbance of the sample solution at 517 nm.

The ABTS radical scavenging activity was measured following the method of Wang et al. [28]. ABTS solution (7 mM, 100mL) was blended with potassium persulfate solution (2.45 mM, 1.6mL) and stored in dark at 25℃ for 14 hours. Then, ethanol was added to get a stock solution with an absorbance of 0.7 ±0.02 at 734 nm. The supernatant (40 μL) was added with the ABTS stock solution (160 μL) and kept in dark at 25℃ for 6 minutes. The absorbance of the sample was recorded at 734 nm, and the ABTS radical scavenging activity was obtained by Equation (3.3):

$$ABTS\ \ radical\ \ scavenging\ \ activity(\%) = \left(1 - \frac{A_1}{A_0}\right) \times 100 \quad (3.3)$$

where A_0 is the absorbance of ABTS in ethanol at 734 nm, and A_1 is the absorbance of the sample at 734 nm.

Ferric reducing antioxidant power (FRAP) assay was performed following the method of Zhou et al. [29] with minor modifications. The FRAP reagent was obtained by adding TPTZ solution (10 mM in 40 mM HCl) and $FeCl_3$ $6H_2O$ solution (20 mM) to acetate buffer (300 mM, pH 3.60) with the ratio of 1:1:10. The blueberry extraction (10 μL) was mixed with the FRAP reagent (190 μL) and incubated for 30 minutes, and the absorbance was recorded at 593 nm. The total antioxidant capacity was calculated as the concentration of $FeSO_4$ standard solution.

3.3.5. Statistical Analysis

The tests were repeated in triplicate, and the results were displayed as mean ± standard deviation. The statistical analysis was conducted by using analysis of variance (ANOVA) and Duncan's multiple range test. All the analyses were carried out with SPSS statistical program (IBM, New York, NY, USA), and the significant differences were determined at $p < 0.05$.

3.4 RESULTS AND DISCUSSION

3.4.1 Cellulose/Zein Composite Film Structure

The surface and cross-section of RC-20G films with and without zein coating were observed by SEM. As shown in Figure 3.1, no obvious fibers were observed on the surface or in the RC-20G film, suggesting that the acid-hydrolyzed pine pulp was successfully dissolved in NaOH/urea aqueous solution. The RC films showed a dense structure. This was because the hot-pressing process not only quickly dried the wet films but also led to the decreased free space and cavities in the RC films [14, 30]. The surface of RC-20G films with zein coating was smooth, and the coating (underside in Figures 3.1F, 3.1G, and 3.1H) attached well to the RC film, indicating the good compatibility. The thickness of the zein coating was uniform and increased with the concentration of zein (less than 5 microns).

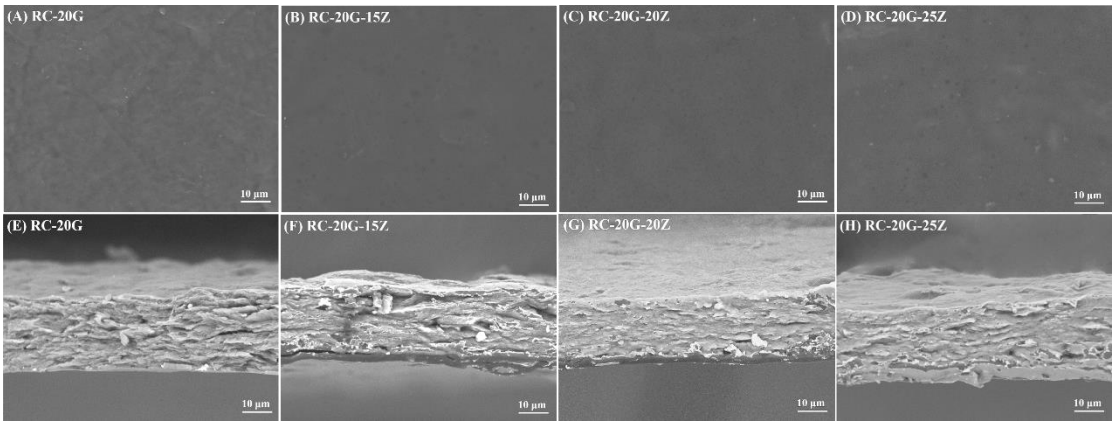

Figure 3.1 SEM images of modified RC films: surface (A-D) and cross-section (E-H).

The FT-IR spectra of modified RC films are shown in Figure 3.2. The typical peak in the range of 3050-3550 cm^{-1} was attributed to the O-H stretching and -NH_2 stretching vibrations [31-33], and the peaks at 2944 cm^{-1} and 2868 cm^{-1} were attributed to the C-H stretching vibration [34]. The peak near 1440 cm^{-1} was attributed to the C-OH in-plane bending [35]. The amide I band at 1637 cm^{-1} belonged to the stretching of the amide carbonyl group C=O, and the amide II band at 1528 cm^{-1} corresponded to the angular deformation vibration of the N-H bond and the stretching vibration of the C-H bond. In addition, the symmetric stretching of COO- was observed at 1440 cm^{-1} [21, 32]. There was no doubt that glycerol could be well dispersed in the composite films, and the addition of zein coating didn't change the molecular structure of regenerated cellulose.

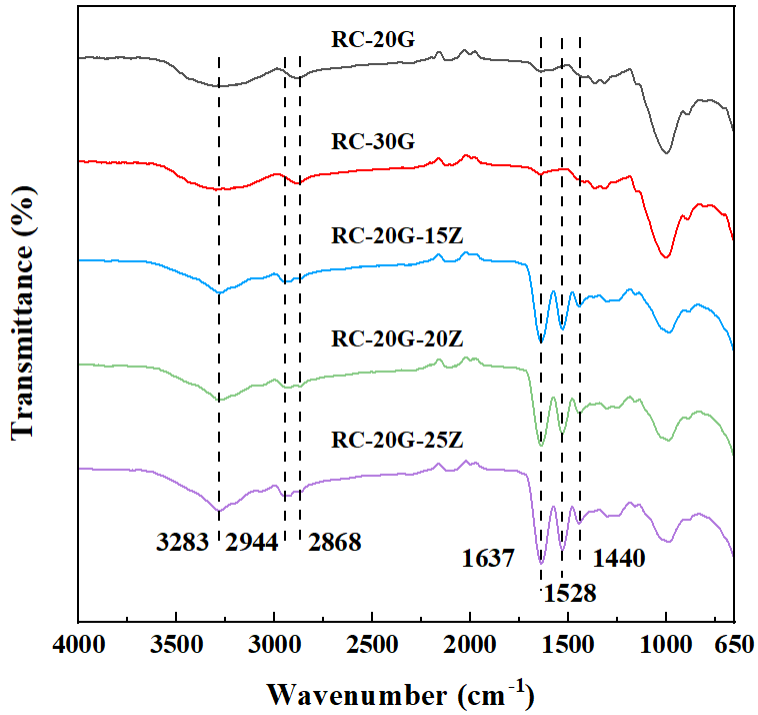

Figure 3.2 FT-IR spectra of modified RC films.

