Recovery of Pectin from Plantain Peels and Transformation into Composite Films Using A Green Approach

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ABSTRACT

The growing global population and its associated industrial activities have increased the generation of by-products from food harvesting and processing in the Agri-food industry. Current methods of disposal of such waste lead to harmful environmental impacts; thus, stimulating much attention in research to develop innovative waste management approaches to help mediate the waste disposal problem by transforming them into profitable high value-added products. Plantain peels are major Agri-food waste in many developing countries with limited commercial value. Pectin, a major polysaccharide in plantain peels, is a biopolymer with great potential for application in food, biomaterials, biomedicine, and etc. Various green extraction methods of pectin have been widely studied in several sources including citrus peels, apple pomace, mango peels, cocoa husks, corn cobs, and others. However, similar studies on plantain peels are yet to be established despite the high volume of this waste produced in many developing countries. Pectin film is an emerging food-related application of pectin in recent years, but the functionality is limited by its low mechanical properties and poor moisture resistance. Beeswax solid lipid nanoparticles (BSLNs) has proved to be effective in improving the mechanical and water barrier properties for some polysaccharide films, but it has not yet been investigated in pectin films. In this research, pectin was firstly recovered from plantain peels by green approach with cellulase. The highest yield of pectin on dry basis was 13.38%. The most yield of pure pectin was obtained with 50U of cellulase at 50 ℃ and pH 4.5. Plantain peel pectin had degree of esterification at 43.43–48.23% (< 50%), thus was characterized as a low methoxy pectin and the result shew that the enzymatic treatment did not significantly affect the degree of esterification. The recovered pectin was subsequently transformed into composite films and compared with citrus peel pectin films, for water and light barrier properties, mechanical attributes, conformational change, and microstructure. The plantain peel pectin and citrus peel pectin both produced films with significantly ($P \leq 0.5$) improved water and light

barrier properties with BSLNs incorporation. The addition of different concentrations of BSLNs was shown to enhance some mechanical properties of plantain peel pectin films, such as tensile strength and Young's Modulus but decreased the tensile strength and elongation at break of citrus peel pectin films. This work provides a novel approach to manage plantain peels waste and contributes new knowledge in pectin films for potential food packaging usage and sustainable resource management.

RÉSUMÉ

La croissance de la population mondiale et les activités industrielles qui y sont associées ont augmenté la production de sous-produits issus de la récolte et du traitement des aliments dans l'industrie agroalimentaire. Les méthodes actuelles d'élimination de ces déchets ont des effets néfastes sur l'environnement, ce qui incite la recherche à développer des approches innovantes de gestion des déchets afin de résoudre le problème de l'élimination des déchets en les transformant en produits rentables à haute valeur ajoutée. Les pelures de plantain sont des déchets agroalimentaires majeurs dans de nombreux pays en développement, avec une valeur commerciale limitée. La pectine, un polysaccharide majeur dans les pelures de plantain, est un biopolymère avec un grand potentiel d'application dans l'alimentation, les biomatériaux, la biomédecine, etc. Diverses méthodes d'extraction verte de la pectine ont été largement étudiées dans plusieurs sources, notamment les écorces d'agrumes, le marc de pomme, les écorces de mangue, les coques de cacao, les épis de maïs, etc. Cependant, des études similaires sur les pelures de plantain doivent encore être établies malgré le volume élevé de ces déchets produits dans de nombreux pays en développement. Le film de pectine est une application alimentaire émergente de la pectine ces dernières années, mais sa fonctionnalité est limitée par ses faibles propriétés mécaniques et sa faible résistance à l'humidité. Les nanoparticules lipidiques solides de cire d'abeille (BSLN) se sont avérées efficaces pour améliorer les propriétés mécaniques et de barrière à l'eau de certains films de polysaccharides, mais elles n'ont pas encore été étudiées pour les films de pectine. Dans cette recherche, la pectine a d'abord été récupérée à partir de pelures de plantain par approche verte avec de la cellulase. Le rendement le plus élevé de pectine sur une base sèche était de 13,38%. Le rendement le plus élevé de pectine pure a été obtenu avec 50U de cellulase à 50 °C et pH 4.5. La pectine de la peau de plantain avait un degré d'estérification de 43.43–48.23% (< 50%), elle a donc été caractérisée comme une pectine à faible teneur en méthoxy et le résultat montre que le traitement enzymatique n'a pas

affecté de manière significative le degré d'estérification. La pectine récupérée a ensuite été transformée en films composites et comparée aux films de pectine d'écorce d'agrumes, pour les propriétés de barrière à l'eau et à la lumière, les attributs mécaniques, le changement de conformation et la microstructure. La pectine d'écorce de plantain et la pectine d'écorce d'agrume ont toutes deux produit des films présentant des propriétés de barrière à l'eau et à la lumière significativement améliorées ($P \leq 0.5$) avec l'incorporation de BSLN. L'ajout de différentes concentrations de BSLNs a permis d'améliorer certaines propriétés mécaniques des films de pectine de plantain, telles que la résistance à la traction et le module de Young, mais a diminué la résistance à la traction et l'allongement à la rupture des films de pectine d'agrumes. Ce travail fournit une nouvelle approche pour gérer les déchets de peaux de plantain et apporte de nouvelles connaissances sur les films de pectine pour une utilisation potentielle dans l'emballage alimentaire et la gestion durable des ressources.

CONTRIBUTION OF AUTHORS

This thesis is arranged in the format of manuscripts and consists of one introduction plus three chapters. It starts with a general introduction for the research background on the current issue that our society is facing and opens the concepts of waste reutilization and transformation into value-added products. This part also gives the outline, rationale, and objectives of the research. Chapter I gives a detailed literature review of the current studies done on Agro-waste management, plantain and plantain peels, pectin using various pectin extraction techniques, as well as fabrication of pectin composite films. Chapters II and III are mainly based on the manuscripts in preparation to be published with slight modifications. Connecting statements are provided to show the links among the chapters. The last chapter (Chapter IV) provides the overall conclusions from the study with recommendations for follow-up work.

The author, Jiayu Xie, designed and conducted the experiments, collected and analyzed the data, and prepared the drafts of all the manuscripts. Dr. Benjamin Kofi Simpson suggested the research topic, advised on the direction, content and techniques used for the research, made edition on the manuscripts, and provided laboratory space, materials, and supplies for the experiments. Dr. Yi Zhang provided advice and suggestion for the design and content of the experiments, gave technical guidance and support, and provided help with co-directing and editing the manuscripts. Dr. Yixiang Wang provided advise & expertise on the work reported in Chapter III of the thesis, laboratory space, and equipment. Dr. Zhiming Qi and his student Mr. Ziwei Li provided equipment and helped with the study on the particle size determination and zeta potential measurements reported in Chapter III. Dr. Saji George provided laboratory space and equipment for all the FTIR testing, while Dr. Youssef Chebli provided training, laboratory space and equipment for all the SEM analysis for Chapter III, and Dr. Daniel Chartrand performed analysis and statistical support for XRD measurements for Chapter III.

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ABBREVIATIONS AND ACRONYMS

ASTM ……..….... American Society for Testing and Materials

ANOVA ……...One-Way Analysis of Variance

COVID-19 ……..... Coronavirus Disease of 2019

BSLNs ……..……...Beeswax Solid Lipid Nanoparticles

DE ……..Degree of Esterification

DM ……...Degree of Methoxylation

DNS ……....……Dinitrosalicylic Acid

dwd ……dry weight basis

EB ……..…..Elongation At Break

EE ……...……..Enzymatic Extraction

FAO ……...……..Food and Agriculture Organization

FAOSTAT ……...Food and Agriculture Organization Corporate Statistical Database

FTIR ……Fourier-transform infrared spectroscopy

GalA ……..…...…..Galacturonic Acid

HCE ……..……Hydrodynamic Cavitation Extraction

HGA ……...…...…Homogalacturonan

HMP ……...……..High Methoxy Pectin

IITA …….....…..International Institute of Tropical Agriculture

kHz ……..…….Kilohertz

LMP ……...……...…Low Methoxy Pectin

MAE ……..…….....Microwave-Assisted Extraction

MEO ……...……...Marjoram Essential Oil

OHE ……..……....Ohmic Heating Extraction

PDI ……...……..…Polydispersion Index

PVA ……..…….Poly Vinyl Alcohol

REO ……....Rosemary Essential Oil

RG-I ……..……....Rhamnogalacturonan-I (RG-I)

RG-II ……..….Rhamnogalacturonan-II (RG-II)

RH ……...……...…Relative Humidity

SD ……...……..…Standard Deviation

SDF ……...…..….Soluble Dietary Fibers

SEM ……...…….Scanning Electron Microscopy

SWE ……..……..Subcritical Water Extraction

TGA ……..…Thermo-Gravimetric Analysis

TS ……..Tensile Strength

UAE ……...……....Ultrasound-Assisted Extraction

UAEE ……...Ultrasound Assisted Enzymatic Extraction

UAME ……...……..Ultrasound Assisted Microwave Extraction

UAOH ……...Ultrasound Assisted Ohmic Heating Extraction

UV ……...……..Ultra-violet

WVP ……...……Water Vapor Permeability

XRD ……...……..X-ray Diffraction

YM ……...……..…..Young’s Modulus

INTRODUCTION

The growing global population has created demands for more food production for human consumption, and this growth has been accompanied by more food processing and generation of more food processing residues. The residues are 'food supply chain waste', defined as the lost or degraded organic material produced from human food consumption (Lin et al., 2013). According to the Food and Agriculture Organization (FAO), since 2011, approximately one-third of the food produced for human consumption (~1.3 billion tons) is wasted every year (FAO, 2020). Such waste problem is more severe in the Agro-industry and is mainly produced at the manufacturing stage and retail stage (Lin et al., 2013; Ranganathan et al., 2020). Agro-wastes are commonly disposed by burying in landfills or burning in the air. Substantial burning actions create harmful smokes and increase ambient temperatures, and dumped wastes in open areas lead to water and land pollution, contributing significantly to global warming (Tsai et al., 2020; Zhang et al., 2015). Thus, development of green technologies for innovative waste management to not only solve the waste disposal problem but also produce value-added products from Agro-waste has gathered considerable research interest.

On the other hand, white pollution created by excessive use of non-biodegradable plastic is always a big threat to the environment, natural ecosystems and public health due to the harmful chemicals produced by traditional disposal approaches (Ali et al., 2021; Xanthos & Walker, 2017). During the COVID-19 pandemic, the implementation of public hygiene measures has driven the demand for plastics by 40% for packaging, and by 17% for other applications (Patrício Silva et al., 2021; Prata et al., 2020). Therefore, development of biodegradable substitutes for plastics is of vital importance (Valdés et al., 2016; Xie et al., 2020; Yadav et al., 2019).

Biopolymers are synthesized by living organisms or chemically synthesized from biological

materials. Such polymers have some appealing properties like biocompatibility, biodegradability, and non-toxicity (Jha & Kumar, 2019; Martău et al., 2019; Mellinas et al., 2020). Biopolymers are widely available in plant discards, and biopolymer derived films can be fabricated to have improved properties over conventional plastics. Recovery of biopolymer from Agro-waste into biodegradable material as a substitute for plastics can not only alleviate the environmental threats but also help in creating value-added products which has attracted a lot of interest (Redondo-Gómez et al., 2020).

A lot of studies have focused on the reutilization of commonly available Agro-waste from developed countries. On the other hand, due to the inadequate tools and limited research ability in many developing countries, the abundant Agro-wastes (e.g., plantain peels) have challenges for their disposal and reutilization. The overall goal of this project is to enhance and maximize the value of Agro-waste (i.e., plantain peels) through the recovery of useful components like biopolymers (i.e., pectin) for transformation into biodegradable films.

The objectives and hypotheses for each part of research are:

1. to investigate green extraction of pectin from plantain peels using enzymes.
    - Hypothesis: pectin can be extracted from plantain peels successfully and efficiently by cellulase via enzymatic hydrolysis of plantain peel cell walls.
2. to fabricate plantain peel pectin films and reinforce the films by adding hydrophobic beeswax solid lipid nanoparticles into the films, as well as evaluate the effect of the addition on the structure and properties of pectin based composite films.
    - Hypothesis: plantain peel pectin can be made into films and beeswax solid lipid nanoparticles can improve the mechanical properties and water vapor barrier properties of pectin based composite films.

CHAPTER I

LITERATURE REVIEW

1.1. Plantain and plantain peels

Plantain, as a major source of carbohydrates, is an important staple crop for millions of people in tropical areas. Plantain peel, as the major waste from plantain processing and utilization, contributes to a large amount of Agro-waste in developing countries. The peels are discarded after utilizing the starchy flesh, creating environmental problems during disposal.

1.1.1. Origin and yield

Plantain, originated in Southeast Asia, is an herbaceous plant from the banana family *Musacae* and genus *Musa*, which consists of 30–40 species and more than 700 varieties (Salleh et al., 2016). The FAO and the International Institute of Tropical Agriculture (IITA) use 'plantain' to refer to the *Musa paradisiaca* species, which is starchier and cooked before eating, generally denoted as cooking banana (FAOSTAT, 2020b; IITA, 2020). Most modern edible banana species originated from two seeded wild species—*Musa accuminata* (A genome) and *Musa balbisiana* (B genome). Variation in degree of hybridization creates *Musa paradisiaca* cultivars. Generally, plantain belongs to triploid hybrids AAB group. There are two major types under the plantain subgroup—French plantain type and Horn plantain type. French plantain is a group, grown in different parts of India, Africa and Central America, with characterization of male axis, male flowers and bracts. Under the group, varieties can be classified according to plant size from giant, medium, to dwarf and color. Horn plantain type is characterized by the early degeneration of the male axis and flower parts, mainly produced in India, Africa, Central America, Philippines and the Pacific. This group can be further categorized as French horn, False horn, and True horn with

distinct plant size and color (Noyer et al., 2005; Robinson & Galán Saúco, 2011).

Plantain generally looks very similar to banana, but has longer length, thicker skin and contain more starch in the flesh. It constitutes the major source of carbohydrates for millions of people, especially in Africa, the Caribbean, Latin America, Asia and Pacific (IITA, 2020; Tchango Tchango et al., 1999). Plantain provides food security and income for small-scales farmers and the total global production was about 39,482,163 tons in 2018. Congo (12.0%), Ghana (10.6%), Cameroon (10.0%), Uganda (9.6%), Colombia (9.0%), Philippines (8.1%), Nigeria (7.8%), Peru (5.6%) are the leading producers of plantain (Fig 2.1). According to FAOSTAT, more than 90% of the plantains are consumed domestically (FAOSTAT, 2020a). Post-harvest losses of plantain, which mainly contain fruits with pathological or mechanical damage, discarded fruits, as well as stalk, leaves, and fruit peels waste, are heavy due to poor handling and transport conditions plus inadequate market routes (Hernández-Carmona et al., 2017; IITA, 2020). Around 30% of harvested plantains are wasted every year (FAOSTAT, 2020a). Plantains are usually processed into fried slices, used in the preparation of soups, traditional dishes, etc., leaving the residual peels as consumption waste (Hernández-Carmona et al., 2017) which accounts for approximately 30% of the fruit and is the main by-product of the plantain processing and utilization (Arun et al., 2015). Based on the total production of plantain, up to 12 million tons of plantain peel waste are produced annually.

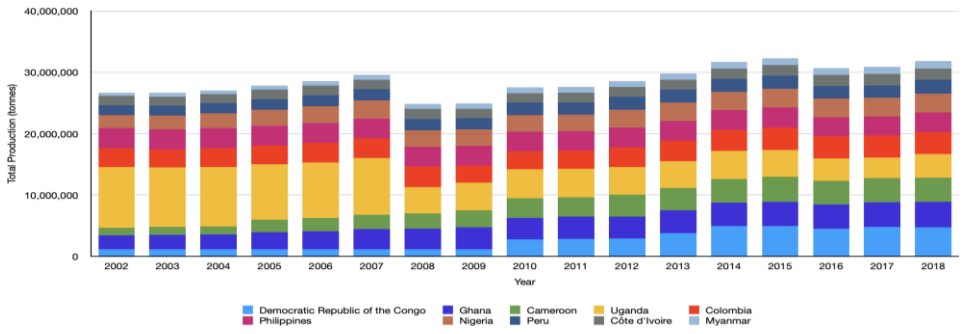

Fig 1.1: Production of plantain in top 10 countries from 2002–2018 (FAOSTAT, 2020a).

1.1.2. Principal components

The main composition of plantain peel waste includes biopolymers ranging from pectin, cellulose, hemicellulose, lignin, to fiber, and some low-molecular-weight molecules like phenolic compounds, minerals (e.g. potassium), vitamins (e.g. folic acid), etc. (Arun et al., 2015). It contains high moisture content (~87% (Hernández-Carmona et al., 2017)) which makes it susceptible to degradation before processing, affecting their handling, transportation, storage, and further application in biorefinery technologies (Pathak et al., 2016).

The percentage of different components of plantain peel have been reported in several studies on a dry matter basis. According to Ighodaro, plantain peel contained approximately 42.95%–48.18% of carbohydrate contents, 6.89%–7.18% crude protein, 17.59%–22.30% ash content, and 3.67%–6.22% crude fat content (Ighodaro, 2012). Another earlier report showed that plantain peel from six varieties contained 40%–50% of total dietary fiber, 8%–11% of protein, 2.2%–10.9% fat, and 32.4%–39.3% starch. (Happi Emaga et al., 2007). Based on other researches, plantain peel had 29.6% of starch (Hernández-Carmona et al., 2017), 8.5% of pectin (Arun et al., 2015), 7.1% of cellulose, 4.5% of hemicellulose and 12% of lignin (Emaga et al., 2011). Variation in the content exists due to different species, seasonal change, maturity state, geographical location and climatic condition (Arun et al., 2015).