3.4.2 Cellulose/Zein Composite Films Properties

The mechanical properties of modified RC films are shown in Figure 3.3. The increased concentrations of zein and glycerol resulted in the decrease of tensile strength and Young's modulus of the RC film. It was reported that the tensile strength of zein film was inferior (5.38 ±1.05 MPa) [36], so the composite films with a thicker zein coating had a lower tensile strength. Additionally, the incorporation of plasticizers weakens the intra- and intermolecular interactions among the polymer molecules [3]; for example, the hydrogen bonding competition between cellulose and plasticizers [37]. However, it was worth noting that all the composite films had the higher tensile strain values than the RC film, although this value decreased with the increase of zein contents. Among them, RC-20G-15Z exhibited the relatively high tensile strength of 49.54 ±0.19 MPa and the elongation of 5.67 ±0.44%, which were better than some of the reported biodegradable packaging films such as zein/β-cyclodextrin films reinforced by cellulose nanocrystals (22.64 ± 1.77 MPa, 4.76 ± 0.11%) [12], zein/cinnamaldehyde films reinforced by nano-cellulose (33.24 ± 0.48 MPa) [36], oleic acid and glycerol modified zein

films (14.37 ±0.91 MPa, 2.36 ±0.02%) [3], and starch films modified by glycerol and cellulose nanoparticle (10.98 MPa, 3.1%) [38].

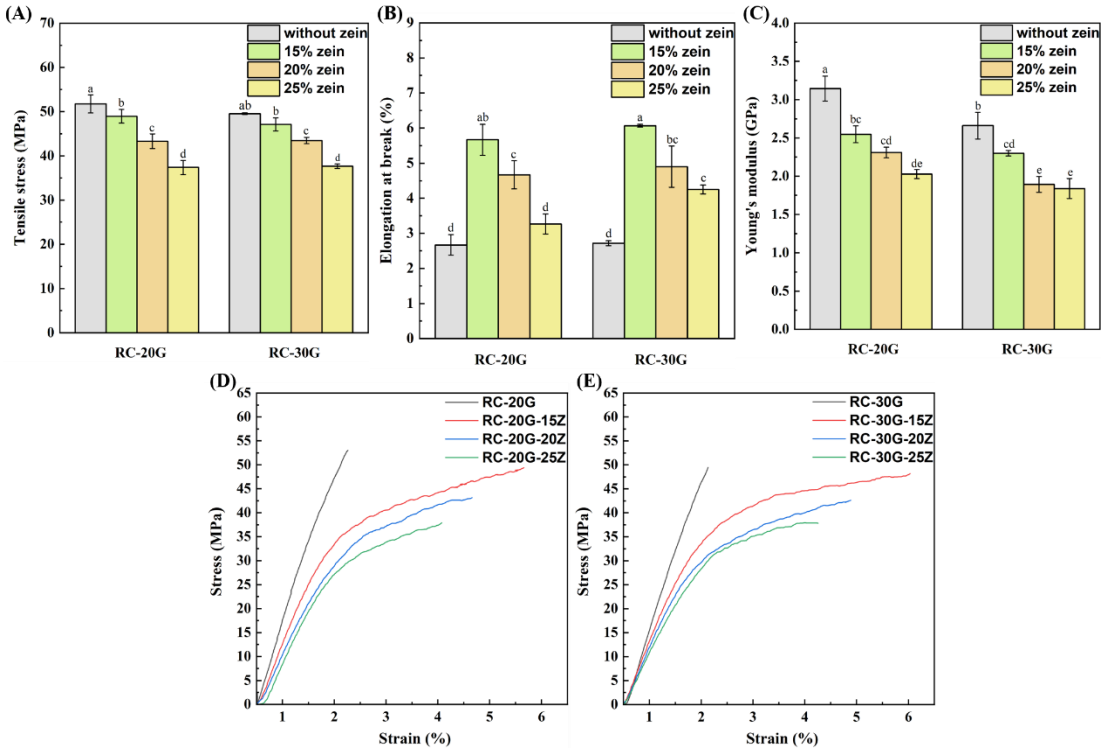

Figure 3.3 Mechanical properties of modified RC films. Different letters on the tops of columns represented the significant difference in terms of zein coating mass ($p < 0.05$).

The WVP and OTR values of the modified RC films are listed in Table 1. As expected, the water vapor barrier property of RC films decreased with the addition of glycerol [16]. The WVP value is generally related to the solubility and diffusivity of water molecules in the material matrix. Glycerol as the plasticizer is highly hydrophilic and is inserted among the polymer chains to enlarge the inter-chain space and facilitate the water adsorption, which cause the accelerated water vapor diffusion and transport through the films [39]. It was observed that zein coating significantly improved the water vapor barrier property of RC films. This could be explained by the hydrophobicity of zein [3, 5].

The OTR value can be determined by the mobility of molecular chains and the free volume in the material, as well as the contents of plasticizers and water [40]. The breakdown of hydrogen bonds enables additional sites for the solubilization of oxygen and boosts oxygen molecule

mobility within the materials [19, 37]. The incorporation of zein coating also significantly improved the oxygen barrier property of RC films. Especially, RC-20G-20Z showed the best performance of 4.83 ± 0.10 cc/m²day, which was better than the commercial polypropylene bags (2000 cc/m²day) and zein coated polypropylene films (59.3 cc/m²day) [41], and zein films reinforced by cellulose nanocrystals (11 cc/m²day) [19].

Table 3.1 Water vapor permeability and oxygen transmission rate of modified RC films. Different letters in the same columns indicate a significant difference within the same sample groups ($p < 0.05$).

Samples	WVP ($\times 10^{-7}$ g m^{-1} h^{-1} Pa^{-1})	OTR (cc/m²day)
RC-20G	5.77 ± 0.16^{cd}	9.41 ± 0.09^{a}
RC-20G-15Z	4.27 ± 0.13^{f}	6.25 ± 0.25^{cd}
RC-20G-20Z	5.09 ± 0.08^{e}	4.83 ± 0.10^{e}
RC-20G-25Z	4.74 ± 0.27^{e}	7.85 ± 0.29^{b}
RC-30G	6.99 ± 0.08^{a}	9.71 ± 0.64^{a}
RC-30G-15Z	5.18 ± 0.08^{de}	7.41 ± 0.37^{bc}
RC-30G-20Z	6.40 ± 0.31^{b}	5.39 ± 0.32^{de}
RC-30G-25Z	5.97 ± 0.2^{bc}	7.33 ± 0.07^{bc}

3.4.3 Cellulose/Zein Composite Films for Blueberry Preservation

3.4.3.1. Heat-sealing Property

Zein-coated RC films with heat-sealing property can be used for food packaging without the need of adhesives. It was worth pointing out that the modified RC film could be easily sealed by a simple tabletop device, and the heat-sealing conditions of 200℃ and 150 kPa recommended by many works or large heat-sealing machines were not necessary [4, 42]. Generally, the heat-sealing capacity test has three types of results, including the separation of

two testing strips, and the material breakage at either the edge of the heat-sealing area or the non-heat-sealing area. For 15% zein-coated RC films, complete separation with no damage to the films occurred, which was caused by the inadequate sealing component [43]. If the films are damaged during the heat-sealing compression or by the stress concentration at the edge of the heat-sealing area, they will break at the edge of the heat-sealing area under the tensile force [42, 43], while a good heat sealability will result in the rupture at the non-heat-sealing area of the film [18]. All the RC films with 20% and 25% zein coatings broke at the non-heat-sealing area during the tensile test. With the overall consideration of mechanical, water vapor barrier, oxygen barrier, and heat-sealing properties, RC-20G-20Z film was selected for blueberry preservation and compared with the commercial Ziploc® package. The unpackaged blueberries showed softening and surface wrinkling on day 6, significant wrinkling on day 9, and a spoiled condition on day 12 (Figure 3.4). In contrast, the blueberries packed in RC-20G-20Z bags maintained the color and hardness on day 6, with only a few wrinkles and no spoilage on day 12, which were similar to the blueberries packaged with Ziploc®.

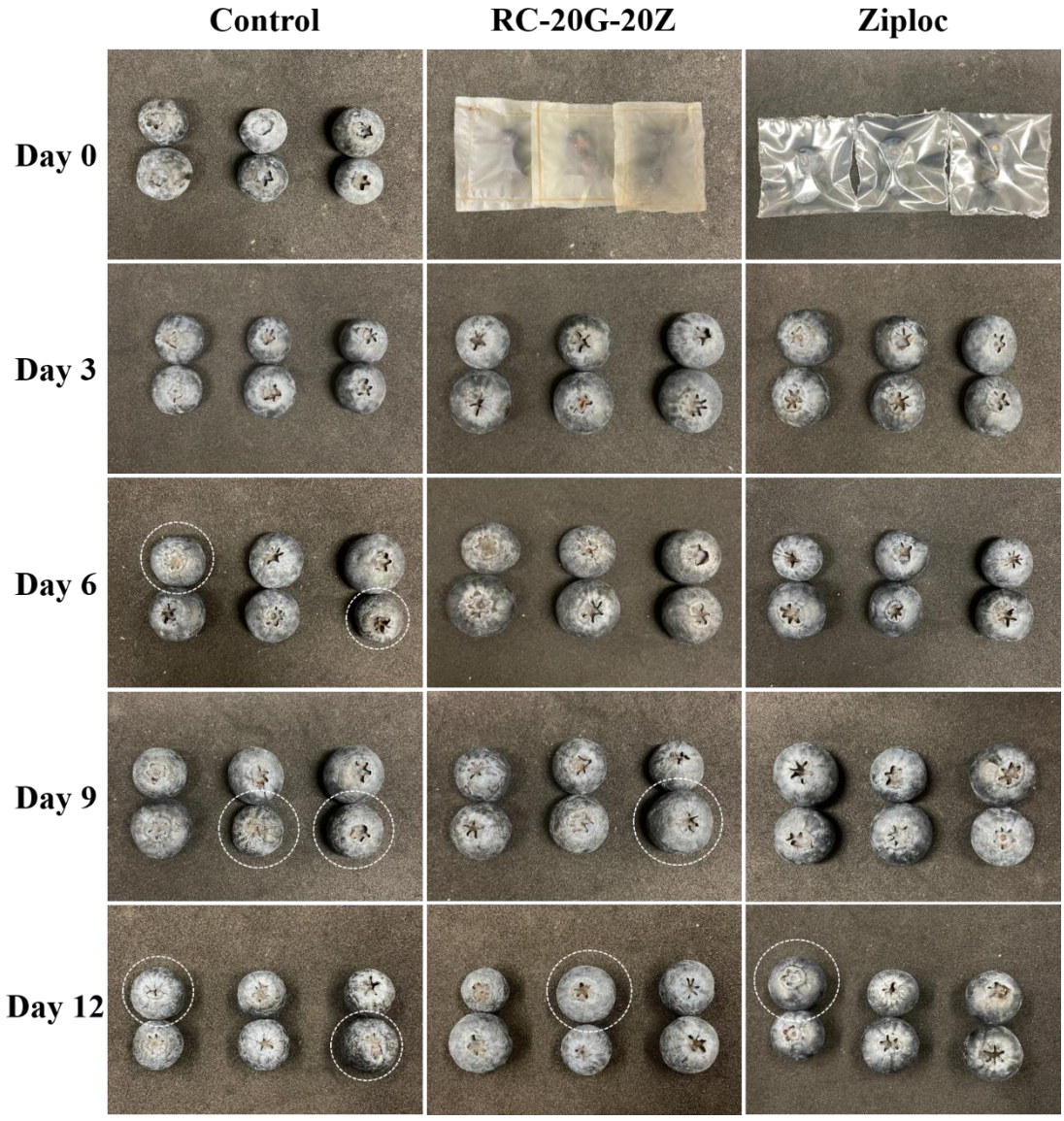

Figure 3.4 Appearance of blueberries under different packaging conditions.

3.4.3.2 Antioxidant Activity

The radical scavenging activity of DPPH has commonly been used to evaluate the antioxidant property of natural compounds [27]. Figure 3.5A presents the DPPH radical scavenging activity of blueberry extracts obtained on different days. The DPPH radical scavenging rate of the unpacked blueberries decreased with time, from 71.48 ± 2.12% on day 0 to 43.73 ± 0.53% on day 12. This activity was associated with the contents of antioxidant compounds existed in blueberries, and it was possible to observe an accelerated decrease during the late storage period [44, 45]. Packaging can slow down the depletion of antioxidant compounds, and RC-

20G-20Z and Ziploc® bags had similar DPPH radical scavenging rates that were obviously higher than the unpackaged blueberries during the whole storage period. The DPPH radical scavenging rates of the packaged blueberries (both in RC-20G-20Z and Ziploc®) showed an increase on day 3 compared to day 0. It was because of that the packaging materials reduced the exposure of blueberry in air, and thus the consumption of organic acids was inhibited [45]. At the same time, the elevation of temperature from refrigeration to room temperature facilitated the accumulation of phenolics and resulted in the improved antioxidant property [29, 46].

To further confirm the antioxidant activity, the ABTS free scavenging activity of the blueberry extracts was determined [29]. As depicted in Figure 3.5B, the ABTS radical scavenging activity of the unpacked and packed blueberries had a similar trend to the DPPH free radical scavenging results [47]. On day 9, the sample packed in RC-20G-20Z even showed a slightly better scavenging effect (70.67 ± 1.32%) than the Ziploc® packed ones (68.92 ± 4.66%). In particular, compared with the results of DPPH assay, the ABTS radical scavenging activity was overall higher. Same observation was reported by Li et al. [48], and it might be because the DPPH reaction fluctuated with the amount of dissolved oxygen and oxygen could be converted to $O_2^{\cdot-}$ by the antioxidant, whereas the reaction between ABTS and $O_2^{\cdot-}$ was more intense [29, 49].

FRAP is a way to test the antioxidant property at low pH conditions through the blue-violet complex formed by ferrous ions and TPTZ [50]. As shown in Figure 3.5C, the result also had a similar trend to the other antioxidant activity tests, with the maximum antioxidant activity of 3.90 ± 0.02 mmol·L^{-1} (RC-20G-20Z) on day 3 and the reduced values of 3.01 ± 0.01 mmol·L^{-1} (control), 3.31 ± 0.05 mmol·L^{-1} (RC-20G-20Z), and 3.42 ± 0.07 mmol·L^{-1} (Ziploc®) on day 12, respectively. Therefore, both RC-20G-20Z and Ziploc® bags could efficiently preserve the antioxidant property of blueberries, and no significant difference was observed between these two packaging materials.