1.1.3. Current usage

Plantain peels in most cases, are disposed in municipal landfill as agricultural waste or composted into fertilizer. The degradation takes time and occupies space. The peel contains large quantities of nitrogen and phosphorus with high water content which makes it susceptible to modification by microorganism, creating environmental problems like the production of

greenhouse-gases, water contamination, etc. (Tchobanoglous et al., 1993). Another more environmentally friendly way of disposing is to process the peel into animal feed. The increasing demand for livestock products has elicited a high demand for feed. In 1991, FAO emphasized the usefulness of multiple parts of plantain as animal feed, including peels which contains various nutrients (Machin et al., 1992). Plantain peel waste, with relatively lower cost, various nutrient content and good availability at local market, was evaluated and proved to be suitable in animal meals like poultry diet (Diarra, 2018). One study successfully examined the performance of plantain peel as a corn maize substitutes for African catfish (Agbabiaka & Okorie, 2013). Other studies concluded that plantain peel could be used as a replacement for wheat offal or maize in broiler production without adverse effect (Aderemi et al., 2016; Uchegbu et al., 2017).

On the other hand, the traditional disposal methods limit the commercial value of plantain peel. In recent years, plantain peels were investigated as potential raw material to produce value–added products like flour (Gutiérrez, 2018). One study found plantain peel flour had the potential to serve as a source of dietary fiber and antioxidant compounds (Agama-Acevedo et al., 2016) while another study developed functional cookies with health benefits from plantain peel flour. (Arun et al., 2015). Blend of plantain peel with cocao pod husk produced a novel green heterogeneous catalyst for biodiesel synthesis (Olatundun et al., 2020). The ash component has been utilized to make biochar with higher heating values (Adekola et al., 2019; Ogunjobi & Lajide, 2015). Plantain peel has also been used to generate high yield of bioethanol (Alonso-Gómez et al., 2020; Parra-Ramírez et al., 2019), or been used as good source of starch which has various application in food (as thickener), polymer industries, water treatment (as coagulant), etc. (Hernández-Carmona et al., 2017).

Another usage of plantain peel is to produce pectin (Akusu & Chibor, 2020; Happi Emaga

et al., 2008; Obele et al., 2019; Oyawaluja et al., 2020). Current production of pectin is mainly based on citrus peel and apple pomace. Some studies investigated the extraction of pectin from banana peels but only few could be found on plantain peels. Considering the large quantity of waste from this commodity, and usefulness of pectin in a plethora of industries, it is worth the effort to review the literature for potential industrial applications.

1.2. Pectin

1.2.1. Structure & properties

Pectin, a complex heteropolysaccharide, is the main multifunctional component of the plant cell wall and is usually associated with other cell wall components like cellulose, lignin or polyphenols (Noreen et al., 2017). The main composition of pectin is α–(1→4)–linked galacturonic acid (GalA) units backbone, which usually accounts for 70%. The carboxyl group of uronic acid residues can present either in the free form or as a salt with sodium, calcium or other small counterions. Depending on the extraction method or pectin source, they can also present as naturally esterified groups (Mohnen, 2008). Pectin's structure can be changed during storage, extraction and processing, affecting its functionalities. Three main polysaccharide domains can be found in pectin: homogalacturonan (HGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (Debra Mohnen, 2008; Munarin et al., 2012).

The degree of esterification (DE) or degree of methoxylation (DM), defined as the percentage of carboxyl groups esterified in the structure of pectin, is an important parameter to classify the type of pectin. If more than 50% of carboxyl groups are methylated, the pectin is regarded as high methoxyl pectin (HMP); less than 50% of DE is low methoxyl pectin (LMP) (Atmodjo et al., 2013; BeMiller, 1986). Generally, when DE increases, the gelation rate improves

and water solubility of the polymer decreases due to the hydrophobicity of the long hydrocarbon chains with esters (Sañudo Barajas et al., 2014). The amount and composition of neutral sugars and the overall molecular weight influence pectin's rheological properties as well (Morales-Contreras et al., 2018).

Pectin is soluble in water. When polymer molecules interactions reach a certain extent, they can form a network that traps solvent and solutes which is called gelation. The gelation mechanism of pectin is mainly dominated by DE. HMP forms gel when sufficient sugar and acid are present while LMP gels in the presence of divalent cations (e.g. calcium ion is used in food application) that provide cross bridges. Since LMP does not require sugar for gelation, it is usually used to make low-sugar jams, jellies and marmalades (Bagal-Kestwal et al., 2019). On the other hand, pectin precipitates as a solid gel when treated with dehydrating agent like alcohol. The negative charge of pectin depends on the number of free carboxyl group which is also responsible for their precipitation (Bonner, 1936)

1.2.2. Current applications of pectin

In the food industry, pectin is traditionally marketed as a gelling agent or thickening agent. It is a hydrocolloid which can form networks to trap water and form gels at low concentrations (Abid et al., 2017; Chan et al., 2016; Chan et al., 2017). New applications of pectin continue to emerge like as a colloidal stabilizer (Fei et al., 2020), texturizer (Abe-Inge et al., 2020), emulsifier (Yang, Nisar, Hou, et al., 2018), edible coating to extend food's shelf–life (Arantzazu et al., 2015), micro and nano-encapsulating material for controlled release of active compounds with specific functionalities (Picot-Allain et al., 2020), and biosensors for food safety control (Anjali et al., 2022). Because of the outstanding availability and biocompatibility, pectin-based biodegradable

films incorporated with other biopolymers or active compounds have gathered a lot of research interest in the field of food packaging. H. Gao et al. reported that pectin based films integrated chitosan and tea polyphenols showed remarkable antioxidant and antiseptic properties which retarded the undesirable color change of fresh beef during 8 days storage (H. Gao et al., 2019). Rodsamran & Sothornvit found the bioactive pectin film retarded soybean oil oxidation during 30 days of storage (Rodsamran & Sothornvit, 2019a).

Non-food applications of pectin range from medical, pharmaceutical, to the cosmetic industry. In the pharmaceutical industry, pectin is used in the formulation of drug for oral delivery, including tablets, gels, hydrogels, aerogels, etc. (Chomto & Nunthanid, 2017). The specific pH characteristic and degradability by colonic enzyme of pectin make it suitable to deliver antitumor drug for colon disease (Khotimchenko, 2020). Kodoth et al. concluded that pectin based silver nanocomposite film was efficient in Donepezil drug delivery (Kodoth et al., 2019). In addition to those, pectin can also be used in cosmetic and personal care products like shampoos, lotions, makeup foundations, hair conditioners due to its thickening and stabilizing properties (Bagal-Kestwal et al., 2019).

In 2015, the global pectin market was estimated as 958 USD million and is expected to grow at the annual growth rate of 7.3% from 2018 to 2023 because of the growing demand for natural products (IndustryExperts, 2019).

1.2.3. Sources

Due to the high utilization potential of pectin biopolymers, the extraction of pectin has been widely studied in various biomass. The most studied one is citrus peel including orange, pomelo, lime, grapefruit, and commercial pectin is also made from citrus peels attributing to their good

properties and high extraction yields (Duwee et al., 2022; Hilali et al., 2019; Hosseini et al., 2019; Jin & Yang, 2020; Liew et al., 2019; Rahmani et al., 2020; Rodsamran & Sothornvit, 2019b; Su et al., 2019). Other Agro-waste sources have also been proposed in last few years, including cocao pod husk (Kley Valladares-Diestra et al., 2022; Pangestu et al., 2020), mango peel (Chaiwarit et al., 2020; Wongkaew et al., 2020), tomato processing waste (Lasunon & Sengkhamparn, 2022; Sengar et al., 2020), walnut processing waste (Asgari et al., 2020a, 2020b), almond hulls waste (Najari et al., 2022), passion fruit (Freitas et al., 2020; Lin et al., 2021), durian rind (Hasem et al., 2019). Several studies have been reported on banana peels as well (Arias et al., 2021; Maneerat et al., 2017; Oliveira et al., 2016; Pagarra et al., 2019b). To the best of our knowledge, there has been only a few reports on the extraction of pectin from plantain peel (Arun et al., 2015; Obele et al., 2019).

1.2.4. Pectin obtained from plantain peels

1.2.4.1. Extraction of pectin from plantain peels

In the literature, very limited references could be found for pectin extraction from plantain peels (Table. 1.1) and all of them applied the conventional hydrothermal method. Happi Emaga et al. applied a three-steps sequential extraction method with extraction medium comprised of water, chelating agent, and hydrochloric acid and found ammonium oxalate was more suitable for pectin extraction from plantain peel (French Clair) than acid. The pectin yield (dry weight basis (dwb)) ranged from 8.9% to 14.6% at different stages of maturation of the plantain peel, and characterized as LMP with 14.2–30.6% DE (Happi Emaga et al., 2008). In another research, Oyawaluja et al. reported pectin yield (dwb) of 4.65% to 6.91% via sulfuric acid extraction (Oyawaluja et al., 2020). The study of Obele et al. extracted 5.07% (dwb) of HMP with 59.52% of DE from ripe plantain

peel via mineral acid extraction (Obele et al., 2019). Arun et al. applied sulfuric acid extraction and yielded 8.5% of pectin in dry basis. (Arun et al., 2015).

Table. 1.1: Literature summary of pectin yield extracted from plantain peels

Sample	Extraction Method	Pectin Yield*	Reference
Unripe PP	sequential	8.9%	(Happi Emaga et al., 2008)
Ripe PP	hydrothermal	14.6%	
Over-ripe PP		11.8%	
PP	conventional hydrothermal	8.5%	(Arun et al., 2015)
Ripe PP	conventional hydrothermal	5.04%	(Obele et al., 2019)
Unripe PP	conventional	4.65%	(Oyawaluja et al., 2020)
Ripe PP	hydrothermal	6.61%	
Over-ripe PP		6.91%	

PP: plantain peel; * pectin yield was calculated on dry weight basis

1.2.4.2. Effect of maturity stage of plantain on pectin content

Stage of maturity can affect the plantain pectin's content (Table. 1.1). In Oyawaluja et al.'s study, plantain peel at overripe stage had the highest pectin yield (6.91%) while ripe plantain peel contained 6.61% pectin, of which the two yields had no obvious difference between each other but were significantly higher than that of unripe plantain peel (4.65%) (Oyawaluja et al., 2020). In Happi Emaga et al.'s study, French Clair plantain peel had the highest pectin yield at stage 5 of maturity which was defined as more yellow than green plantain peel and similar to the 'ripe stage' in Oyawaluja et al.'s study (Happi Emaga et al., 2008). In another maturation effect study on plantain peels conducted by Happi Emaga et al. in 2007, it was found that the concentration of soluble dietary fiber (SDF) reached the highest level at stage 7 of maturation in French Clair

plantain and Big Ebanga plantain. Stage 7 was defined as yellow with a few brown spots for the peel, similar to overripe stage. Another uncommon type Pelipita (ABB species) plantain peels presented highest SDF concentration at stage 5 (Happi Emaga et al., 2007).

1.3. Pectin extraction

In this part, different methods of pectin extraction from different Agro-waste will be summarized. Normally, extraction of pectin is governed by mass transfer into the extraction solvents. The method can be evaluated or assessed based on the yield and the quality of the pectin produced. Various methods, including conventional hydrothermal method, and innovative techniques utilizing ultrasound, microwave, enzyme, cavitation, etc., have been proposed in different studies. Bearing in mind the idea of sustainable development, recent research focus has shifted to green techniques. The conventional hydrothermal extraction usually requires longer extraction time, higher energy, and the use of strong mineral acids which produce acidic waste requiring stringent disposal methods, thus, is unfavorable. Methods that require fewer chemical solvents can be regarded as green. Based on this concept, the extraction technologies can be classified into conventional hydrothermal methods, green single extraction methods (ultrasound-assisted, microwave-assisted, hydrodynamic cavitation, subcritical water extraction, enzymatic method, etc.), and green hybrid extraction methods (ultrasound assisted enzymatic extraction, ultrasound assisted microwave extraction, ultrasound assisted ohmic heating extraction, etc.).

1.3.1. Conventional hydrothermal methods

In the conventional method, pectin is usually extracted in acidic condition using water as solvent. The acid hydrolyzes the plant cell wall to release water-soluble pectin from the network

during extraction. The pH of the solution usually ranges from 1.3–3.0 and the extraction is conducted at high temperature (70 °C–100 °C) for 60–300 min. Different factors like the extraction pH, temperature, time, selection of acidic solvent, solid to liquid ratio, etc., can all affect the yield and quality of the pectin produced (Table. 1.2) (Hasem et al., 2019; Liew et al., 2019; Maneerat et al., 2017; Muñoz-Almagro et al., 2019; Oliveira et al., 2016; Pagarra et al., 2019a). The use of mineral acids, like hydrochloric acid, sulfuric acid, nitric acid is usually associated with environmental pollution requiring disposal or equipment corrosion (Sabater et al., 2018). More environmentally friendly organic acids like citric acids have been widely applied. One study indicated that extraction with citric acids at 85°C produced apple pectin with high molecular weight, apparent viscosity, and high DE compared with other organic acids (i.e., tartaric acid and malic acid). The pectin yields were 6.2%, 5.4%, 5.3% for tartaric, malic, citric acid, respectively, compared with the yield 6.4% for hydrochloric acid, implying the potential of organic acid to replace mineral acid in hydrothermal extraction of pectin (Cho et al., 2019).

Table. 1.2: Conditions for different hydrothermal methods to extract pectin from agro-wastes

Raw Material	Temperature (°C)	Time (min)	pH	Extraction medium	Reference
Durian Rind	85	60	pH 2.5	HCl	(Hasem et al., 2019)
Banana Peel	90	30–120	pH 1.5	Citric acid	(Maneerat et al., 2017)
Cocao pod husk	95	95	pH 3.0	Citric acid	(Muñoz-Almagro et al., 2019)
Pomelo Peel	88	140	pH 1.8	Citric acid	(Liew et al., 2019)
Banana Peel	70–90	120–240	pH 2.5–4.5	Citric acid	(Oliveira et al., 2016)
Banana Peel	60–100	90	pH 1.3–2.7	HCl	(Pagarra et al., 2019a)

1.3.2. Green single extraction methods

The emerging green single extraction methods can be classified into 3 categories based on

the extraction medium used, i.e., chemical, water, and enzymatic. These green methods either decreased the quantity of chemicals used, or shortened the time of extraction compared with conventional methods by increasing mass-transfer, heat transfer, etc.

1.3.2.1. Chemical medium

Several methods utilize physical assistance to enhance the extraction efficiency. Similar to conventional methods, all these techniques still use the chemical solvent (i.e., commonly used acids) as extraction medium, but in lower quantity or shorter extraction time.

Ultrasound-assisted extraction (UAE) applies ultrasonic waves ranging from 20 kHz–100 kHz in pectin extraction. The pressure fluctuations of acoustic waves generates microbubbles which collapse to form microjets to disrupt the cellular structures and enhancesolvent penetration and mass transfer kinetics (Picot-Allain et al., 2020). Guandalini et al. applied ultrasound for 10 min at 85 °C yielded higher pectin content as 8.1% from mango peel waste as compared to 5.4% with conventional extraction (85 °C for 30 min) while maintaining the quality (i.e., no significant effect on purity and DE) (Guandalini et al., 2019). Such method has also been successfully applied on other Agro-waste like orange peel (Hosseini et al., 2019), tomato processing waste (Sengar et al., 2020), and walnut green husk (Asgari et al., 2020a).

Microwave-assisted extraction (MAE) utilizes microwave field to promote energy release and generate higher amount of heat to the dielectric material (solvent-sample matrix), which enhances the diffusion rate, therefore, improves the extraction yield (Picot-Allain et al., 2020; Su et al., 2019). It was observed in Sengar et al.'s study, that as the microwave power increased from 540 W to 900 W with extraction time within 5 min, the pectin yield extracted from tomato peel increased from 20.83% to 25.42% (Sengar et al., 2020). Similar result was observed in Asgari et

al.'s study, the pectin yield dramatically increased as the microwave power elevated from 300W to 500W in 3 min extraction (Asgari et al., 2020b). Similar to ultrasound-assisted extraction, microwave-assisted extraction also uses shorter processing time and less solvent to produce high yield of pectin, that preserved or improved the quality compared with conventional method (Su et al., 2019). For example, Rahmani et al. reported that extracted pectin via microwave-assisted method under optimal conditions (i.e., 700 W and 3 min) was high in GalA (60%), high in molecular weight and presented good emulsifying property and antioxidant activity (Rahmani et al., 2020). Similarly, microwave assisted extraction produced higher pectin yield from tomato processing waste than ultrasound assisted extraction (25.42% compared with 15.21%) with higher energy requirement (Sengar et al., 2020).

Ohmic heating extraction (OHE) is an alternative fast method that applies uniform heating which is generated when alternating electrical current is passed through the matrix with electrical resistance. Based on Saberian et al.'s research, ohmic heating assisted extraction at 90 °C could produce higher pectin yield than conventional method due to the more intensive rupturing of the cell wall, and the extracted pectin presented good emulsifying activity and stability (Saberian et al., 2017). However, compared with ultrasound and microwave assisted methods (25.42% and 15.21% respectively), pectin yield with ohmic heating method was a bit lower (10.65%) (Sengar et al., 2020).