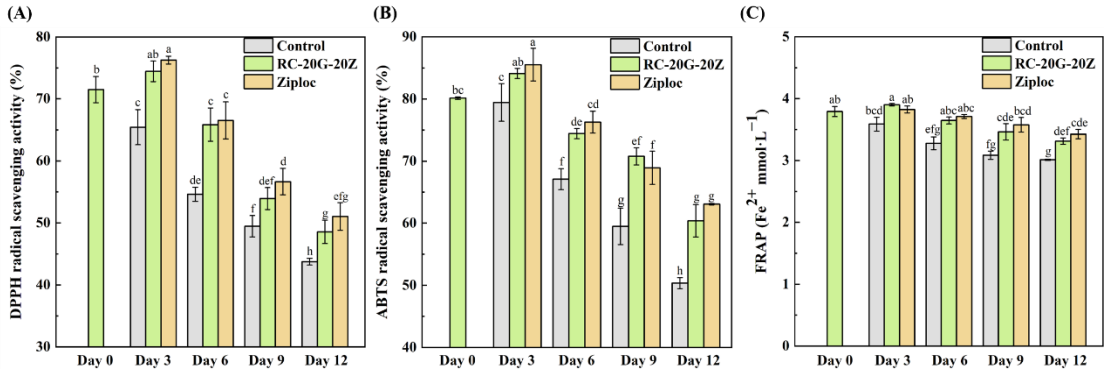

Figure 3.5 Antioxidant activities of blueberries under different preservation conditions. Different letters on the tops of columns represented the significant difference ($p < 0.05$).

3.5 CONCLUSION

In this study, by adding a thin layer of zein, the RC films prepared from pine pulp dissolved in NaOH/urea aqueous system were successfully converted into heat sealable packaging materials. When the concentration of coating solution was 20%, the composite films exhibited good mechanical properties (tensile strength of 46.49 ± 0.94 MPa, elongation at break of 5.30 ± 0.38%), gas barrier properties (WVP of 5.09 ± 0.08 × 10^{-7} g m^{-1} h^{-1} Pa^{-1}, OTR of 4.83 ± 0.10 cc/m^2day), and heat sealability. The packaging bags could be easily fabricated from the RC/zein composite films by a tabletop heat sealer without using high temperature or high pressure. The blueberries sealed in RC-20G-20Z and commercial Ziploc® bags showed similar texture and antioxidant activity after a 12-day storage and were much better than the unpacked blueberries. Thus, this work suggests a facile way to endow cellulose films with heat-sealing property and promotes the development of biodegradable food packaging materials.

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CONNECTING STATEMENT II

In **Chapter 3**, zein coating was applied on the surface of the RC films, resulting in good heat-sealing property and barrier properties. It is noteworthy that the blueberries packed in the heat-sealed cellulose/zein sachets were protected from oxidation and spoilage during a 12-day storage period. In **Chapter 4**, the zein-coated RC films were further modified by the incorporation of sodium stearate. The effect of sodium stearate content on water vapor permeability, biodegradability, and food preservation property of the modified RC films was investigated.

CHAPTER 4. IMPROVEMENT OF WATER VAPOR BARRIER PROPERTY OF REGENERATED CELLULOSE/ZEIN FILMS BY SODIUM STEARATE

4.1 ABSTRACT

Petroleum-based materials are widely used in packaging, medical, and other fields, and increasing concerns about the environment have facilitated the development of eco-friendly packaging. Cellulose, as the most abundant and renewable natural polymer, has been extensively studied as an ideal substitute for plastics due to its low-cost, excellent biocompatibility, and renewability. In the previous study, zein coating was applied on the surface of the RC film to endow it with heat-sealing property and reduced oxygen permeability. Herein, sodium stearate was added during the film preparation, aiming to improve the water vapor permeability of the RC films. The resultant composite films showed good mechanical properties and better barrier properties compared to zein-coated RC films and exhibited a similar capacity as the Ziploc® during the preservation of blueberries for up to 12 days. Therefore, this study demonstrates a feasible strategy to fabricate heat-sealable RC films with good performance for biodegradable food packaging.

Keywords: regenerated cellulose, sodium stearate, water vapor permeability, compost disintegrability, food packaging

4.2 INTRODUCTION

Petroleum-based materials with favorable physical and barrier characteristics are widely used and play an important role in our daily life. Global plastic production in 2020 is reported to have increased by 36% since 2010, resulting in a large amount of plastic waste (Nanda et al., 2022). Approximately 140 million tons of petroleum-based polymers are produced and used, and subsequently enter the ecosystem as industrial waste products each year (Fesseha & Abebe, 2019). In the past few years, growing concerns about global pollution and the negative impact on ecosystems are accelerating the development of biodegradable materials as an alternative to petroleum-based plastics. Biodegradable materials are based on natural polymers such as cellulose (Cazón & Vázquez, 2021; Xing et al., 2020), starch (Cortés-Rodríguez et al., 2020; Hiremani et al., 2021), proteins (Li et al., 2021; Oymaci & Altinkaya, 2016; Xavier et al., 2021), and so on, which have less environmental impact compared to petroleum-based materials. However, there is a challenge in the development of natural polymer-based materials combined with good mechanical and barrier properties (Kou et al., 2014).

Cellulose is considered as the most widely distributed and inexhaustible natural polymer with the attractive structure and suitability for eco-friendly products (Tu et al., 2021). Cellulose can be made into transparent films by dissolution and regeneration and can be applied as packaging materials after modification, such as the incorporation of tannin in RC film for sustainable active packaging (Huang et al., 2022), cellulose-polyvinyl alcohol-glycerin films with favorable mechanical properties, barrier properties and thermal stability (Cazón et al., 2019), and regenerated cellulose film with end-amino hyperbranched polyamide anchored nano-silver as a safe antimicrobial food packaging material (2019). However, cellulose is not a thermoplastic, adhesives are usually required to seal the regenerated films. In order to overcome this drawback, zein coating was applied on the surface of the RC films, and the resultant composite films showed good heat-sealing, mechanical, and oxygen barrier properties. However, the water vapor permeability of the zein-coated RC films can be better improved. In recent years, many additives such as graphene (Xu et al., 2019), zinc oxide nanoparticles (Saedi

et al., 2021), and nano-SiO_2 (Reddy et al., 2018) have been used to greatly improve the water vapor barrier performance of RC films. To ensure that the modified RC film was still a low-cost and sustainable material, sodium stearate was chosen to incorporate with RC films in this study. Stearic acid is derived from animal and vegetable fats and oils and is a mono-functional molecule with long hydrophobic hydrocarbon chains (Chen et al., 2020), which is generally used as a waterproofing agent in the paper-making process. Sodium stearate can be formed by the reaction between stearic acid and sodium hydroxide solution. He et al. (2013) and Sobhana et al. (2017) have used it to prepare highly hydrophobic cellulose films. However, there is no evaluation of RC films with both heat-sealing property and excellent water vapor barrier property for food packaging applications.

To prepare the biodegradable heat-sealable food packaging materials with high water vapor barrier properties, this study used sodium hydroxide/urea aqueous solution system to prepare RC films, endowed the RC film with heat-sealing properties by zein coating, and improved water vapor permeability by incorporating sodium stearate. The structure and properties of the modified RC films were characterized, and the optimized films with good mechanical and barrier properties were selected for preserving fresh blueberries.

4.3 MATERIALS AND METHOD

4.3.1 Materials

The bleached kraft pulps of pine were kindly provided by FPInnovations (Pointe-Claire, QC, Canada). Sulfuric acid (95.0%-98.0%) was purchased from Sigma-Aldrich (Oakville, ON, Canada). Ethyl alcohol anhydrous was purchased from Greenfield Global (Brampton, ON, Canada). Sodium hydroxide (≥97.0%), urea (≥99.6%), stearic acid (≥97.0%), and glycerol (≥99.5%) were purchased from Fisher Scientific (Ottawa, ON, Canada). Zein F4400C-FG was purchased from FloZein Products (Ashburnham, MA, USA). Fresh blueberries were purchased from a local supermarket in Montreal and were harvested in Laredo (TX, USA).

4.3.2 Preparation of Cellulose/Zein/Sodium Stearate Composite Films

Pine pulp was placed in 30% w/w H_2SO_4 solution with continuous stirring at 200 rpm for 48 hours at 25℃ to reduce the molecular weight. After acid hydrolysis, the material was rinsed thoroughly with deionized water and dried in an oven at 100℃, and then dissolved (4% w/w) in an aqueous solution comprising NaOH/urea/H_2O (Cai & Zhang, 2005). Sodium stearate solution was prepared by incorporating molten stearic acid into 7% w/w sodium hydroxide solution and was added to the cellulose solution with the sodium stearate contents of 10, 15, and 20% w/w. The mixture was pre-cooled to -12℃ overnight and stirred at 2000 rpm for 20 minutes in an ice bath. The solution was centrifuged at 1000 rpm for 5 minutes at 25℃ to degas and precipitate the insoluble fraction, and then cast and coagulated to make RC films. The wet films were dried by a hot press machine (Carver 3895, Wabash, IN, USA) at 90℃ and 0.4 MPa for 10 minutes, followed by hot pressing at 120℃ and 0.6 MPa for another 10 minutes. The dried RC films were coated on one side with a solution of zein (20% w/w) and glycerol (20% w/w of the dry weight of the zein) dissolved in ethanol solution (80% w/w). The regenerated cellulose film with zein coating was coded as zein-coated RC film, and the RC films with different sodium stearate contents were labelled as 10% SS, 15% SS, and 20% SS, respectively.

4.3.3 Characterization

4.3.3.1 Cellulose/Zein/Sodium Stearate Composite Film Structure

The structure of the sodium stearate-modified RC films was characterized by FT-IR spectrometer (Cary 630, Agilent Technology, Santa Clara, CA, USA), and the spectra were recorded in the range of 4000-650 cm^{-1}. The morphology of the films was observed using a Hitachi TM1000 scanning electron microscope (SEM) (Schaumburg, IL, USA), operating at an acceleration voltage of 4 kV. The film samples were sputtered with 4 nm gold-platinum by a Leica EM ACE200 low vacuum coater (Vaughan, ON, Canada) prior to observation and photographing.

4.3.3.2 Cellulose/Zein/Sodium Stearate Composite Film Properties

The modified RC films were tested after being placed at 25℃ and 43% RH for 48 hours. The mechanical properties of the modified RC films were tested by an ADMET MTEST Quattro eXpert 7601 single-column testing machine (Norwood, MA, USA) at 25℃ according to standard ASTM D-882.

The WVP of the modified RC films was observed based on the ASTM E96-92 standard (ASTM, 1995). Sodium stearate-modified RC films were sealed on the top of glass flasks containing anhydrous calcium chloride. The sealed glass flask was then placed in a desiccator with water. The weight change of the flask was recorded periodically at 25℃. The WVP (g m^{-1} h^{-1} Pa^{-1}) of films was calculated by the Equation (4.1):

$$WVP = \frac{\Delta m \times k}{A \times \Delta T \times \Delta P} \tag{4.1}$$

where Δm is the weight change of the flask (g), k is the thickness of the modified RC films (m), A is the exposed area of the film, ΔT represents the time, and ΔP is the partial pressure difference that existed between the two sides of the film (Pa).

The OTR (cc/m^2day) of the modified RC films was characterized by AMETEK MOCON OX-TRAN 2/22 OTR Analyzer (Brooklyn Park, MN, USA) at 23℃ and 0% RH.

The disintegration performance of sodium stearate-modified RC/zein composite films was tested by compost according to the ISO-20200 standard (ISO, 2015). Briefly, solid synthetic waste was prepared by mixing 40% sawdust, 30% rabbit feed, 10% ripe compost, 10% corn starch, 5% saccharose, 4% corn oil, and 1% urea. The dry waste was mixed with water in a 45:55 ratio and aerobic status was stabilized by blending it gently. The composite films were cut into 40 mm × 40 mm and buried at 6 cm depth in the perforated composting reactor containing reconstituted wet waste. After that, the reactors were put in an air-circulation oven at 58 ± 2℃, and water was added periodically to maintain the humidity in the compost. The films were recovered from the reactor by tweezer at different times (3, 6, 9 and 12 days).

4.3.4 Study of Shelf Life

The preservation effect of modified RC films on blueberries was evaluated. Fresh blueberries with similar size and hardness and without blemishes were selected and sealed in commercial storage bags (Ziploc®) and 20% SS films by KF-300HC 8-inch Impulse Sealer with Cutter (St-Laurent, QC, Canada). The blueberries without any packaging were used as the control. All blueberries were stored at 25℃ and 50% RH for 12 days to observe changes in appearance and weight loss on days 3, 6, 9 and 12. The evaluation of blueberry samples was based on color, hardness, wrinkling, and decay. The weight loss of the blueberries was calculated using Equation (4.2):

$$Weight\ \ loss\ \ (\%) = \frac{W_i - W_f}{W_i} \times 100\% \tag{4.2}$$

where W_i and W_f were the initial and final sample weight (g), respectively.

4.3.5 Statistical Analysis

The experiments were performed in triplicate, and data were presented as mean ± standard deviation. Analysis of variance (ANOVA) was used for the statistical analysis, followed by multiple comparison tests using Duncan's multiple range test. All the analyses were performed through SPSS statistical software with significant differences within samples at $p < 0.05$.

4.4 RESULTS AND DISCUSSION

4.4.1 Structure of Cellulose/Zein/Sodium Stearate Composite Films

The surface and cross-sectional morphologies of the modified RC film were observed by SEM. As shown in Figure 4.1, all the films presented a smooth and uniform surface without cracks or holes (characterization was done on the non-zein-coated side). No obvious changes were found after the addition of sodium stearate, indicating that sodium stearate was completely dissolved and uniformly distributed in the RC films. Figure 4.1(E)-(H) show the cross-sectional features. The hot-pressed RC film had a consistent thickness and dense structure, and a uniform

zein coating was observed at the lowermost part of the films. The thicknesses of the composite films were determined by the cross-sectional view and were 36 ± 1 μm.

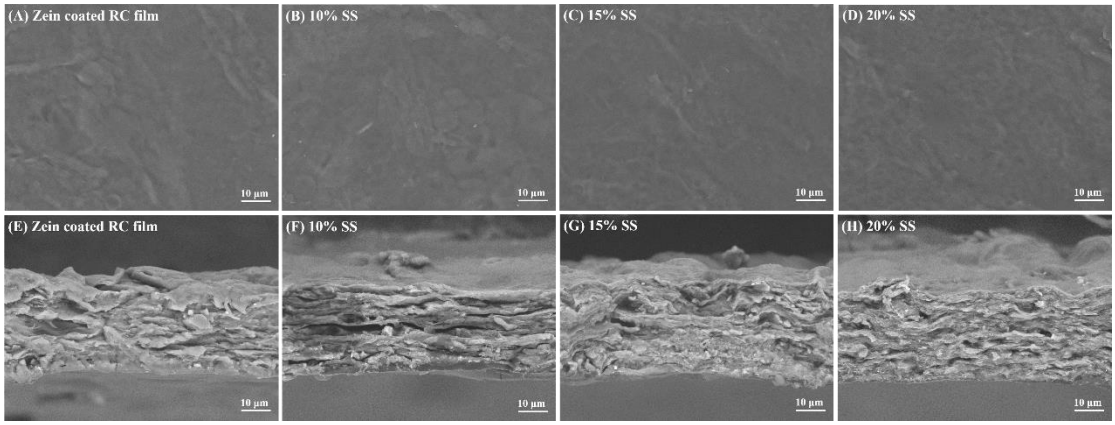

Figure 4.1 SEM images of sodium stearate modified RC films: surface (A-D) and cross-section (E-H).