1.3.2.2. Water medium

Subcritical water extraction (SWE) applies high-pressure liquid water as a solvent. The high pressure brings the solvent to higher temperature without phase change. During the process, the temperature of water is within the range of boiling point at 1 atm (100°C) to the critical point

(374°C). This method has the advantage of time-saving and sustainability with zero pollution as no chemical is used (Freitas et al., 2020). Muñoz-Almagro et al. found that SWE produced higher yield of pectin with greater purity as compared with conventional extraction with citric acid (Muñoz-Almagro et al., 2019). Liew et al. made comparison among conventional extraction, SWE, ultrasound-microwave-assisted extraction, and found that SWE was the suitable method to produce LMP in a single step instead of having extra processing procedures used in traditional industrial production which firstly extracted HMP before conversion to LMP. However, the method produced pectin with relatively lower molecular weight (low quality) with amorphous structure, using the most energy which might limit its application (Liew et al., 2018; Liew et al., 2019).

Hydrodynamic cavitation extraction (HCE) is initiated by pumping a liquid through one or more constrictions with suitable geometry like venturi tubes or orifice plates to create microbubbles. The bubbles subsequently collapse under pressure and create localized hot spots with extremely high energy density. Similar to SWE, water is used during hydrodynamic cavitation as solvent. Meneguzzo et al. reported that hydrodynamic cavitation successfully extracted high quality (high molecular weight) pectin in semi-industry scale, implying its potential for use in industrial operations (Meneguzzo et al., 2019).

1.3.2.3. Enzymatic medium

Enzymatic extraction (EE) of pectin utilizes enzymes with specificity and selectivity to disrupt the plant cell wall and help the release of pectin. Advantages of this method includes mild extraction conditions, lower energy cost, higher pectin quality, and no usage of corrosive acids (Picot-Allain et al., 2020). In one study, the pectin extracted from artichoke by-product with commercial Celluclast enzyme solution presented high GalA content with good yield (17.5%, dwb)

under optimal conditions (Sabater et al., 2018). Dranca and Oroian found that during enzymatic extraction, the pectin yield was strongly influenced by enzyme dose while the temperature and extraction time had little or no effect. Despite that, prolonged extraction would result in pectin with higher DE. It was highlighted in the study that enzymatic method successfully met the parameters for industrialization, i.e., less or no effect on the quality of pectin, mild extraction environment, sustainable extraction process, less energy and capital cost, and no equipment corrosion (Dranca & Oroian, 2019).

1.3.3. Green hybrid extraction methods

A hybrid of different techniques, taking advantages of distinct features, is of recent research interest which include ultrasound assisted microwave extraction (UAME), ultrasound assisted ohmic heating extraction (UAOH), and ultrasound assisted enzymatic extraction (UAEE). According to Sengar et al., UAME presented highest dissolution rate, and produced pectin with lower degradation (i.e., better quality), higher GalA (i.e., better purity), high DE% and clearer color compared to UAOH and UAE extractions (Sengar et al., 2020). Another study found pectin extracted via UAEE method had high GalA content and a well-preserved molecule chain, and UAEE presented high potential for industrial production of high quality pectin (Yang, Wang, et al., 2018). Hybrid methods generally produce higher pectin yields with better quality than each single method as it combines the advantage of every method and optimizes the extraction procedure.

Table. 1.3: Summary of green extraction methods

Sample	Method	Temperature (°C)	Time (min)	Other Variables	Extraction medium	Reference
Tomato processing	UAE	60	9	pH 1.5, 600 W	HCl	(Sengar et al., 2020)

waste						
Tomato processing waste	MAE	89	3	pH1.5, 900 W	HCl	(Sengar et al., 2020)
Tomato processing waste	OHE	81	5	pH 1.5, 60 V	HCl	(Sengar et al., 2020)
Tomato processing waste	UAME	85	8	pH 1.5 450–540 W	HCl	(Sengar et al., 2020)
Tomato processing waste	UAOH	69	10	pH 1.5 450 W (UA) 60 V (OH)	HCl	(Sengar et al., 2020)
Walnut green husk	UAE	N/A	10	pH 1.5, 200 W	Citric acid	(Asgari et al., 2020a)
Sour orange peel	UAE	< 30	10	pH 1.5, 150 W	Citric acid	(Hosseini et al., 2019)
Mango peel	UAE	85	10	pH 2.0, 500 W	Nitric acid	(Guandalini et al., 2019)
Mango peel	MAE	N/A	20	pH 1.5, 500 W	1.0M HCl	(Chaiwarit et al., 2020)
Walnut green husk	MAE	N/A	3	pH 1.5, 500 W	HCl, H_2SO_4, citric acid	(Asgari et al., 2020b)
Orange peel	MAE	N/A	7	pH 1.2, 400 W	HCl	(Su et al., 2019)
Sweet lemon peel	MAE	N/A	3	pH 1.5, 700 W	Citric acid, HCl	(Rahmani et al., 2020)
Orange juice waste	OHE	90	30	pH 2, 75 V	HCl	(Saberian et al., 2017)
Cacao pod husk	SWE	121	30	103 bar	Water	(Muñoz-Almagro et al., 2019)
Pomelo peel	SWE	120	N/A	30 bar	Water	(Liew et al., 2018)
Orange peel	HCE	15 – 95	270	N/A	Water	(Meneguzzo et al., 2019)
Sisal Waste	EE	50	1200	pH 4, 88 U/g	Celluclast 1.5 L	(Yang, Wang, et al., 2018)
Sisal Waste	UAEE	50 – 70	1260	pH 4, 88 U/g, 450 W	Celluclast 1.5 L, HCl, ammonium oxalate	(Yang, Wang, et al., 2018)
Artichoke by-product	EE	50	1632	pH 5, 10 U/g	Celluclast 1.5 L	(Sabater et al., 2018)

Apple pomace	EE	48	1094	pH 4.5, 43 μL/g	Celluclast 1.5 L	(Dranca & Oroian, 2019)	

1.3.4. Summary

The innovation of green extraction techniques offers new possibility for better utilizing Agro-waste and pursuing sustainable development goals. Methods like ultrasound-assisted extraction, microwave-assisted extraction, subcritical water extraction, etc., have relative high capital cost than conventional methods at the beginning of the production. But they can produce pectin with higher yield and better quality at less processing time, which makes the unit cost for production lower for long-term consideration. On the other hand, for small companies that want to reduce the capital cost, enzymatic extraction could be their method of choice considering its high yield and low cost as lesser or no sophisticated instruments are needed.

1.3.5. Current methods on extraction of pectin from plantain peels

As mentioned in section 1.3.4.1., limited information could be found in the literature for pectin extraction from plantain peels and all these studies used hydrothermal extraction (Table. 1.4). The highest pectin yield (14.6%, dwb) was achieved using the three-steps sequential hydrothermal extraction method. The production of pectin was more complex than normal hydrothermal extraction but extracted more pectin (Happi Emaga et al., 2008). The remaining methods all applied conventional hydrothermal methods utilizing strong mineral acids as solvent which were non-sustainable and could lead to environmental problem during chemical waste disposal and equipment corrosion in long-term usage. Furthermore, the pectin yield and quality were not optimized using those methods (Arun et al., 2015; Obele et al., 2019; Oyawaluja et al., 2020). Investigation on green extraction of pectin from plantain peel is yet to be established.

Table. 1.4: Summary of pectin extraction methods in plantain peels

Sample	Method	Temperature (°C)	Time (min)	Other Variables	Pectin yield / properties	Extraction medium	Reference
Ripe PP	sequential hydrothermal	60–80	180	N/A	14.6% LMP	0.5% ammonium oxalate, 0.05M HCl	(Happi Emaga et al., 2008)
Ripe PP	conventional hydrothermal	88.7	60	pH 4	6.61%	Sulfuric acid	(Oyawaluja et al., 2020)
Ripe PP	conventional hydrothermal	40	30	pH 1.5	5.04% HMP	0.5% HCl	(Obele et al., 2019)
PP	conventional hydrothermal	85	60	pH 1.5	8.5%	1M sulfuric acid	(Arun et al., 2015)

PP: plantain peel

1.4. Pectin-based films

1.4.1. Pectin films

Pectin-based film is one of the innovative applications of pectin in the food industry based on its biodegradability, biocompatibility, edibility, gelation properties, and selective gas permeability. On the other hand, the low mechanical properties and poor moisture resistance limit its function as a film (Šešlija et al., 2018). Thus, vast studies have focused on the enhancement of pectin film by integrating pectin with other ingredients or functional additives to improve their functionality. Based on the incorporated materials, the pectin-based composite films can be categorized into pectin-protein films (Chakravartula et al., 2019), pectin-lipid films (Chalapud et al., 2020; Manrich et al., 2017), pectin-polysaccharides films (Akhter et al., 2019; H. Gao et al., 2019), and pectin-active additives films (Table. 1.5) (Almasi et al., 2020; Chiarappa et al., 2018).

1.4.2. Pectin-based composite films

Addition of natural polysaccharides can improve the physical and mechanical properties of

pectin-based films. Šešlija et al. stated in their study that carboxymethyl cellulose (CMC) improved the tensile strength and elongation at break of pectin films by increasing the intemolecular forces and forming a denser chain packing. Based on thermo-gravimetric analyses (TGA), it was inferred that the strong physical interactions between polar groups of pectin and CMC enhanced thermal stability of the film (Šešlija et al., 2018). Multiple studies utilized chitosan, usually together with other active additives (e.g., polyphenols, essential oils) to form a continuous delivery system within the film which has special functionality (e.g., antioxidant, antimicrobial) (Akhter et al., 2019; H. Gao et al., 2019; Yeddes et al., 2020). Incorporation of alginate into pectin film was shown to increase the thermal, mechanical and gas barrier properties (Chakravartula et al., 2019).

Regarding addition of protein, whey protein is the most used. Whey protein can impact physico-chemical, rheological, optical, and water permeability of the pectin films. Multiple studies proved that increasing whey protein concentration incorporated into the films decreased their water vapor permeability, while preserving the hydrophilic character of the surface, making it suitable for food wrapping applications (Chakravartula et al., 2019; Oliveira et al., 2021). However, Chakravartula et al. reported that whey protein incorporated films showed higher opacity, lower gas barrier values, denser structure, and were easy to form aggregates (Chakravartula et al., 2019).

Incorporation of lipids can improve the water resistance of the film. The effect of sunflower wax, candelilla wax and cutin on performance of pectin-based film has been studied. Chalapud et al. found sunflower wax increased the water resistance (i.e., lower swelling index and water absorption) significantly and distributed evenly within the film network. The film presented satisfactory properties as an alternative to conventional food packaging materials (Chalapud et al., 2020). Manrich et al. investigated the effect of tomato cutin on the pectin-based films which

produced pectin film with uniform hydrophobic surface resulting from the lipophilic effect of cutin and greater stiffness, showing potential to be used as a water-resistant plastic wraps (Manrich et al., 2017). Like other lipids, candelilla wax also imparted good water vapor barrier properties to the pectin films. As suggested by Lozano-Grande et al., candelilla-pectin film exhibited good water vapor barrier properties, and was successfully applied in postharvest conservation for oyster mushroom to extend the shelf-life of the product (Lozano-Grande et al., 2016).

Various active additives have also been incorporated into pectin film to provide specific functionalities. A new antioxidant delivery system based on adding ascorbic acid into LMP formed a consistent and homogeneous pectin composite film in which calcium chloride was added as a crosslinking agent (Chiarappa et al., 2018). H. Gao et al. reported that incorporation of chitosan into pectin films together with cross-linking agent (calcium ion) and tea polyphenols successfully generated a network structure with remarkable slow-release effect for tea polyphenols which possessed excellent free-radical scavenging capacity to prevent food spoilage and to inhibit color deterioration of meat (H. Gao et al., 2019).

Essential oil is another popular type of active additives. Examples include marjoram, mint, and rosemary essential oils. Almasi et al. investigated the marjoram essential oil (MEO) additives and found that the encapsulation of MEO with pickering emulsions provided slow-release profile for pectin-based films. The functional film exhibited good antioxidant and antimicrobial properties which provided an innovative active food packaging system to enhance the quality and shelf-life of food (Almasi et al., 2020). In Yeddes et al.'s study, rosemary essential oil (REO) incorporated in chitosan-gelatin-pectin composite films had higher inhibition property against the activity of bacteria *Bacillus subtilis, Staphylococcus aureus, Enterococcus aerogenes, Enterococcus faecalis* and *Escherichia coli* to better protect the packaged food. Moreover, the optimized

composite film presented good mechanical properties, color property, water vapor barrier, and resistance to ultraviolet light (Yeddes et al., 2020). Akhter et al. added REO and mint essential oils, nisin and lactic acid in inexpensive chitosan-starch-pectin composite films which significantly improved the water barrier properties, tensile strength, antioxidant properties, and thermal stability of the films. Furthermore, Fourier-transform infrared spectroscopy (FTIR) analysis revealed that addition of these active additives increased the microstructural heterogeneity and opacity (Akhter et al., 2019). Active additives also endow the pectin-based films with attributes of intelligent packaging. Red cabbage extract incorporated into HMP based film had high sensitivity for gaseous amines with an apparent colorimetric change (purple to yellow upon exposure) at low concentrations (1 ppm). This intelligent film could successfully identify degraded fishery and meat samples as the colorimetric change due to the increasing concentration of total volatile basic nitrogen (key indicator of spoilage) corresponded to the results measured by standard methods (Dudnyk et al., 2018)

1.4.3. Film making methods

1.4.3.1. Conventional method—casting

Based on the Table. 1.5, we can see casting is the most frequently used technique to form films. Pectin solution (usually around 2–3% by weight) is mixed with appropriate amount of plasticizer (e.g., glycerol) and stirred until complete dissolution. Then the film forming solution is spread on a mold and dried under controlled temperature and humidity to form a thin film. The drying process usually takes 1–2 days (Akhter et al., 2019; Dudnyk et al., 2018; H. Gao et al., 2019). Despite the simplicity of the procedure, the process is time consuming. Problems like shrinkage or break due to unstable loss of moisture or fluctuation of temperature may occur during

drying. Thus, getting a flat and intact film via casting is not easy.

1.4.3.2. New method: Thermo-compression molding

Considering the drawbacks of conventional casting method, a new approach was developed. In Gouveia et al.'s study, thermos-compression molding was applied to produce thermoplastic pectin films which had desirable visual property with semi-transparent appearance and was suggested as potential alternative for plastics to be used in packaging dry/low moist food products. During thermos-compression molding, appropriate amount of film-formulation was placed inside a circular mold which was placed in between two stainless steel plates covered with aluminum foils. The mold was then removed, allowing the molded paste to be thermos-compressed with hydraulic press. The hot film produced was cooled down to the room temperature before being removed. A temperature of 120 °C with compression load of 196.1kN for 20–25 min compression time was used to generate the film (Gouveia et al., 2019). Application of such method reduces the film forming time and can produce uniform films with desirable characteristics, but the high capital cost of the machine makes it less suitable for small-size businesses.

Table. 1.5: Summary of pectin composite films incorporated with different ingredients

Category	**Polymer Matrix**	**Additive**	**Film formation**	**Application**	**Reference**
Polysaccharides & Active additives	Pectin / chitosan	Tea polyphenols	Casting	Antioxidant, prevent food spoilage	(H. Gao et al., 2019)
Active additives	HMP	Marjoram essential oil	Casting	Antioxidant	(Almasi et al., 2020)
Polysaccharides & protein &	Pectin / gelatin / chitosan	Rosemary essential oil	Casting	Antibacterial property, enhance shelf–life	(Yeddes et al., 2020)

active additives					
Lipid	LMP / sunflower waxes	N/A	Casting	Satisfactory properties for food packaging material	(Chalapud et al., 2020)
Polysaccharides & protein	Pectin / alginate / whey protein	N/A	Casting	Edible film, improve shelf life for bakery food	(Chakravartula et al., 2019)
Polysaccharides, active additives	Pectin / chitosan / starch	Rosemary, mint essential oil, nisin, lactic acid	Casting	Antioxidant, antimicrobial, extend shelf–life	(Akhter et al., 2019)
Polysaccharides	HMP / carboxymethyl cellulose	N/A	Casting	Improved mechanism property, thermal stability	(Šešlija et al., 2018)
Protein	Pectin / Whey protein	N/A	Thermo–compression molding	Improve water barrier property	(Oliveira et al., 2021)
Lipid	LMP / cutin	N/A	Casting	Edible film, coating for fruits and vegetables, plastic wraps	(Manrich et al., 2017)
Lipid	LMP / candelilla wax	N/A	Casting	Good barrier to water vapor exchange, use in postharvest conservation of oyster mushroom	(Lozano-Grande et al., 2016)
Active additive	LMP	Ascorbic acid	Casting	Antioxidant	(Chiarappa et al., 2018)
Active additives	HMP	Red cabbage extract	Casting	Freshness indicator for meat & seafood as intelligent packaging	(Dudnyk et al., 2018)

1.5. Conclusion

To summarize, researchers and scientists have achieved outstanding success in the recovery of pectin from various Agro-waste materials via different extraction methods. By incorporating different ingredients, biodegradable films with desirable properties can be obtained as potential

substitutes for traditional plastics; thus, acting as a solution for the dilemma of waste disposal and white pollution, in consonance with the goal of sustainable development. The high volume of plantain peel waste produced in many developing countries is still disposed in the traditional way, threatening the environment. Pectin is the main carbohydrates in plantain peels but very limited studies about its extraction were found in the literature and only conventional extraction methods were investigated with drawbacks of non-sustainability, low yield, and poor quality. No studies were reported on green approaches to either recover pectin from plantain peels or strengthen the plantain peel based pectin films. Considering the high volume of waste amount, it is worthwhile to conduct research in this area to fill these knowledge gaps.

CHAPTER II

CONNECTING STATEMENT

Based on the previous review conducted in Chapter I, a knowledge gap regarding the green extraction of pectin from plantain peels was found. Plantain peel, as one of the most common Agro-waste in developing countries produced from plantain peel processing industry, is underutilized, and disposed via incineration, landfilling, composting, or feeding to animals. This chapter details the recovery of pectin from plantain peel using green approach, i.e., enzymatic extraction which was chosen due to its recyclability, mild extraction process, simplicity, and potential for industrial use. Various concentrations of enzymes were used to extract pectin and their effects on pectin yield and quality were assessed. The characteristics of plantain peel pectin were investigated. The extraction techniques were designed in a way that maximized the utilization of material and economic benefits and minimized any waste or pollutions that might be produced during the process. The success of this research can not only bring solution to deal with the bulk plantain peel waste, but also bring new inspiration to industrial production of pectin, helping achieve sustainable development.

CHAPTER II

RECOVERY OF PECTIN FROM PLANTAIN PEELS USING A GREEN APPROACH

2.1. Abstract

Pectin was successfully extracted from plantain peels via enzymatic extraction with cellulase at various concentrations (0–800 U) and analyzed for efficiency in terms of pectin yield (%) and quality (i.e., galacturonic acid (GalA)). The yield of pectin was significantly higher ($P < 0.05$) with cellulase treatment versus without cellulase, i.e., 12.04–13.38% on dry basis against 6.25%, respectively. Similar result was found for GalA content, i.e., 110.3–150.1 mg on dry basis versus 62.0mg. As the concentration of cellulase increased, the yield and GalA content was relatively steady, indicating that the amount of cellulase used had little effect on the yield of pectin. A level of 50 U of cellulase was found to produce the most quantity of pectin with highest GalA content. The study further revealed that plantain peel pectin was low methoxyl pectin and the cellulase treatment had no significant effect on degree of esterification (DE), and these results were confirmed with FTIR analysis.

2.2. Introduction

The growing global population has been associated with demands for increased food production. This trend has elicited increased by-products generation from food harvesting and processing in Agro-industry that pose concerns to society by way of the challenges to waste management and the harmful environmental impacts (Ranganathan et al., 2020). Agro-wastes are traditionally disposed via several routines like burning in air, composting, feeding to animals, or landfilling. Extensive burning has created harmful smokes and increased the ambient temperature;

composting and farming have created greenhouse gases; and wastes dumped in the environment has led to water and land pollution. All these conditions contribute significantly to global warming (Tsai et al., 2020; Zhang et al., 2015). Thus, strategies of innovative waste management to solve waste disposal problem have gathered considerable research interest. Recent studies have developed many innovative methods for Agro-waste reutilization by recovering biopolymers from waste and transforming them into high value-added products which has wide applications in food industry, biomaterials, and biomedicine.

Pectin is composed of linear chain of α–(1–4) linked D-galacturonic acid units as the backbone, and is a complex polysaccharide found in plant cell walls together with other polysaccharides (e.g., cellulose and hemicellulose) to form a strong biopolymer matrix (Castro e Silva et al., 2020; Noreen et al., 2017). Pectin has wide applications in the food industry as a gelling agent, thickening agent, stabilizer, emulsifier, edible coating, etc., due its bioavailability, gelation capacity, selective gas permeability, and edibility (Bagal-Kestwal et al., 2019; Fei et al., 2020; Yang, Nisar, Hou, et al., 2018). Commercial pectin is mainly extracted from citrus peels or apple pomace, via conventional hydrothermal methods which usually use strong mineral acids or weak organic acids and heat to release soluble pectin from plant cell wall material. Such methods are either environmentally damaging due to the pollution created during chemical disposal, or time consuming due to the lower rates of reactions (Cruces et al., 2021; Guo et al., 2021; Kumar et al., 2020). Thus, various green methods, like ultrasonic assisted extraction (Asgari et al., 2020a), ohmic heating assisted extraction (Saberian et al., 2017), enzymatic extraction (Yang, Wang, et al., 2018), etc., are widely studied these years aimed at either decreasing treatment time or the amount of chemicals used. Alternative agricultural waste other than citrus peel or apple pomace like cocoa pod husk (Muñoz-Almagro et al., 2019), mango peel (Wongkaew et al., 2020), tomato processing

waste (Sengar et al., 2020), etc. have also attracted a lot of attention as sources of pectin.

Plantain, a major source of carbohydrate for millions of people, is an important staple crops in tropical countries with total of about 39 million tons produced in 2018 worldwide. Plantain peels, account for about 30% of the plantain fruit and contribute to an estimated 12 million tons of waste per year (Arun et al., 2015; FAOSTAT, 2020a). Despite the fact that pectin is the major polysaccharides in plantain peels (Arun et al., 2015) and the waste quantity is enormous, very few studies were found in the literature on the extraction of pectin from plantain peels and the few reported all applied conventional methods (Emaga et al., 2008; Obele et al., 2019).

Enzymatic extraction is one of the green extraction method of pectin which utilizes the enzymes with specificity and selectivity to disrupt the plant cell wall and help release of pectin from the biomass (Picot-Allain et al., 2020). As reported by Sabater et al., the pectin extracted from artichoke by-product with commercial Celluclast enzyme preparation presented high GalA content and high yield (Sabater et al., 2018). It was highlighted in another study that enzymatic extraction successfully met the important conditions for industrial use, i.e., producing high quality pectin, sustainable extraction process, cheap enzyme preparation, mild reaction condition, equipment-friendly, and minimum pollution and waste (Dranca & Oroian, 2019). Considering the drawbacks of conventional extraction methods and the fact that green extraction method like enzymatic extraction is not yet established on pectin recovery from plantain peels, it was decided to conduct this study.

The main objectives of this study were to: (1) design a green approach to extract pectin from plantain peels using enzymes; (2) evaluate the effect of different concentrations of cellulase on pectin yield and purity; and (3) characterize the plantain peel pectin.

2.3. Materials and methods

2.3.1. Materials

Fresh plantains were obtained from a local market in Kumasi, Ghana. The plantains were washed with distilled water to remove dirt. The plantain peels were collected, cut into 2×2 cm pieces, and dried at 50 ℃ for 3 days until constant weight. The dried plantain peels were powdered and sieved with 1 μm mesh to obtain fine plantain peel powder.

Pectin from citrus peel (Galacturonic acid ≥ 74.0% at dried basis), cellulase (from *Aspergillus niger*), sulfuric acid (95–98%), dinitrosalicyclic acid were purchased from Sigma-Aldrich (Missouri, USA). Citric acid, sodium citrate dihydrate, sodium tetraborate, sodium potassium tartrate, hydrochloric acid, anhydrous ethanol and microcrystalline cellulose were purchased from Fisher Scientific (Geel, Belgium). Sulfamic acid (99%), and 3-phenylphenol (90%) were purchased from Acros Organics (Geel, Belgium). Sodium hydroxide from EM Science (Darmstadt, Germany), and D-galacturonic acid from (97%) from Alfa Aesar (Massachusetts, USA) were purchased.

2.3.2. Recovery of pectin from plantain peel

The plantain peel powder was subjected to pre-treatment and cellulase-assisted extraction under pre-determined conditions (see section 2.3.2.2) to obtain the pectin extract to which ethanol was added (see section 2.3.2.3) to precipitate pectin, followed by filtration, drying, and grinding to obtain the pectin powder. The whole recovery process as shown in Fig. 2.1, was designed to be green due to two improved processes. Firstly, cellulase enzyme in small amounts was used as an alternative to conventional chemicals in pectin extraction. Secondly, the ethanol used was recycled for reuse.

2.3.2.1. Determination of cellulase activity

Cellulase activity was measured by determining the amount of reducing sugars released via dinitrosalicylic acid (DNS) method with slight modifications (Miller, 1959; Sabater et al., 2018). Reaction mixture containing 1% (w/v) of cellulose and 0.5% (w/v) of cellulase enzyme in citrate buffer (pH 4.5) were mixed and incubated at 50 ℃ for 1 h under constant shaking at 200 rpm in a water bath (Precision Scientific, Chicago, Il, US). A standard curve was prepared using glucose. One unit (U) of cellulase activity was defined as the amount of enzyme required to release 1 mg of glucose at pH 4.5, 50 ^{o}C per h.

2.3.2.2. Cellulase-assisted extraction of pectin from plantain peel

An amount of 5 g plantain peel powder was soaked in 0.05 M citrate buffer (pH 4.5) for 48 h, and the mixture was boiled for 5 min to loosen the biopolymer network. Cellulase prepared in citrate buffer (pH 4.5) was added to the mixture at different concentrations (0, 25, 50, 75, 100, 200, 400, 600, and 800 U) with a solid-to-liquid ratio of 1:9 (w/v). The cellulase-assisted extraction was conducted at 50 ℃ for 18 h with constant shaking (200 rpm) in a water bath.

2.3.2.3. Ethanol-facilitated precipitation of pectin

After extraction, the mixture was cooled down to room temperature 25 ℃, filtered three times on a 4-folded cheese cloth, and centrifuged at 4000 rpm for 10 min (Centrifuge 5430, Eppendorf, Germany) to obtain the supernatant. Ethyl alcohol pre-cooled at 4 ℃ was added to the supernatant at a final concentration of 70% to precipitate pectin. The precipitation was performed at 4 ℃ for 10 h. Afterwards, the pectin precipitates were filtered, washed with 70% of ethyl alcohol for three times and filtered, and finally vacuum dried at 25 ℃ until constant weight. The yield of

pectin (%) extracted from the plantain peels was calculated using the Equation (1).

$$\text{Yield of pectin (\%) in dry basis} = \frac{\text{Weight of dried pectin (g)}}{\text{Weight of dried plantain peel powder (g)}} \times 100 \qquad (1)$$

2.3.2.4. Recovery of ethanol

The waste solution generated from the filtration in section 2.3.2.3 containing ethanol was collected for ethanol recovery. The liquid was firstly filtered using 0.22 μm membrane (Stericup vacuum filtration system, USA), and the ethanol in the filtrate was evaporated, condensed, and collected with a rotary evaporator with heating at 40 ℃ and spinning rate at 60 rpm. The alcohol recovery rate was measured to be 93.94 ± 2.26%. The recycled ethanol was re-used for further recoveries to achieve a greener and relatively cheaper process.

2.3.3. Characterization of recovered pectin

2.3.3.1. Determination of galacturonic acid content

Sulfamate/*m*-hydroxydiphenyl method was used to determine the galacturonic acid content of recovered pectin (Melton & Smith, 2001). In brief, four hundred (400) μL of 0.12 % (w/v) pectin solution was added with 40 μL of 4 M sulfamic acid/potassium sulfamate solution (pH 1.6), and 2.4 mL of 75 mM sodium tetraborate in sulfuric acid in a borosilicate glass tube. Next, the mixture was boiled for 20 min and cooled in an ice bath for 10 min. Reaction control was prepared following the same method but replacing pectin with H_2O. Eighty (80) μL of 0.15% m-hydroxydiphenyl solution in 0.5% NaOH was added to sample, while 80 μL of 0.5% sodium hydroxide was added to the control. Absorbance at 525 nm was read between 10 min to 30 min versus the control. Sample absorbances were subtracted with the sample control absorbances. A standard curve of D-galacturonic acid was made for each batch of sample analysis.

2.3.3.2. Determination of esterification degree (DE)

The DE of pectin was determined using titrimetric method with slight modifications (Yang, Wang, et al., 2018). Pectin dissolved in CO_2-free H_2O (0.5%, w/v) was firstly titrated with 0.1 N NaOH (V_1) in the presence of three drops of phenolphthalein solution to give a reddish color, which determined the non-esterified carboxylic groups. Then, 10 mL of 0.1 N NaOH was mixed with sample at 300 rpm and 25 ^oC for 1 h to saponify the esterified groups, and 10 mL 0.1 N HCl was added until pink color disappeared. Afterward, phenolphthalein was added, and sample was titrated against 0.1 N NaOH (V_2) to give a reddish color, which determined the esterified carboxylic groups. The DE (%) was calculated using the Equation (2).

$$Degree\ of\ esterification\ (\%) = \frac{V_2}{V_1+V_2} \times 100 \qquad (2)$$

2.3.3.3. FTIR analysis

The FTIR spectra of self-recovered and commercially purchased pectins were measured by Nicolet 6700 spectrophotometer (Fisher Scientific, USA). Dried samples were recorded at spectral range of 500–4000 cm^{-1} in 72 scans with resolution of 2 cm^{-1}, against the background spectrum with empty diamond-ATR (Attenuated Total Reflectance) accessory at 25 ^oC.

2.3.4. Statistical Analysis

The experimental results of pectin recovery were shown as means of three replicates, expressed as mean ± SD (standard deviation). Statistical evaluation was performed by SPSS 28.0 software (Chicago, USA), using one-way analysis of variance (ANOVA) with significant level of $P \leq 0.05$. The differences between means were assessed by the Duncan's multiple range test.

2.4. Results and discussion

2.4.1. Yield & purity of pectin recovered using cellulase

The activity of the used cellulase enzyme was measured at 1.20 U/mg at pH 4.5 and 50 ℃. The effect of enzyme addition (0–800 U per 5 g plantain peel powder) on the yield of pectin is shown in Fig. 2.2A. Natural pectin conventionally is extracted from agricultural biomass using hydrothermal method with acidic pH 1.5.0–4.5 at 60–100 ℃ for 30–240 min. The control treatment was conducted at pH 4.5, at 50 ℃ for 18 h, of which the degree of hydrolysis achieved should be equivalent to the traditional extraction method. With the addition of cellulase, the yield of pectin was significantly higher than that with no enzyme ($P \leq 0.05$). For example, the exaction with 25 U cellulase led to a pectin yield of 12.04%, almost twice as high as the control sample which obtained a yield of 6.24 % ($P \leq 0.05$). The result indicated that cellulase could break down the cellulose-based polymer network in plantain peel to release pectin. However, the yield of pectin showed a relatively constant trend for all the cellulase-assisted extractions ($P > 0.05$), suggesting the amount of cellulase used had little effect on the yield of pectin. Addition of cellulase at 75 U per 5 g plantain peel produced the highest yield of pectin with 13.38%, which was similar to the yield produced with 25 U cellulase ($P > 0.05$). The yield achieved in this study was much higher than those previously-reported for pectin extractions from plantain peels with yields of 4.65–6.91% (Oyawaluja et al., 2020), 5.04% (Obele et al., 2019), 8.5% (Arun et al., 2015), respectively, using conventional hydrothermal methods. The yield was also comparable with the one 8.9–14.6% achieved with the more complex 3 steps sequential hydrothermal method which used water, acid, and chelating agent as extraction solvent (Happi Emaga et al., 2008). Thus, adding a low amount of cellulase effectively improved the yield of pectin from plantain peels via a simpler process.

The purity of pectin recovered from plantain peel can be assessed using the content of

galacturonic acid (GalA). Since 70% of pectin has α–(1–4)–linked galacturonic acid units backbone (Noreen et al., 2017), a higher GalA content indicates a higher purity. The pectin recovered using cellulase at different concentrations (Fig. 2.2B) had significantly higher GalA content than the pectin extracted with no cellulase ($P \leq 0.05$), which corresponded to the result of pectin yield, indicating the enzymatic extraction was an effective method. Cellulase can hydrolyze cellulose existing in the cell wall to release pectin from plantain peel into the aqueous phase, which is represented by the increase of GalA content measured in pectin (Cui et al., 2021). As the cellulase addition increased to 50 U, GalA content of recovered pectin attained the highest value of 150.1 mg, then fluctuated from 75 U to 800 U. GalA contents at 75 U, 200 U, and 400 U were not significantly different from that of 50 U, possibly indicating a saturated substrate concentration for the catalysis starting from 50 U. The enzyme levels of 100 U, 600 U, and 800 U showed a relatively lower GalA content, the possible reason could be that the high level of cellulase hydrolyzes polysaccharide domains in pectin (e.g. galactans linked with β–(1–4)–glycosidic bonds that maybe cleaved by cellulase in rhamnogalacturonan chains (Yuliarti et al., 2011; Gawkowska et al., 2018)). Loss of hydrophilic saccharide side chains might result in a decreased hydrophilicity/solubility of pectin, thus reducing the pectin yield and GalA content in pectin. This study indicated that the addition of cellulase at 50–75 U per 5 g plantain peel powder was preferred to obtain a high yield and purity of pectin.