To further investigate the structure of the composite films, FT-IR spectra of zein-coated RC films and sodium stearate-modified films (the uncoated side) were collected. As shown in Figure 4.2, the RC films had the characteristic peaks in the regions of 3400-3000 cm^{-1} and 1645 cm^{-1}, which were due to -OH stretching from intramolecular hydrogen bonding and O-H bending from adsorbed water, respectively (Poletto et al., 2014; Sobhana et al., 2017). In addition, two new absorption peaks at 2916 and 2850 cm^{-1} were attributed to the stretching vibrations of $-CH_3$ and $-CH_2$ in the sodium stearate-modified films (Yan Chen et al., 2017; Zhu et al., 2016). This was the result of the combination of sodium stearate and cellulose. It was supposed to be the moderate intensity symmetric stretching vibration of the C-H band in the $-CH_3$ in sodium stearate and the C-H stretching vibration of the methyl and methylene groups in cellulose (Chen et al., 2020). The intensities of these two peaks increased with the increasing concentration of sodium stearate, suggesting that sodium stearate and cellulose were well integrated. Besides, the peak at 1431 cm^{-1} was the absorption band of crystalline cellulose due to the asymmetric $-CH_2$ bending vibration, while the peak at 895 cm^{-1} was the -C-O-C- bridge stretching, representing the amorphous region and the crystal structure of cellulose (Ciolacu et al., 2011; Md Salim et al., 2021).

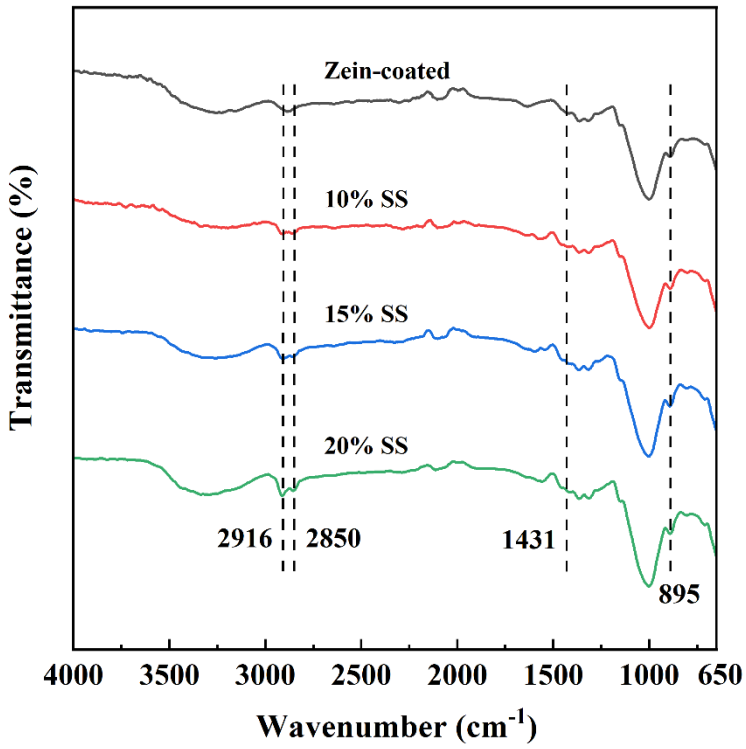

Figure 4.2 FT-IR spectra of sodium stearate modified RC films.

4.4.2 Properties of Cellulose/Zein/Sodium Stearate Composite Films

4.4.2.1 Mechanical Properties

The mechanical properties of the modified RC films were investigated, and Figure 4.3(A) shows the tensile stress and tensile strain of the composite films. In general, the addition of stearic acid or stearate has a negative effect on the mechanical properties of the films. The samples containing 10%, 15%, and 20% sodium stearate showed decreasing values of tensile strength, which were in agreement with the results of cassava starch films and whey protein films containing stearic acid (Anker et al., 2002; Chen et al., 2020). The decrease in tensile strength with the addition of sodium stearate might be attributed to the presence of inhomogeneous microphases in the composite films and the weakened polymer network (Bertan et al., 2005; Schmidt et al., 2013). Tensile strain also decreased with the increased concentration of sodium stearate. As reported by Péroval et al. (2002), the addition of stearic acid in the arabinoxylan films reduced the tensile strain, which might be attributed to the

inability of lipids to form a viscous matrix with polysaccharides (Schmidt et al., 2013). The mechanical test results showed a similar trend to those of sodium stearate-modified starch films (14.19 MPa, 0.56%) (Schmidt et al., 2013), soybean isolate protein films (8.6 MPa, 0.7%) (Lodha & Netravali, 2005), and peanut starch films (25.42 MPa) (Oyeyinka et al., 2017).

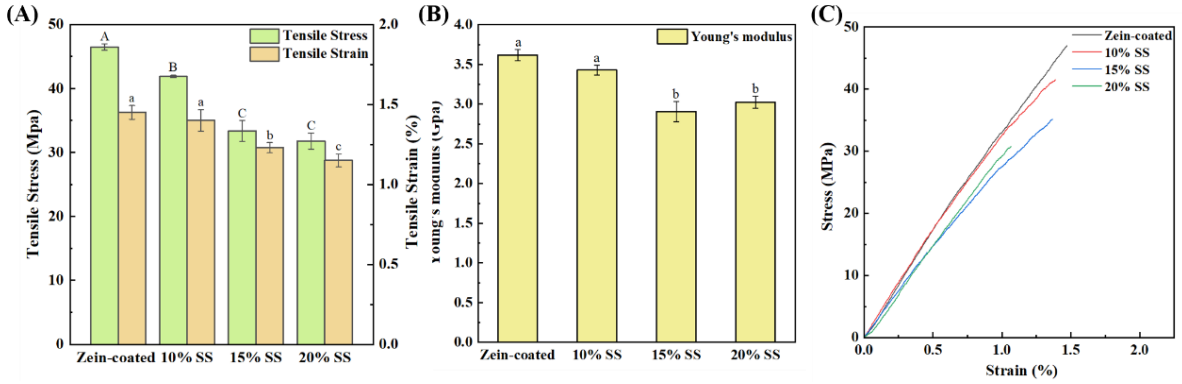

Figure 4.3 Mechanical properties of sodium stearate modified RC films. Different letters on the tops of columns represented the significant difference in terms of sodium stearate mass ($p < 0.05$).

4.4.2.2 Barrier Properties

The WVP and OTR of the modified RC films are shown in Table 4.1. The incorporation of sodium stearate resulted in a lower WVP compared to the zein-coated neat RC films, which was expected due to the hydrophobic feature of the added stearate (Schmidt et al., 2013). Meanwhile, the high amount of sodium stearate might lead to the increased roughness of the film surface, which also restricted the pass-through of water vapor (Oyeyinka et al., 2017). With the addition of 20% sodium stearate, the WVP was reduced by almost 50% and reached 1.56×10^{-7} g m^{-1} h^{-1} Pa^{-1}. Similarly, WVP showed a decrease after the addition of stearic acid or sodium stearate to corn starch films (Jiménez et al., 2012; Ortega-Toro et al., 2014), gelatin-based films (Bertan et al., 2005), and gluten coatings (Tanada-Palmu & Grosso, 2005).