2.4.2. Properties of recovered pectin

2.4.2.1. Degree of esterification (DE)

The degree of esterification (DE), measuring the percentage of carboxyl groups esterified on the galacturonic acid backbone of pectin, is an important parameter to differentiate the type of

pectin. Pectin with DE value greater than 50% is regarded as high methoxyl pectin while with DE value less than 50% is assigned as low methoxyl pectin (Giacomazza et al., 2018). Table. 2.1. presents the DE of different pectin samples. The commercial pectin from citrus peel had DE of 78.80% which was characterized as high methoxyl pectin and consistent with the 68–86% reported in the literature (Ciriminna et al., 2017), and validating the testing method used in this study. Plantain peel pectin extracted with or without cellulase had DE of 43.43% and 48.23% respectively with no significant difference ($P > 0.05$) and was characterized as low methoxyl pectin. Thus, cellulase-assisted extraction may not affect the DE of pectin possibly due to the mild nature of such method and specificity of the catalytic activity.

2.4.2.2. FTIR spectra

FTIR spectra of the pectin samples were shown in Fig. 2.3 which was similar to the spectra reported in other studies of citrus peel pectin (Hu et al., 2021) and banana peel pectin (Oliveira et al., 2016). All samples presented similar functional groups and structure with differences in the transmittance intensity. The absorption peak at 1740 cm^{-1} corresponded to the stretching vibration of the methyl esterified carboxyl C=O groups while the absorption peak at 1610 cm^{-1} represented the stretching vibration of COO^- ionic carboxyl groups. Based on literature, the methyl esterified carboxyl band was more intense for high methoxyl pectin while the ionic carboxyl band was stronger for low methoxyl pectin (Gopi et al., 2014). In this study, citrus peel pectin had larger peak for methyl esterified carboxyl band compared to the carboxyl groups, indicating its high methoxyl nature, which corresponded to the literature and DE measurements results in section 2.4.2.1. For plantain peel pectin extracted with or without cellulase, the peaks of ionic carboxyl band were much larger than that of the methyl esterified carboxyl group which indicated their low

methoxyl nature, corresponding to the DE results. Bands around 2930 cm^{-1} represented the C–H stretching while the broad absorption peak at 3433 cm^{-1} was attributed to the stretching vibration of O–H group (Deng et al., 2020). The cellulase recovered plantain peel pectin showed the highest intensity for O–H peak, which could be explained by the higher glucose content in the pectin sample from the enzymatic hydrolysis of cellulose. Apart from that, two small peaks at 1410 cm^{-1} and 1300 cm^{-1} were found for both plantain peel pectin samples and were attributed to the C–H bending of glucose and ring vibration of pectin (Balan et al., 2019; Hu et al., 2021).

2.5. Conclusions

A simple, feasible, efficient, and green process was developed to recover pectin from plantain peels, which has high possibility to be scaled-up and industrialized. Pectin was successfully recovered from plantain peel, and the extraction process involved the use of cellulase enzyme and alcohol recovery step to achieve sustainability. Pectin with high yield and purity was obtained by adding 50 U of cellulase per 5 g powder. The plantain peel pectin was characterized as low methoxyl pectin and the cellulase extraction did not have significant effect on the degree of esterification of pectin. The FTIR results corresponded to DE measurements and confirmed the composition of pectin samples. This study explored novel and sustainable strategies for agricultural waste management, that may help solve the disposal problem in many African countries.

2.6. Acknowledgements

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Figures & Tables

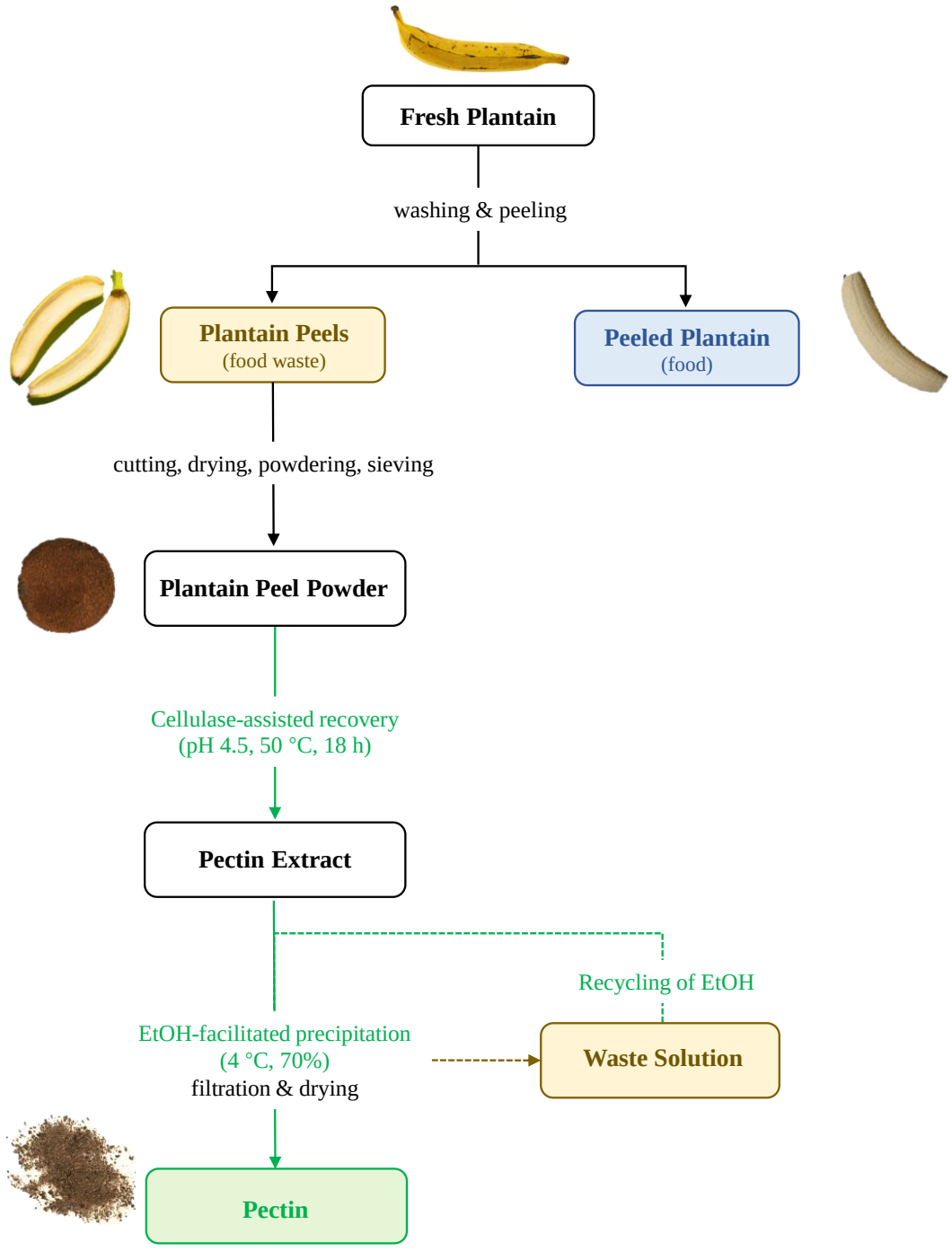

Fig. 2.1: Scheme of the recovery process of pectin from plantain peels via a green approach.

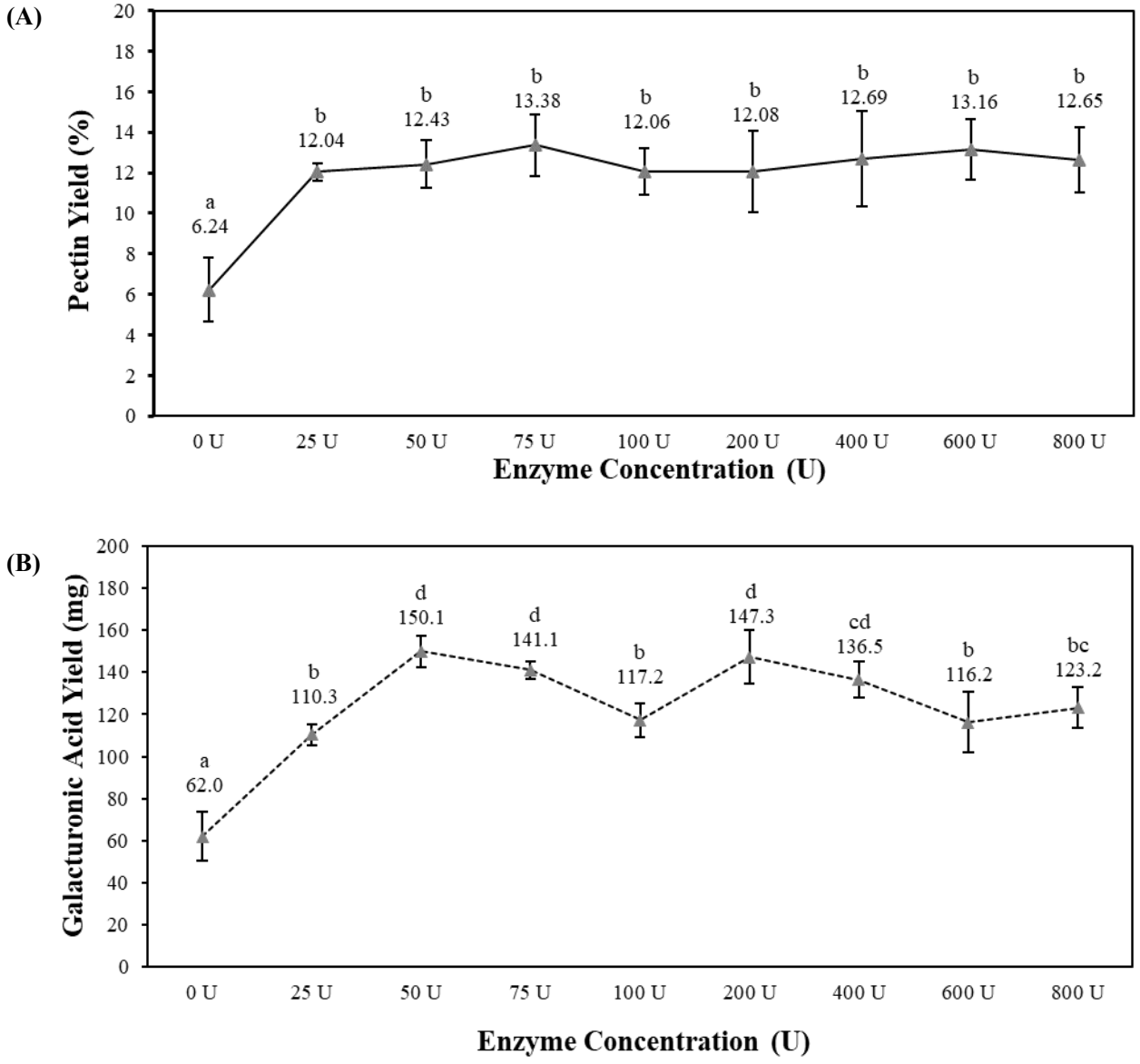

Fig. 2.2: The yield (A) and galacturonic acid content (B) of pectin extracted from plantain peel using cellulase with amounts of 0 (control), 25, 50, 75, 100, 200, 400, 600, 800 U per 5 g plantain peel powder. Different letters in the same group represent significant differences among the different samples ($P \leq 0.05$).

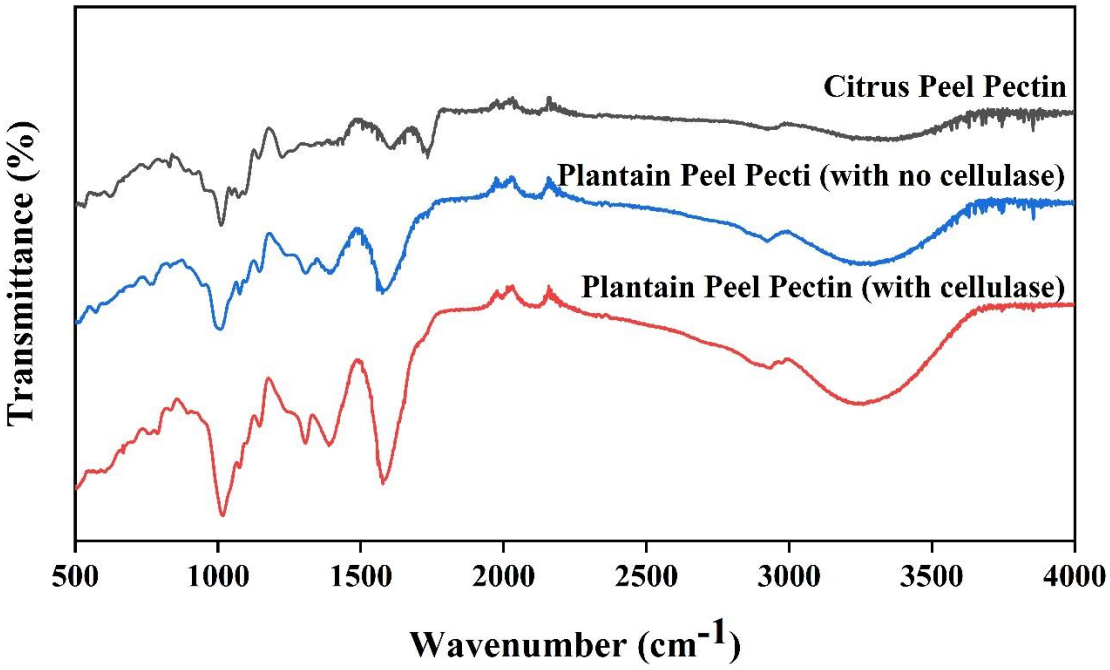

Fig. 2.3: The FTIR spectra of citrus peel pectin, and pectin recovered from plantain peel with and without cellulase.

Table. 2.1: Degree of esterification of different pectin samples: citrus peel pectin, plantain peel pectin extracted with no cellulase, and plantain peel pectin extracted with cellulase. Different letters represent significant differences among the samples ($P \leq 0.05$)

Pectin Sample	DE (%)
Citrus Peel Pectin	78.80 ± 2.72^{a}
PP Pectin (extracted with no cellulase)	48.23 ± 6.87^{b}
PP Pectin (extracted with cellulase)	43.43 ± 2.22^{b}

PP: plantain peel

CHAPTER III

CONNECTING STATEMENT

In the previous chapter (II), the recovery of pectin from plantain peels via green approach was investigated and the effect of cellulase concentration on the yield and quality of pectin were assessed. Pectin is a common biopolymer that has a plethora of uses in food industry. One of its applications is acting as the polymeric matrix for film generation due to its properties like selective gas permeability, biocompatibility, gelation property, edibility, and biodegradability. On the other hand, pectin films have low mechanical strength and poor water barrier properties; thus, enhancement of these properties by adding functional material is necessary to produce better films. The study in this chapter was intended to utilize the greenly recovered plantain peel pectin to make a biodegradable film which was reinforced in terms of its mechanical & water barrier properties by adding a hydrophobic material—beeswax solid lipid nanoparticles (BSLNs). The effect of BSLNs in different concentrations on the properties of pectin films were evaluated. A parallel film group made with commercial citrus peel pectin was conducted for comparison. This study explodes a novel reutilization of plantain peel waste and provides new knowledge in pectin films reinforcement.

CHAPTER III

TRANSFORMATION OF PECTIN FROM PLANTAIN PEELS INTO COMPOSITE FILMS—A COMPARATIVE STUDY WITH CITRUS PEEL PECTIN

3.1. Abstract

The recovered plantain peel pectin was transformed into composite films by incorporating with different concentrations of beeswax solid lipid nanoparticles (BSLNs) for comparison with citrus peel pectin composite films. Addition of BSLNs significantly increased ($P \leq 0.5$) the water & light barrier properties of both types of films, and improve the mechanical property of plantain peel pectin films while impaired that of citrus peel pectin films. Increases in the amount of BSLNs significantly ($P \leq 0.5$) enhanced the tensile strength (TS) and Young's Modulus (TS) of plantain peel pectin films, making them stronger with preserved elasticity, but significantly ($P \leq 0.5$) decreased the TS and elongation at break (EB) of the citrus peel pectin films, making them stiffer and easier to break. X-ray diffraction results suggested the possible existence of structural strain within the film network of the citrus peel pectin films incorporated with higher amount of BSLNs, which might explain the decreases in TS and EB. FTIR spectra studies confirmed the composition of films, and the microstructure revealed more homogeneous and uniform distribution of hydrophobic BSLNs in the citrus peel pectin films compared with their plantain peel pectin counterparts.

3.2. Introduction

Food packaging protects food products from surrounding factors such as UV light, oxygen, moisture, bacteria, etc. Thus, it plays a key role in food shelf-life extension and food quality / safety preservation (Zhang et al., 2018). Synthetic plastics, currently the most widely used for food

packagin, are poorly recyclable or biodegradable, which poses a major threat to environment health and protection (Cruces et al., 2021; Liu et al., 2020; Zheng et al., 2019). Packaging films made of biodegradable polymers are more environmentally sustainable alternatives to conventional plastics because of their superior attributes in terms of biodegradability, biocompatibility, functionality, and versatility (Jha & Kumar, 2019; Martău et al., 2019; Mellinas et al., 2020). The well-recognized biopolymers used in food packaging include polysaccharides (Huang et al., 2021; Mujtaba et al., 2019), proteins (Aydogdu et al., 2019; Ortiz et al., 2018), and lipids (Romani et al., 2020; Syahida et al., 2020), into which some functional compounds (e.g., polyphenols (H. Gao et al., 2019; Riaz et al., 2018), essential oils (Geranpour et al., 2020; Roy & Rhim, 2021b)) can be integrated to enhance their functionality as packaging films (Mir et al., 2018). Pectin, mainly made of linear chain of α–(1–4) linked D-galacturonic acid units, is a natural and hydrophilic polysaccharide found in plant cell walls as one of the most commonly present biopolymers in terrestrial plants (Castro e Silva et al., 2020; Noreen et al., 2017). Food packaging films made with pectin have good bioavailability, gelation capacity, gas permeability, and edibility (Bagal-Kestwal et al., 2019). Most of the pectin films are made with commercially available pectin from citrus peels (Guo et al., 2021; Kumar et al., 2020), and recent researches have developed pectin films made with other Agro-waste including watermelon rind (Guo et al., 2021; Han & Song, 2021), mango peels (Chaiwarit et al., 2020), pineapple peel (Rodsamran & Sothornvit, 2019c), as well as pumpkin seeds and peels (Lalnunthari et al., 2020). This study investigated plantain peel as pectin source.

Plantain is an important staple crop that is consumed by millions of people in tropical countries, due to its high carbohydrate content. The global production was estimated at 39 million tons in 2018 (FAOSTAT, 2020a). Plantain peel is the major waste from plantain processing

industry with production conservatively estimated at 12 million tons per year (Arun et al., 2015). It contains pectin (Arun et al., 2015) but very few studies were found from the literature on the preparation of pectin from plantain peels (Emaga et al., 2008; Obele et al., 2019). To the best of our knowledge, there is a paucity of research on the preparation of pectin films from plantain peels based on green chemistry.

Pectin films have a range of merits but their functionality is limited by low mechanical properties and poor water resistance (Cazón et al., 2017; Espitia et al., 2014). Hydrophobic materials are usually incorporated to improve these drawbacks. Beeswax, a natural food-grade wax produced by worker honeybees, is a mixture of saturated and unsaturated monoesters, free fatty acids, various long–chain alcohols, and other minor substances. It is a yellowish solid at room temperature with a melting temperature of 62–64 °C (Cruces et al., 2021; Zhang et al., 2018). Previous studies have shown that the incorporation of melted beeswax into citrus peel pectin films improved the film water vapor permeability but had adverse effects on the mechanical property (Cruces et al., 2021; Maftoonazad et al., 2007). Solid lipid nanoparticles (SLNs), a new type of nanocarriers, composed of solid lipid phase dispersed and stabilized in continuous water phase using surfactants, exhibit advantages such as long-term stability, controlled release kinetics, good reproducibility, feasibility for large-scale production, and biodegradability (Duan et al., 2020; Ghosh et al., 2021). The SLNs prepared by a mixture of carnauba wax and candelilla wax successfully improved the mechanical (e.g. tensile strength and elongation at the breakpoint) and water vapor barrier properties of polysaccharide films (García-Betanzos et al., 2016). Thus, we hypothesized that incorporating beeswax SLNs (BSLNs) into plantain pectin packaging films could improve their properties.

The objectives of this study were to transform recovered plantain peel pectin into films and

reinforce the films with BSLNs at different concentrations, as well as to understand the effect of BSLNs on various properties (i.e., barrier properties, mechanical properties, conformational changes, microstructure, etc.) by comparing with unmodified pectin films. A parallel group, i.e., commercially available citrus peel pectin was made into film for comparison with the plantain peel pectin films.

3.3. Materials and methods

3.3.1. Materials

Pectin from plantain peel was extracted via enzymatic method using 50 U cellulase from *Aspergillus niger* at 50 °C for 18 h. The extracted pectin was precipitated, filtered, washed, vacuum-dried, and powdered for this study.

Pectin from citrus peel (galacturonic acid ≥ 74.0%) and beeswax were purchased from Sigma-Aldrich (Missouri, USA). Glycerol was purchased from Bio Basic (Toronto, Canada). Poly vinyl alcohol (98.0–98.8% hydrolyzed, M.W. approx. 31,000–50,000) was purchased from Fisher Scientific (Geel, Belgium).

3.3.2. Preparation of SLNs

BSLNs were prepared using the hot high-stirring shear method (Zambrano-Zaragoza et al., 2013). In brief, 100 g/L of beeswax was melted into liquid at 70 °C on a hot plate, and 60 g/L poly vinyl alcohol (as the stabilizer) was dissolved in distilled water at 90 °C and cooled to 70 °C to obtain aqueous phase. The beeswax in the liquid phase was dispersed into the poly vinyl alcohol on a high shear stirrer with continuous heating (Ultra-Turrax T10, IKA®, Staufen, Germany with a S10N–8 G, IKA disperser element) at 14,000 rpm per 5 min (1 cycle) for a total of 4 cycles with

5 min repose between cycles. Afterwards, an oil-in-water nano-emulsion was obtained and subsequently cooled down to room temperature (25 °C) as BSLNs dispersions.

3.3.3. Determination of BSLNs and film-forming (pectin-BSLNs) dispersions properties

3.3.3.1. Particle size and polydispersion index (PDI)

Particle size distribution and PDI of stock BSLNs sample were measured at 25 °C with a fixed angle of 90° on a dynamic light scattering instrument (NanoBrook Omni, Brookhaven Instrument, USA) according to the procedure outlined by Y. Gao et al. (Y. Gao et al., 2019). The BSLNs dispersion sample was diluted by 500-fold using distilled water before measurements to obtain a clear and transparent analyte. The diluted sample was pipetted into a plastic cuvette and loaded into the machine. After 60 s equilibration, the data were collected in triplicates at a rate of 60 s per cycle. Particle sizes were reported as the average particle diameter (nm) calculated from the particle size distribution. PDI was calculated automatically using the build-in algorithms of the operating software (Chang et al., 2021).

3.3.3.2. Zeta potential (ζ)

The zeta potential of stock BSLNs sample and film-forming (pectin-BSLNs) dispersion samples were measured at 25 °C using a dynamic light scattering instrument (NanoBrook Omni, Brookhaven Instrument, USA) based on method of Chang et al. (Chang et al., 2021) with slight modifications. The samples were diluted 1000-fold using distilled water before measurements. The diluted sample was placed into a cuvette equipped with an electrode and settled for 60 s to equilibrate. Results were collected three times at a rate of 60 s per cycle. The data were converted from electrophoretic mobility results into zeta-potential value via build-in Smoluchowsky

mathematical model of the operating software.

3.3.3.3. Morphological analysis using microscopy

Morphological observation of the BSLNs was performed using scanning electron microscopy (SEM) (Hitachi TM-1000, Hitachi High-Technologies Corporation, Tokyo, Japan). For SEM analysis, the BSLNs samples were first centrifuged at 10,000 rpm 3 times for 30 min per sample to remove excess stabilizer. Then, 3–4 drops of concentrated BSLNs dispersion were vacuum dried at room temperature (25 °C). The dried sample was placed on stubs and coated (Leica EM-ACE200, Leica Microsystems, Germany) with gold/platinum layer (~4 nm thickness) and then observed under high vacuum SEM.

3.3.4. Preparation of film–forming dispersions

A 4% (w/v) pectin solution was prepared in warm distilled water at 40 °C, followed by the addition of 1.5% (w/v) glycerol as plasticizer. The prepared BSLNs dispersion (Section 3.3.3) at a concentration of 100 g/L was further diluted to obtain a series of BSLNs dispersions at 10, 20, 30, 40, and 50 g/L. Afterwards, the pectin solution was added into each of the BSLNs dispersions drop-wise under ultrasound treatment for 25 min, followed by a 10 min degassing treatment (Jiang et al., 2020). Six film formulations were prepared for each type of pectin (plantain peel pectin and citrus peel pectin), as shown in Table. 3.1, to study the effect of BSLNs concentration on the properties of pectin films. Twenty (20) mL of each film forming solution were poured into a casting plate (diameter of 9.5 cm) and dried at 25 °C to form the film. The films were conditioned in a sealed desiccator at 25 °C to obtain a constant weight before analyses.

3.3.5. Determination of film properties

3.3.5.1. Light transmission

Light transmission of films was measured using a UV/visible spectrophotometer (DU 800, Beckman Coulter Inc., Brea, CA, USA) for the wavelength range from 200 to 800 nm, and reported as transmittance (%). Film samples were cut into 1×1 cm and fixed in a holder for measurement. The spectrophotometer was blanked with air.

3.3.5.2. Water solubility

Water solubility was measured based on the method of Zhang et al. (Zhang et al., 2018) with slight modifications. Film pieces (1×4 cm) were cut using scissors and knife, vacuum-dried for 6 h and placed in a sealed desiccator for 24 h. The initial weight of films was recorded (W_0). Films were then immersed in 20 mL distilled water at 25°C for 1 h. The undissolved parts were collected and vacuum-dried until constant weight (W_1). The water solubility was calculated using Equation (1).

$$\text{Water solubility (\%)} = \frac{W_0 - W_1}{W_0} \times 100 \qquad \text{Equation (1)}$$

3.3.5.3. Water vapor permeability (WVP)

The WVP of films was measured using the modified cup method from American Society for Testing and Materials (ASTM) Standard Method E96/E96M–10 (ASTM, 2020) with slight modifications. The film sample was cut into circles with radius of 1.4 cm and vacuum dried for 6 h and placed in sealed desiccator for 24 h. The dried film sample was mounted on top of a glass cup containing 6 g of anhydrous calcium chloride as a desiccant (0% RH). The test cup was then placed in a desiccator that contains saturated sodium chloride solution (75% RH) at 25 °C. The

weight gain of each cup was measured every 2 h for a total of 24 h. The thickness of each film was measured using a digital micrometer with precision of 0.01 mm (Digital Traceable Calipers 3415CC, Traceable® Products, USA). The WVP of films was calculated using Equation (2).

$$\mathrm{WVP} = \frac{\Delta \mathrm{w} \times x}{\mathrm{A} \times \Delta \mathrm{t} \times \Delta \mathrm{P}} \qquad \text{Equation (2)}$$

where Δw is the weight gain of the cup (g) during the time change Δt (h), *x* is the thickness of film (m), A is the area of the exposed area of the film surface (7.85×10^{-5} m^2), and ΔP is the partial water vapor pressure difference between two sides of the film (Pa).

3.3.5.4. Mechanical properties

The tensile strength (TS), elongation at break (EB), and Young's Modulus (YM) of films were tested using an universal testing machine (Instron 5967, Instron Corp., MA). Film samples were cut into 1×3 cm (width×length) strips and pre-conditioned at 25 °C and 40% RH for 48 h. The thickness of each strip was measured using digital micrometer with precision of 0.01 mm (Digital Traceable Calipers 3415CC, USA). The initial grip distance of machine was set as 20 mm and the crosshead speed was set at 5 mm/min. The TS, EB, and YM values were calculated using Equation (3–5).

$$\mathrm{TS\ (Mpa)} = \frac{\mathrm{L_{max}}}{l \times x} \qquad \text{Equation (3)}$$

where Fmax is the maximum load/tensile force of film (N), l is the initial film width (mm), and *x* is the average thickness (mm).

$$\mathrm{EB\ (\%)} = \frac{\mathrm{L_1}}{\mathrm{L_0}} \times 100 \qquad \text{Equation (4)}$$

where L_1 is the increase of film length after stretching until the film breaks, L_0 is the initial grip distance.

$$\text{YM(Mpa)} = \frac{\text{Stress}}{\text{Strain}} = \frac{\text{Stress}}{\Delta L/L_0} \qquad \text{Equation (5)}$$

where ΔL is change in film length after stretching, L_0 is the initial grip distance.

3.3.5.5. FTIR spectroscopy

The FTIR spectra of films were measured by Nicolet 6700 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA) equipped with an attenuated total reflectance accessory. Dried film samples were scanned at a spectral range of 500–4000 cm^{-1} for 72 scans with resolution of 2 cm^{-1}, against the background spectrum with empty accessory at 25 °C.

3.3.5.6. X–ray diffraction (XRD) analysis

The XRD patten of each film was collected on a multipurpose diffractometer (Empyrean 3, Malvern Panalytical Ltd., UK) from 3 to 51° (2θ) using radiation of Cu Kα (λ = 1.54178 Å) at speed of 0.8°/min. The data were processed with Highscore v.4.9.

3.3.5.7. Scanning electron microscope (SEM) analysis

Films were observed on a SEM (Hitachi TM-1000, Japan). Dried film samples were cut into small pieces and placed on double-sided carbon tape which was then stuck onto aluminum stub. The stub with sample on it was put into sealed desiccator for 24 h. The sample was coated with gold/platinum layer (~4 nm thickness) and observed under high vacuum SEM.

3.3.6. Statistical analysis

The experimental results for BSLNs characterization were shown as means of three replicates, and for film characterization as the mean of five replicates, expressed as mean ± SD

(standard deviation). Statistical evaluation was performed by SPSS 28.0 software (SPSS Inc., Chicago, IL, USA), using one-way analysis of variance (ANOVA) with level of significant at $P \leq 0.05$. The differences between means were assessed by the Duncan's multiple range test.

3.4. Results and discussion

3.4.1. Properties of BSLNs and film-forming (pectin-BSLNs) dispersions

3.4.1.1. Particle size and polydispersion index (PDI) of BSLNs

The particle size of stock BSLNs dispersion was 700 ± 15 nm on average, with a PDI of 0.309 ± 0.024. Thus, the prepared BSLNs dispersion was within the size of solid lipid nanoparticles (i.e., 50 to 1000 nm) (Vitorino et al., 2011). The PDI measures the heterogeneity of the nanoparticles based on size. A value of PDI at around 0.3 or lower than 0.3 indicates a homogenous dispersion of small and large particles in the system (Li et al., 2022). Thus, the prepared BSLNs were dispersed well with relatively low tendency to agglomerate.

3.4.1.2 Zeta potential (ζ) of BSLNs and film-forming (pectin-BSLNs) dispersions

Zeta potential suggests the repulsive force among adjacent and similar charged particles in the system (Zambrano-Zaragoza et al., 2013). As shown in Table. 3.2., the stock BSLNs dispersions prepared had an average negative zeta potential at − 35.31 ± 2.32 mV, consistent with the zeta potential value of beeswax SLNs reported by Shakeri et al. (Shakeri et al., 2019) and Dantas et al. (Dantas et al., 2018) into which distinct surfactants like lecithin with Tween 80 and Tween 80 with phospholipon 80 were added instead of PVA used in this study.

The film-forming (pectin-BSLNs) dispersions presented zeta potential value in the range of − 43.39 ± 0.76 to − 55.88 ± 2.07 mV. Addition of plantain peel pectin or citrus peel pectin to

BSLNs did not change the negative sign of BSLN's zeta potential, but significantly increased the absolute value which indicated a higher degree of electrostatic repulsion among particles, thus created a more stable dispersion system to prevent possible aggregation of nanoparticles during storage (Cacua et al., 2019). This may be probably due to the higher repulsive force between hydrophilic pectin molecules and hydrophobic BSLNs molecules. Increase in BSLNs concentration did not have significant effect on the zeta potential values of most film forming dispersions.

3.4.1.3. Scanning electron microscopy (SEM)

The micrographs of the BSLNs dispersions in Fig. 3.1. showed that the nanoparticles were in spherical shape, and their average particle size (approximately 689 nm) matches with the size measured using dynamic light scattering method. At high concentration, the BSLNs stacked and overlapped (Fig. 3.1a.), while at low concentration, BSLNs presented a clear distribution of the particles, suggesting a relative uniform shape and no aggregation (Fig. 3.1b.).

3.4.2. Properties of pectin and pectin-BSLNs films

3.4.2.1. Light barrier properties

Barrier to light transmission is an important attribute of biomaterial-based films to function as a packaging material. Films with good light barrier properties can help avoid photo-oxidation to maintain a long shelf-life of food products (Priyadarshi et al., 2021). The transmittance values of pectin and pectin-BSLNs films made with plantain peel (Fig. 3.2A) and citrus peel (Fig. 3.2B) at wavelength 200–800 nm are presented. The smaller area under the light transmission curve at 200–800 nm, the higher light barrier property. As shown in Table.3.1. and Fig. 3.2, the light barrier

of the films increased in this order: CP < PP < CP1 < PP1 < PP2 < CP2 < CP3 < PP3 < CP4 < PP4 < CP5 < PP5. Most of the films from plantain peel group showed stronger light barrier properties compared with the citrus peel samples at the same concentration of BSLNs. CP film was observed as transparent which differed from PP film which was brownish. The intrinsic brown color of plantain peel pectin can reflect varying ratio of red and green lights; thus, fewer lights are able to pass through the PP film, leading to lower light transmittances. As the concentration of BSLNs increased, both groups of films presented greater light barrier properties which was probably due to the opacity of beeswax. For example, within the visible light range (400–800 nm), the transmittances for PP film and CP film were 68–9% and 85–50% respectively, and for PP1 film and CP1 film were 44–6% and 37–15% respectively, indicating a big decrease in light transmittance. In the UV range particularly at 240–340 nm, plantain peel pectin films presented extremely low transmittance (< 0.8%) compared with citrus peel pectin films (< 8%). All plantain peel pectin films plus CP3, CP4, CP5 are, therefore, suitable (transmittance < 1%) to pack foods rich in bioactive proteins and peptides because several aromatic amino acids in protein/peptides can absorb UV light below 310 nm that damages the peptide bonding, resulting in protein breakdown (Zhang et al., 2018). Furthermore, films made with plantain peel pectin are more effective light resistant materials to package protein rich foods compared with films made with citrus peel pectin, preventing deterioration of bioproducts caused by light exposure, and addition of BSLNs further increased the light resistance of pectin films.