The OTR of the composite films was significantly decreased after the addition of sodium stearate compared to the zein-coated RC film, where 20% SS had the lowest value of 1.36 ± 0.06 cc/m^2·day. Sodium stearate effectively improved the oxygen barrier property of the RC

films, which might be due to the enhanced phase solubility between the two polymers (Khanoonkon et al., 2016; Srinivasa et al., 2007). In addition, the effective closure of superficial pores of the films by sodium stearate was also responsible for the lower OTR value (Costa & Simões, 2022).

Table 4.1 Water vapor permeability and oxygen transmission rate of sodium stearate modified RC films. Different letters in the same columns indicate a significant difference within the same sample groups ($p < 0.05$).

Sample	WVP ($\times 10^{-7}$ g m^{-1} h^{-1} Pa^{-1})	OTR (cc/m^2·day)
Zein-coated RC film	3.07 ± 0.07 ^{a}	2.83 ± 0.04 ^{a}
10% SS	2.58 ± 0.01 ^{b}	1.84 ± 0.01 ^{b}
15% SS	1.90 ± 0.08 ^{c}	1.55 ± 0.02 ^{c}
20% SS	1.56 ± 0.06 ^{d}	1.36 ± 0.06 ^{d}

4.4.2.3 Compost Disintegrability

RC films can be used as a fully degradable and sustainable food packaging material (Saedi et al., 2021). Hereby, the effects of applying zein coating together with sodium stearate modification on RC film disintegration under composting conditions were investigated and compared with commercial Ziploc®. As shown in Figure 4.4, no particular morphological alterations were observed for all samples after 3 days. The composite films began to disintegrate after day 6, and it was difficult to weigh and calculate the weight loss due to the difficulty of completely separating the reconstituted wet waste and the modified RC film residue. The addition of sodium stearate slightly affected the fragmentation of the composite films, but all of them were fully dissociated after 12 days, compared to the Ziploc® package which had no change. It demonstrated that sodium stearate had no significant impact on the biodegradability of RC films (Allasia et al., 2019; He et al., 2013; Karnnet et al., 2005).

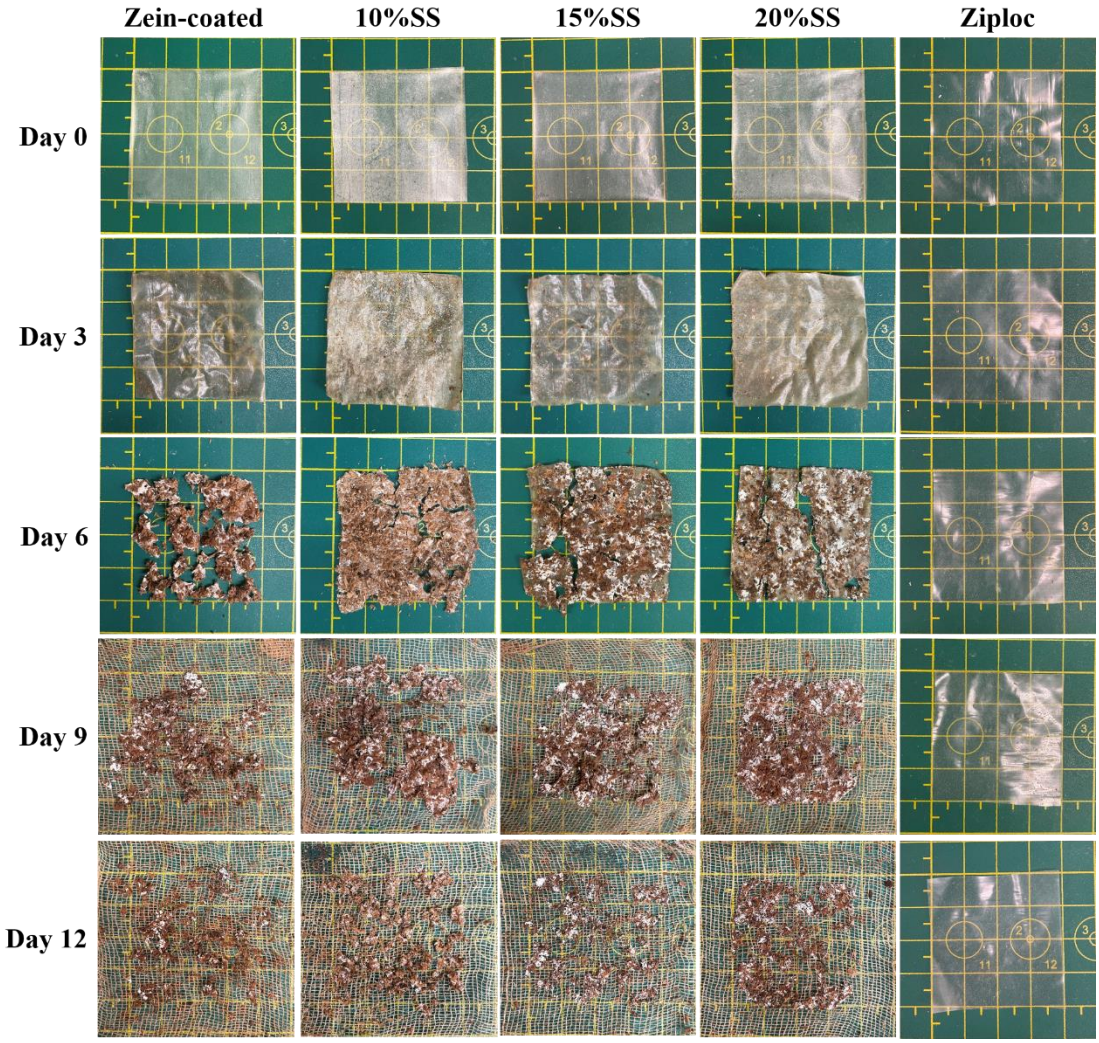

Figure 4.4 Photos of neat zein-coated RC film, sodium stearate modified RC films and Ziploc® after incubating under composting conditions.

4.4.3 Food Preservation

The composite films with heat-sealing property were made into small sachets without using any adhesives. Packaging materials with favorable mechanical and barrier properties can contribute to the protection of food from physical damage, oxidation, and dehydration, thus extending the shelf life of food (Pandey et al., 2022). The 20% SS films with overall good mechanical and barrier properties were chosen for shelf-life test and compared with the commercial Ziploc® plastic bag. As shown in Figure 4.5(A), no obvious changes were observed after 3 days for all the samples. The unpackaged blueberries showed surface wrinkling and

softening on day 6 of storage, and the wrinkling became more pronounced on day 9 while the blueberries became duller in color. To be noted, the control samples showed deterioration and a spoilage condition on day 12. In contrast, the blueberry samples packed with 20% SS and Ziploc® maintained excellent color and firmness after 9 days of storage, and the surface wrinkling occurred on day 12, but without any deterioration. It was reported that the weight loss of blueberries was the major cause of firmness change during postharvest storage (Chen et al., 2017; Paniagua et al., 2014). The weight loss showed a similar trend as the changes in blueberry appearance (Figure 4.5(B)). All the blueberry samples exhibited a gradual increase in weight loss. According to Bovi et al. (2018), respiration and transpiration have long been identified as the primary sources of weight loss in fresh fruit and vegetables. Food packaging can effectively reduce the respiration and transpiration of fruits, so a significantly higher weight loss rate of unpackaged blueberries was observed than the packaged ones. As shown in Figure 4.5(B), after 12 days, the weight losses of 20% SS and Ziploc® packed samples were about 4.25% and 3.21%, respectively, against 20.22% for the control sample. In general, blueberries packed with Ziploc® were able to maintain the appearance and a low weight loss rate due to the good water vapor barrier property of Ziploc® ($1.07 \pm 0.29 \times 10^{-9}$ g m^{-1} h^{-1} Pa^{-1}) (Dias et al., 2013), and 20%SS had the slightly higher weight loss during the 12 days of storage, but both of them were clearly superior to the unpackaged blueberries.