3.4.2.1. Water resistance properties

Water solubility and water vapor permeability (WVP) are used to determine the water/moisture resistance properties of food packaging films, for which a lower values of water

solubility and WVP indicates a stronger water resistance (Omar-Aziz et al., 2021).

Based on Fig. 3.3., both types of pectin films incorporated with BSLNs (PP1–5 and CP1–5) showed a significant decrease in water solubility and WVP compared to unmodified films (PP and CP) ($P \leq 0.05$), suggesting that the addition of BSLNs into the pectin films could help conserve the integrity of the films and block entry of moisture from the outside. In general, the plantain peel pectin based films showed higher water solubilities and WVP than the citrus peel pectin based films. The possible reasons lie in the different types of pectin. As discussed in 'Chapter II', the pectin recovered from plantain peel was low methoxyl pectin, while the citrus peel pectin used in this study was high methoxyl pectin, exhibiting high hydrophobicity from the long hydrocarbon chains that have low water solubility and moisture permeation (Mellinas et al., 2020). In addition, when the concentration of BSLNs was increased, a decreasing trend for water solubility and WVP was observed, indicating that the more addition of BSLNs resulted in higher water resistance of the films. The added BSLNs are hydrophobic in nature (Soazo et al., 2011), and the hydrophilic to hydrophobic ratio of components in film matrix was inversely related with water resistance properties (Mendes et al., 2019; Wang et al., 2015). Increased BSLNs content decreased the hydrophilic to hydrophobic ratio, which resulted a stronger water resistance property of the films. At extremely high BSLNs concentration, films (e.g., CP4 and CP5) networks were loaded with maximum/saturated beeswax solid particles of which the extra portion could no longer fit into the space. This explains the insignificant difference ($P > 0.05$) between films incorporated with high amount of BSLNs. From the perspective of production, it was concluded that PP4 and CP4 would be the most suitable films with optimized water barrier properties at low cost of materials.

3.4.2.2. Mechanical properties

The mechanical properties reveal the ability of packaging films to maintain integrity to allow them to contain / retain the food products without physical damage (Mir et al., 2018). Figure 3.4. shows the mechanical properties, i.e., tensile strength (TS), elongation at break (EB), and Young's Modulus (YM) of the fabricated films. The plantain peel pectin based films exhibited distinct TS, EB, and YM compared with citrus peel pectin based films.

The pure plantain peel pectin film (PP) had TS of 0.04 MPa, EB of 442%, YM of 6.78 MPa, suggesting the PP film was weak in tensile strength but excellent in elasticity, which was different from other reported pectin films that were inelastic (Meerasri & Sothornvit, 2020; Priyadarshi et al., 2021). The recovery of pectin from plantain peel co-produced glucose from the cellulase hydrolysis, which was probably the reason for high elasticity for plantain peel pectin film. Addition of BSLNs significantly ($P \leq 0.05$) increased the TS and YM, but decreased EB for the films, as shown in Figs. 3.4A, B and C (left), respectively, suggesting that the addition of BSLNs enhanced the strength and reduced the elasticity for plantain peel pectin based films. The more BSLNs dispersion added, the more hydroxyl groups in the poly vinyl alcohol (PVA) used as surfactants, leading to the stronger force of hydrogen bond formed with the hydroxyl groups in free glucose (Bao et al., 2021; Nguyen et al., 2021) presented in the recovered pectin. The tight interaction between PVA and glucose could probably explain the enhancement of strength. On the other hand, the addition of BSLNs contributed to the brittle and stiff attributes, reflected from the decreasing YM. The beeswax particles brought weak van der Waals forces to create imbalanced force that negatively affected the film integrity, which made the films easier to break, explaining the decreasing elongation at break after addition of BLSNs.

For the citrus peel pectin based films, TS significantly decreased ($P \leq 0.05$) for the BSLNs

incorporated films (CP1–5) compared to the unmodified CP film (Fig. 3.4A (right)). The value of EB also decreased with increasing addition of BSLNs, and the reduction was significant ($P \leq 0.05$) when BSLNs was added at relatively high concentrations (e.g., 2% and 2.5% of BSLNs) (Fig. 3.4B (right)). As for the YM, there was no obvious change observed among the pure citrus peel pectin film (CP), and the various pectin-BSLNs films (CP1–5) (Fig. 3.4C (right)). The data for these fabricated pectin films corresponded to a previous research finding on another beeswax-incorporated high methoxyl pectin (HMP) (Maftoonazad et al., 2007), with the same type of pectin as the citrus peel pectin that was used in this study. In general, the addition of BSLNs into pure citrus peel pectin film had adverse influence on the mechanical properties especially for the TS and EB. The slight change in the mechanical parameters with the BSLNs addition was probably due to the interruption and discontinuity of the pectin intermolecular structure triggered by the polymer network of beeswax lipid phase (Norcino et al., 2020; Sánchez-González et al., 2011). To further improve the flexibility and decrease the brittleness of the citrus peel pectin-BSLNs films, various types of plasticizers such as polyethylene glycol, sorbitol, xylitol, or other low-molecular-weight sugar could be added and their effects could be studied (Garavand et al., 2022; Gheribi et al., 2018; Zhang et al., 2020).

Apart from those findings, it is worth noting that the value of TS and YM for plantain peel pectin based films were generally lower while the value of EB was quite higher than those for citrus peel pectin based films, due to the different type and composition of the pectin. As mentioned before, the presence of glucose in plantain peel pectin acts as extra plasticizer to produce films with much higher elasticity, thus the plantain peel pectin based films can be a better candidate for film that requires good flexibility.

3.4.2.3. FTIR spectra

Figure 3.5. shows the FTIR spectra of films made with plantain peel pectin (Fig. 3.5A) and citrus peel pectin (Fig. 3.5B). Both groups of films displayed the typical peaks at wavenumbers of 1615 and 1735 cm^{-1} which were attributed to the stretching vibration of ionic carboxyl group (COO-) and esterified carbonyl group (C=O), respectively, on the galacturonic acid unit backbone of pectin. With the increase of BSLNs addition, the 1735 cm^{-1} peak tended to be sharper and more obvious which corresponded to the presence of more esterified carbonyl groups from beeswax (Omar-Aziz et al., 2021). Two characteristic peaks at 2850 and 2916 cm^{-1} which were due to the stretching vibration of C–H group (CH_2 and CH_3 respectively) could be found in both spectra (Cruces et al., 2021). As the addition of BSLNs increased, these two peaks became sharper with higher intensity which was attributed to the abundant presence of long hydrocarbon chains from fatty acid in beeswax structure. A tiny peak at 719 cm^{-1} in pectin composite films (PP1–5, CP1–5) was associated with the C–H rocking mode presented in beeswax (Cruces et al., 2021), and sharper peaks were observed when the BSLNs content was increased.

3.4.2.4. X-ray diffraction (XRD) analysis

XRD provides information about the confirmational change and crystallinity of polymers. Figure 3.6. shows the diffraction patterns of plantain peel pectin based films (Fig. 3.6A), and citrus peel pectin based films (Fig. 3.6B). One broad peak at 21.51° and two sharp but extremely low peaks at 15.05° and 30.10° were detected in the PP film while one broad peak at 21.36° and four low peaks at 12.72°, 15.10°, 16.30° and 30.10° were detected in CP film. All these corresponded to the characteristic peaks for pure pectin, apple pectin hydrogel, and citrus pectin films crystallinities reported in literature (Nisar et al., 2018; Pandey et al., 2021; Yang, Nisar, Liang, et

al., 2018). These peaks were either broad or low in intensity, suggesting both PP and CP films had a tremendously amorphous structure. Between the two, PP film showed less number of peaks than CP film, representing a lower level of crystallinity.

With the addition of BSLNs, the pectin composite films showed changes in the crystalline structure based on the introduction of two intense and sharp peaks at 21.70° and 23.95° that are attributed to the beeswax (Cruces et al., 2021; Dantas et al., 2018). These two peaks indicated the strong crystallinity of the pectin composite films caused by the intrinsic ordered arrangement of the hydrocarbon chain in beeswax (Cruces et al., 2021). In addition, four short peaks were observed at 30.02 °, 36.50°, 41.62°, and 44.07° in the pectin composite films, which were associated with the amorphous or semi-crystalline structure of beeswax (Shirvani et al., 2022; Tian et al., 2021; Zhang et al., 2021). The increased crystallinity incorporated a more ordered lattice structure in the pectin composite films, which should lead to a strong force between the adjacent polymers. This explains the enhanced TS measured in the plantain peel pectin composite films (section 3.4.2.2). Furthermore, obvious shifts of beeswax peaks were observed in the citrus peel pectin films (Fig. 3.6B). Different degrees of shifting to higher diffraction angles occurred in CP2, CP3, CP4, CP5 films which suggested possible compression in the crystal lattice, prompting an unstable stress and strain response. The imbalance of the force within the structure made the film easier to break, thus effecting TS and EB negatively which corresponded to the mechanical test results for citrus peel pectin based films in section 3.4.2.2.

3.4.2.5. Microstructure

The microstructure of pure pectin films (PP and CP) and pectin composite films (PP1–5 and CP1–5) are shown in Fig. 3.7. Both PP film (Figs. 3.7A & A') and CP film (Figs. 3.7a & a') had

smooth and uniform surfaces which were similar to observations reported in other studies (Meerasri & Sothornvit, 2020; Roy & Rhim, 2021a).

The incorporation of BSLNs in the pectin film changed the morphological characteristics. Circular microstructures were perceived in different sizes and numbers on the surface of PP1–PP5 films (Fig. 3.7B–F). As the concentration of BSLNs increased, the number and size of round microstructures increased, possibly due to the aggregate of nanoparticles, especially for PP5. The lack of uniform microstructure led to the low elongation at break (EB) as discussed in section 3.4.2.2. For CP1–5, spherical structures of BSLNs particles were observed in both front and back of films, suggesting a homogeneous dispersion of BSLNs in the films (Figs. 3.7b–f & b'–f'). As the concentration of BSLNs increased, the surface of the films was found with more compact BSLNs. The particle sizes of BSLNs in the films were observed within nanoparticle scale, which corresponded to the result measured on the dynamic light scattering instrument (section 3.1.1). The front sides of the films (Figs. 3.7b–f) had larger particles with relatively irregular shapes compared to the back sides of these films (Figs. 3.7b'–f'). A possible explanation is that the BSLNs aggregated and stacked on the front surface easily by themselves during film formation while for the back side, the surface tension of film casting mold prevented that from happening.

The microstructure features of the films also correlated with their performance in water resistance. Briefly, the increase of BSLNs added evenly on the film surface enhanced the hydrophobicity of films, and thus, prevented water and moisture from penetrating through the films, as reflected from the reduction of water solubility and WVP (section 3.4.2.1).

3.5. Conclusion

Plantain peel pectin was successfully transformed into films that can potentially replace the overused single use plastics. Pure pectin films made with either plantain peel pectin or citrus peel pectin can be reinforced by adding BSLNs for food packaging. The BSLNs prepared using hot high stirring shearing method had homogeneous nanoparticle distribution, for which the high absolute zeta potential indicated a good stability for long-term storage at room temperature. All the pectin-BSLNs film forming dispersions presented higher absolute zeta potentials, suggesting the formation of stabler systems. It is also simple to prepare BSLNs in large quantity for industrial use. Incorporation of BSLNs into pure pectin films improved their water/moisture and light barrier properties, and the higher concentration of BSLNs added, the higher barrier properties obtained. Furthermore, BSLNs addition elicited different effects on mechanical properties of pectin films. It improved that of plantain peel pectin film by increasing the tensile strength and Young's Modulus but weakened that of citrus peel pectin film by decreasing the tensile strength and elongation at break. Films produced with plantain peel pectin showed much higher elasticity than films made from citrus peel pectin, which enables flexibility during packaging to be able to cover the food products thoroughly, making them a better candidate for edible film application. This study explored novel and sustainable strategies for agricultural waste management and value-addition.

3.6. Acknowledgements

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Figures & Table

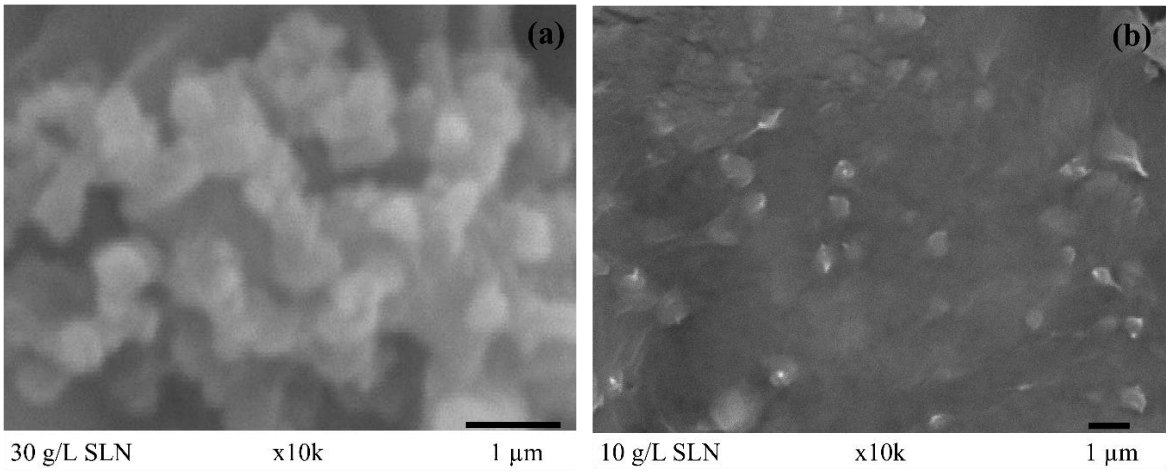

Fig. 3.1: Scanning electron micrographs of BSLNs dispersion samples at concentration of (a) 30 g/L; (b) 10 g/L. BSLNs is beeswax solid lipid nanoparticles.

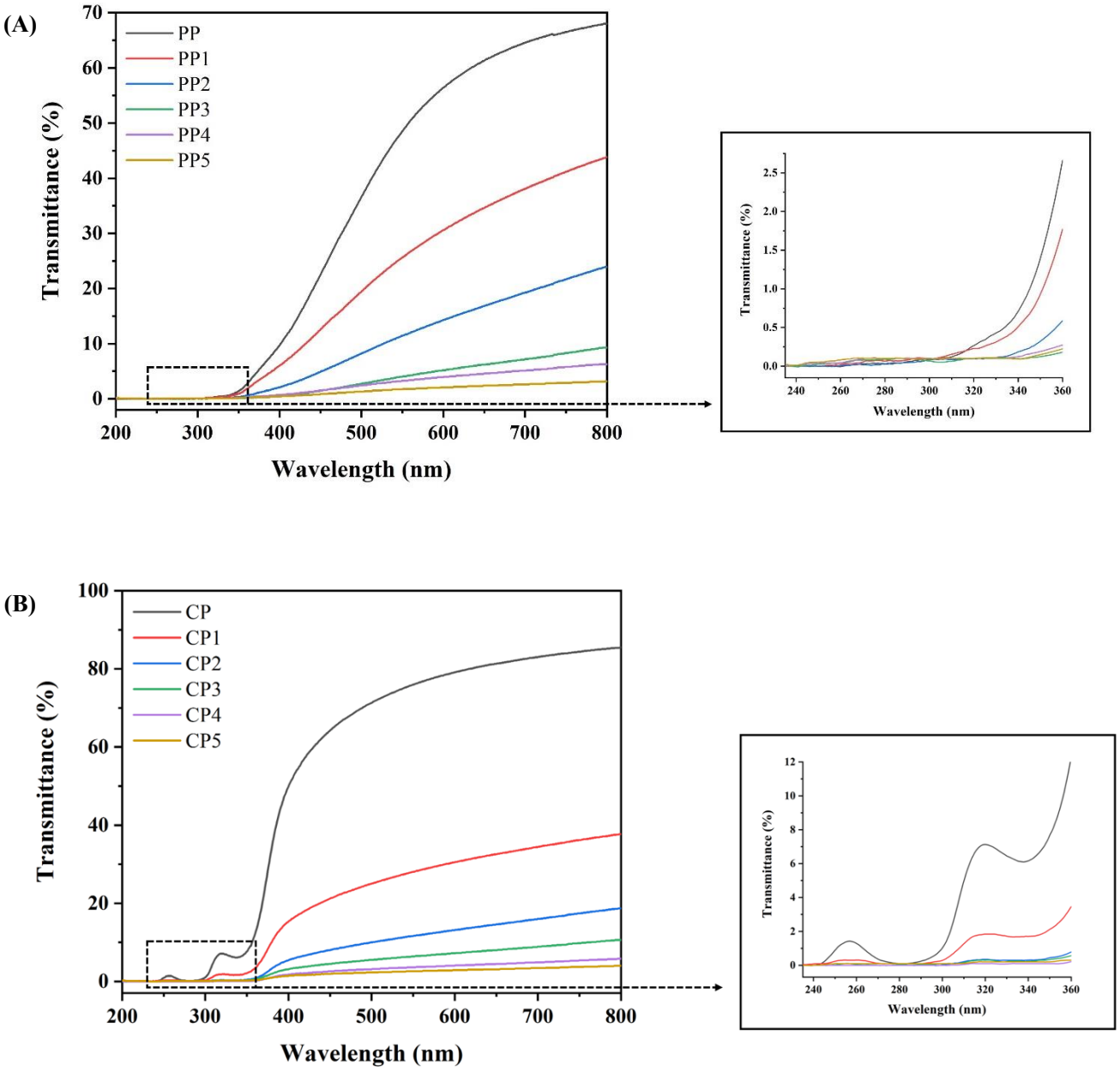

Fig. 3.2: Light transmission (%) at 200–800 nm of the **(A)** plantain peel pectin (PP) film, and plantain peel pectin-BSLNs (PP1, PP2, PP3, PP4, PP5) films, **(B)** citrus peel pectin (CP) film, and the citrus peel pectin-BSLNs (CP1, CP2, CP3, CP4, CP5) films. BSLNs is beeswax solid lipid nanoparticles.