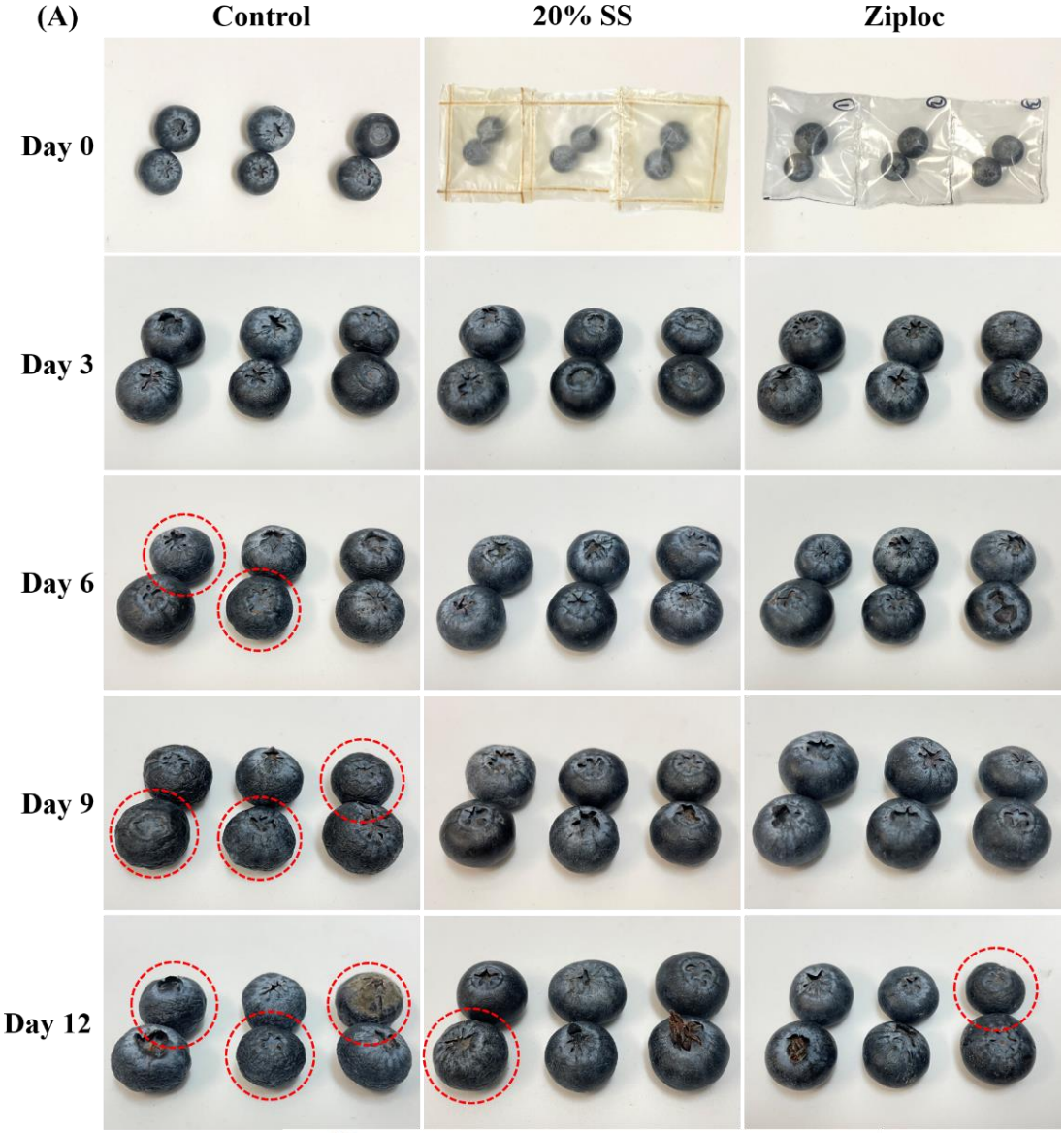
(A)
Control
20% SS
Ziploc
Day 0
Day 3
Day 6
Day 9
Day 12
(B)
Control
20% SS
Ziploc
Weight loss (%)
Storage days (d)
0
5
10
15
20
25
0
3
6
9
12

Figure 4.5 (A) Photos and (B) weight loss of blueberries under different packaging conditions in 12 days.

4.5 CONCLUSION

In this study, RC/zein films were additionally modified with sodium stearate. Compared with zein-coated RC films, the incorporation of sodium stearate resulted in better water vapor and oxygen barrier properties. The resultant composite films could be completely dissociated in 12 days under composting conditions. In particular, the 20% SS films showed overall good mechanical (tensile stress of 33.47 ± 1.21 MPa and tensile strain of 1.15 ± 0.03%) and barrier properties (WVP of 1.56×10^{-7} g m^{-1} h^{-1} Pa^{-1} and OTR of 1.36 ± 0.06 cc/m^2·day), and could effectively prevent the weight loss and spoilage of the packaged blueberries within 12 days, which was comparable to the commercial plastic packaging Ziploc®. Therefore, the modification of regenerated cellulose films with zein and sodium stearate has considerable potential for the preparation of heat-sealable food packaging films with excellent properties.

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CHAPTER 5. GENERAL SUMMARY AND CONCLUSION

5.1 GENERAL SUMMARY

While bringing convenience to life, petroleum-based non-biodegradable plastics have caused serious environmental problems. Therefore, it is emerging to develop biodegradable materials from renewable resources to partially replace petroleum-based plastics. In the literature review, biodegradable materials derived from natural resources in recent years have been summarized. Especially, the types of raw materials, production methods, major properties, and potential applications as biodegradable packaging (films and coatings) were reviewed. Herein, we aimed to develop cellulose-based films with good performance from a 'green' aqueous solution. As cellulose is not a thermoplastic, zein coating was adapted to endow it with heat sealability. The results showed that the composite films had good mechanical (tensile stress of 46.49 $\pm$ 0.94 MPa and tensile strain of 5.30 $\pm$ 0.38%) and oxygen barrier properties (4.83 $\pm$ 0.10 cc/m ^{2}day), and the blueberries packed in the heat-sealed cellulose/zein bags were protected from oxidation and spoilage during a 12-day storage period. Furthermore, the cellulose/zein composite films were modified with sodium stearate to improve their water vapor barrier property (1.56 $\times 10^{-7}$ g m^{-1} h^{-1} Pa^{-1}) and oxygen barrier property (1.36 $\pm$ 0.06 cc/m ^{2}day), which could effectively preserve the blueberries with a comparable effect to the Ziploc® packaging.

5.2 SUGGESTIONS FOR FUTURE WORK

This study provides a promising strategy for the preparation of regenerated cellulose composite films with high performance and heat sealability. In particular, the novel heat-sealing property and excellent barrier properties offered the possibility of the modified RC films as biodegradable food packaging materials. Since this research work focused on the heat-sealing and barrier properties of regenerated cellulose films, further research based on the effects of different biodegradable coatings should be carried out to improve the heat-sealing property. Additionally, new strategies should be investigated to enhance the flexibility of regenerated cellulose films without any negative effects on the water vapor barrier property and

biodegradability. Based on these results, active food packaging derived from regenerated cellulose films can be considered to further increase the potential applications.

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