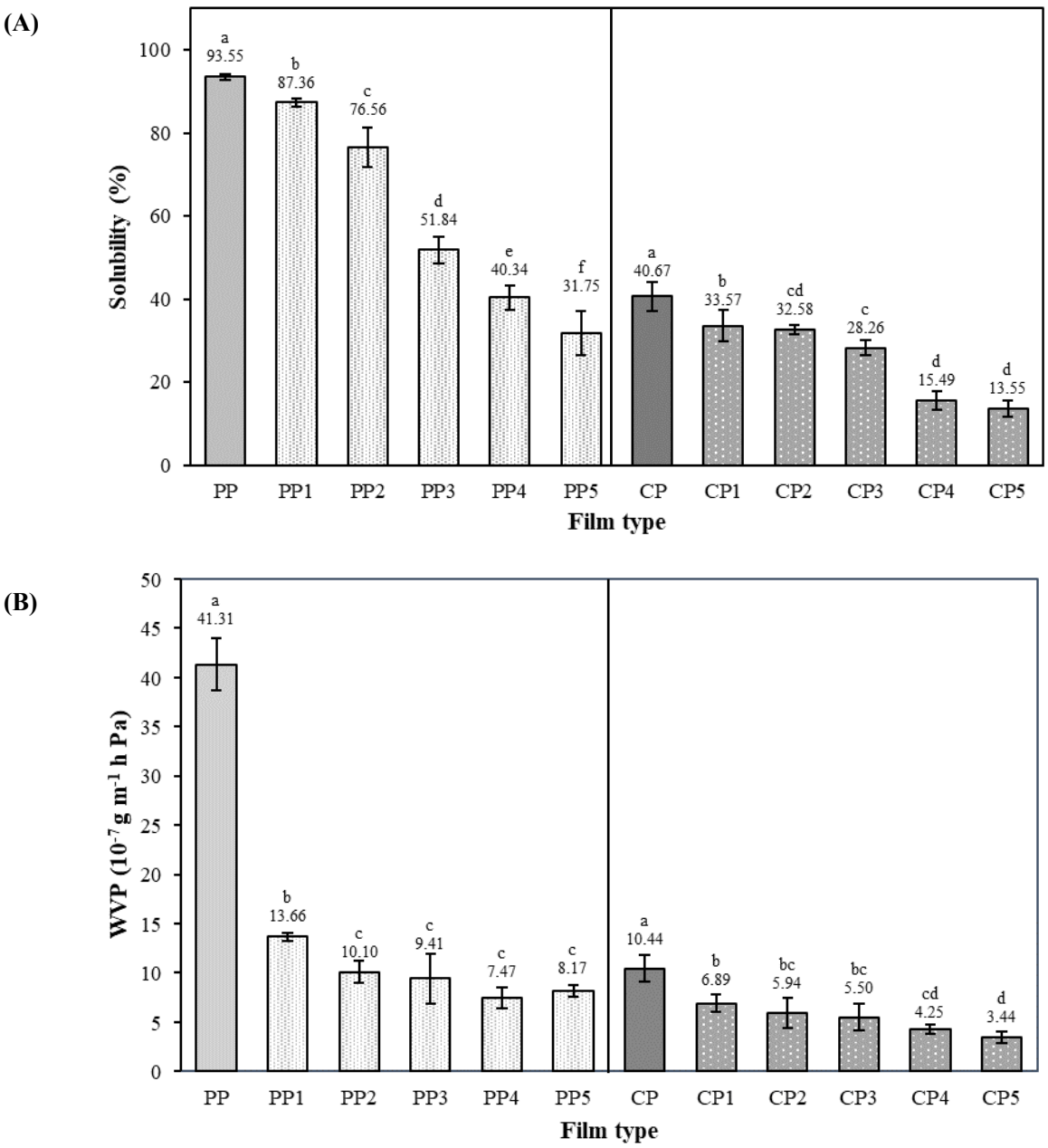

Fig. 3.3: Water resistance properties: The **(A)** water solubility and **(B)** water vapor permeability (WVP) of the plantain peel pectin (PP) film, plantain peel pectin-BSLNs (PP1, PP2, PP3, PP4, PP5) films, citrus peel pectin (CP) film, and the citrus peel pectin-BSLNs (CP1, CP2, CP3, CP4, CP5) films. BSLNs is beeswax solid lipid nanoparticles. Different letters in the same group of film bars represent significant differences among the different films ($P \leq 0.05$).

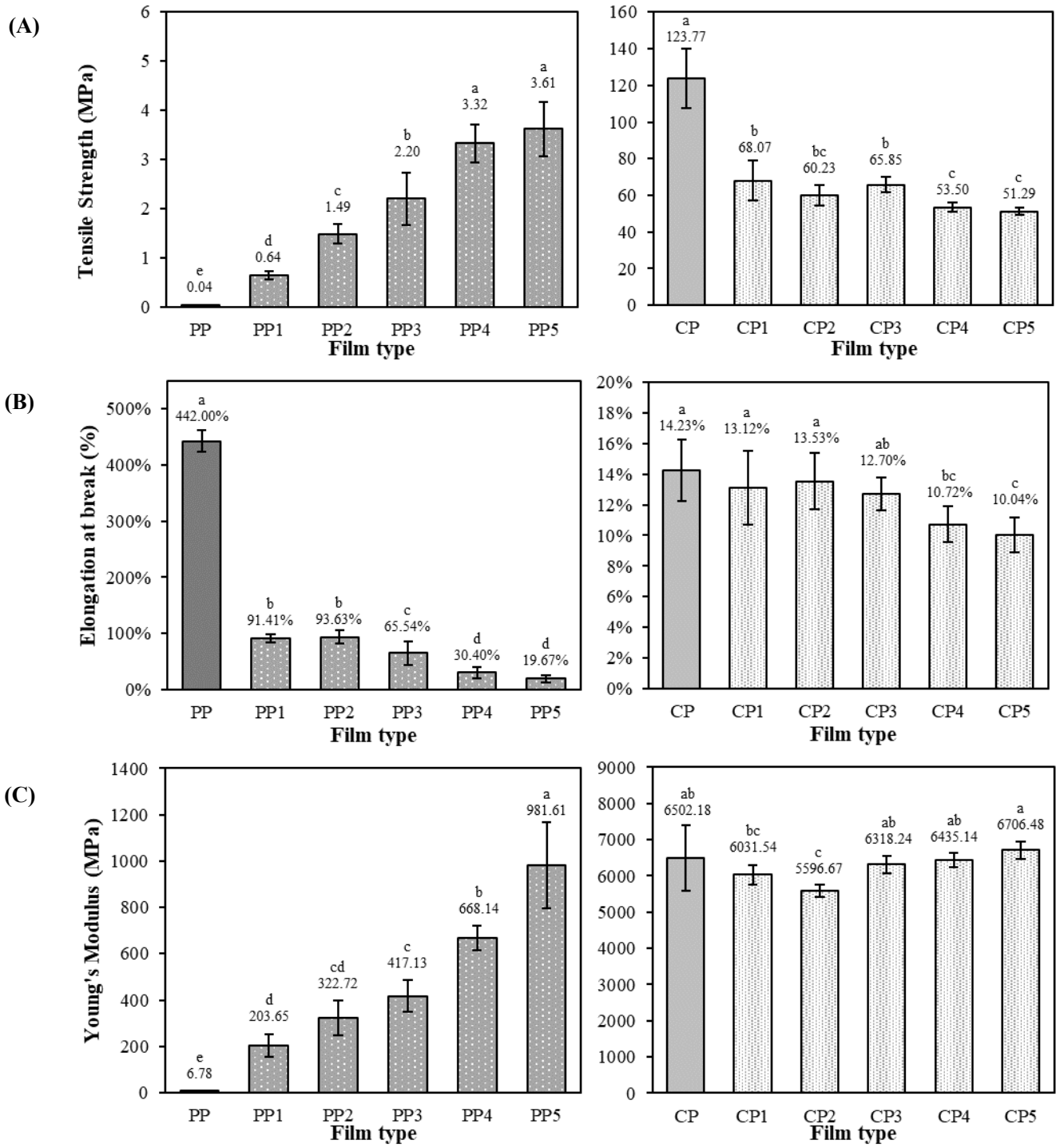

Fig. 3.4: Mechanical properties: The **(A)** tensile strength (MPa), **(B)** elongation at break (%), and **(C)** Young's Modulus of the plantain peel pectin (PP) film, the plantain peel pectin-BSLNs (PP1, PP2, PP3, PP4, PP5) films, the citrus peel pectin (CP) film, and the citrus peel pectin-BSLNs (CP1, CP2, CP3, CP4, CP5) films. BSLNs is beeswax solid lipid nanoparticles. Different letters in the same group of bars represent significant differences among the different films ($P \leq 0.05$).

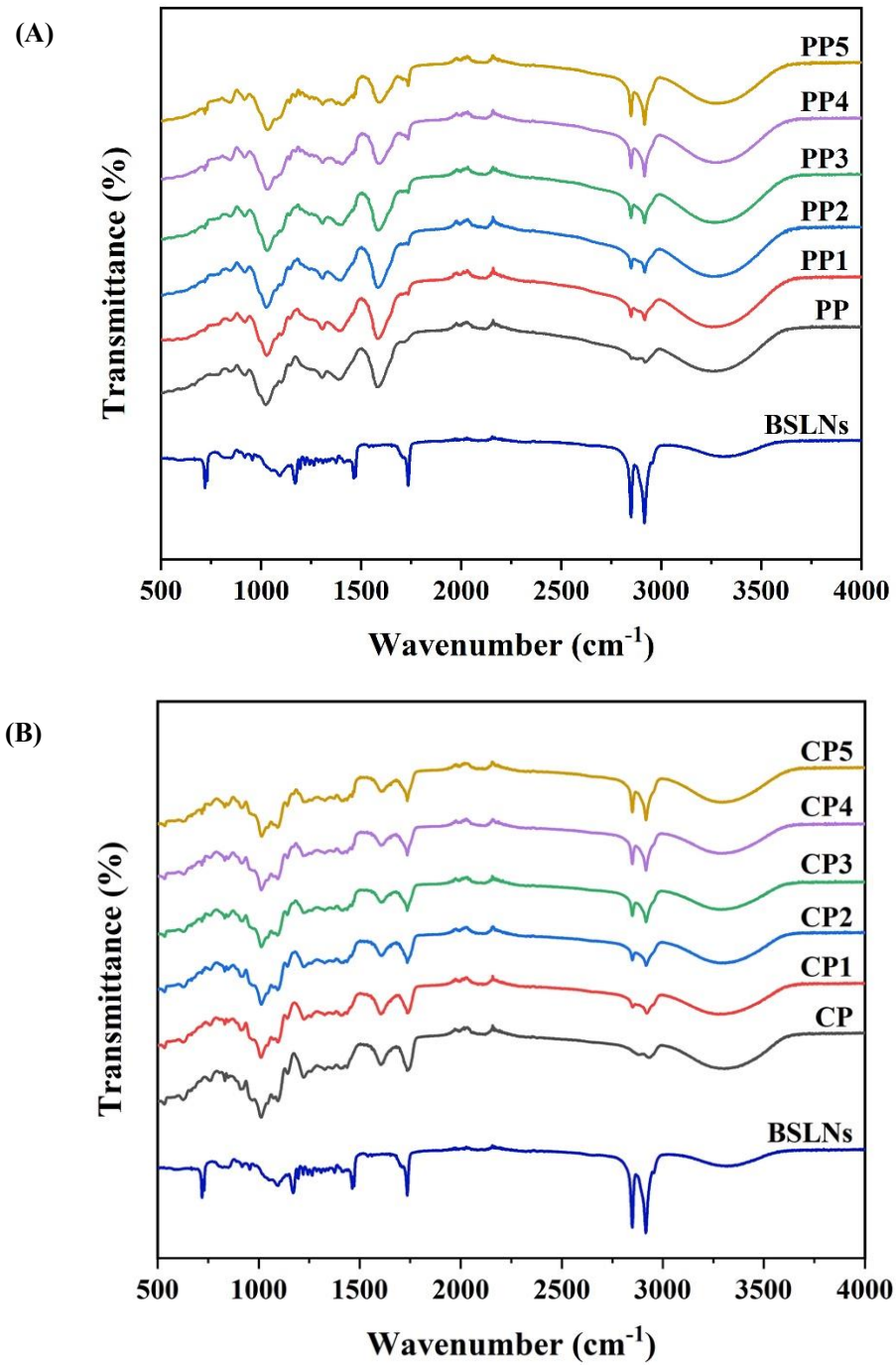

Fig. 3.5: FTIR spectra of the BSLNs with **(A)** plantain peel pectin (PP) film, plantain peel pectin-BSLNs (PP1, PP2, PP3, PP4, PP5) films, and **(B)** citrus peel pectin (CP) film, and the citrus peel pectin-BSLNs (CP1, CP2, CP3, CP4, CP5) films. BSLNs is beeswax solid lipid nanoparticles.

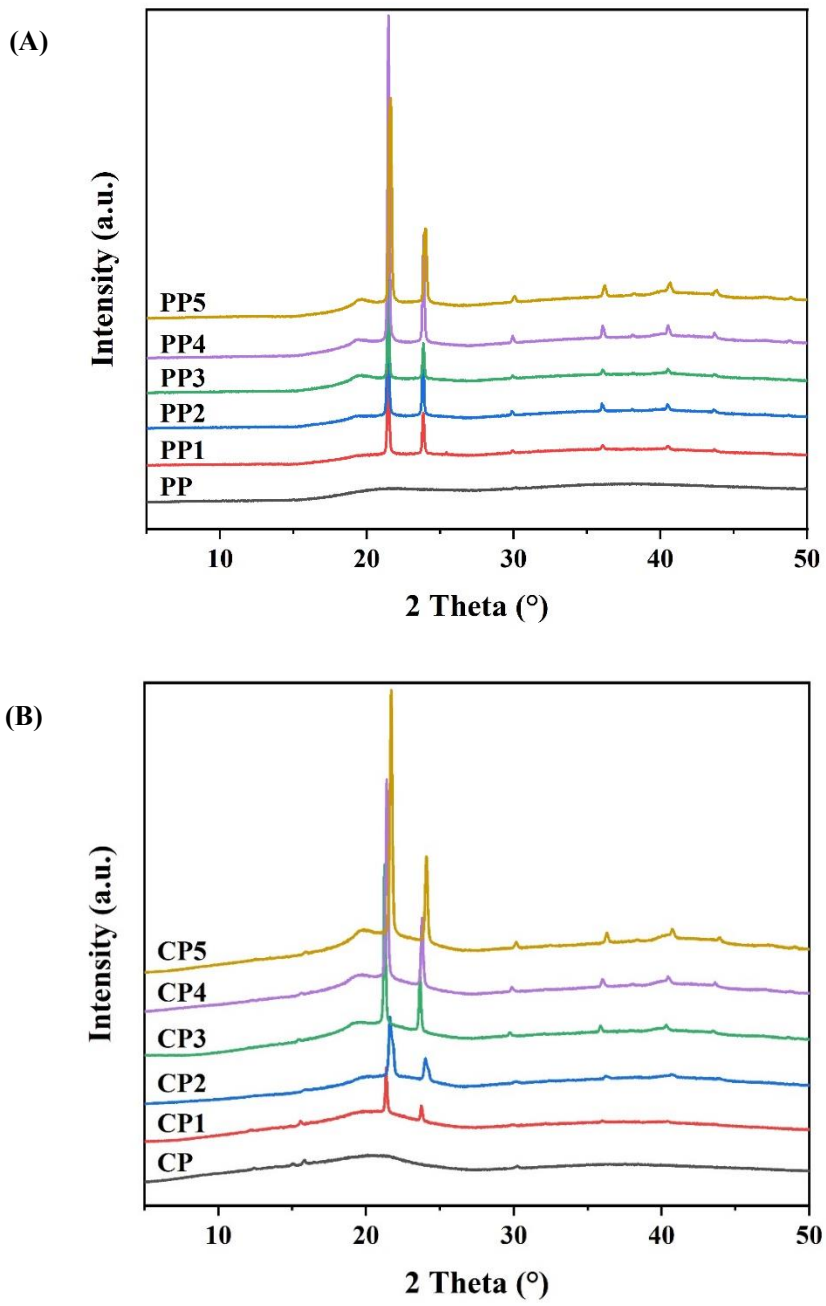

Fig. 3.6: XRD spectra of the **(A)** plantain peel pectin (PP) film, plantain peel pectin-BSLNs (PP1, PP2, PP3, PP4, PP5) films, and **(B)** citrus peel pectin (CP) film, and the citrus peel pectin-BSLNs (CP1, CP2, CP3, CP4, CP5) films. BSLNs is beeswax solid lipid nanoparticles.

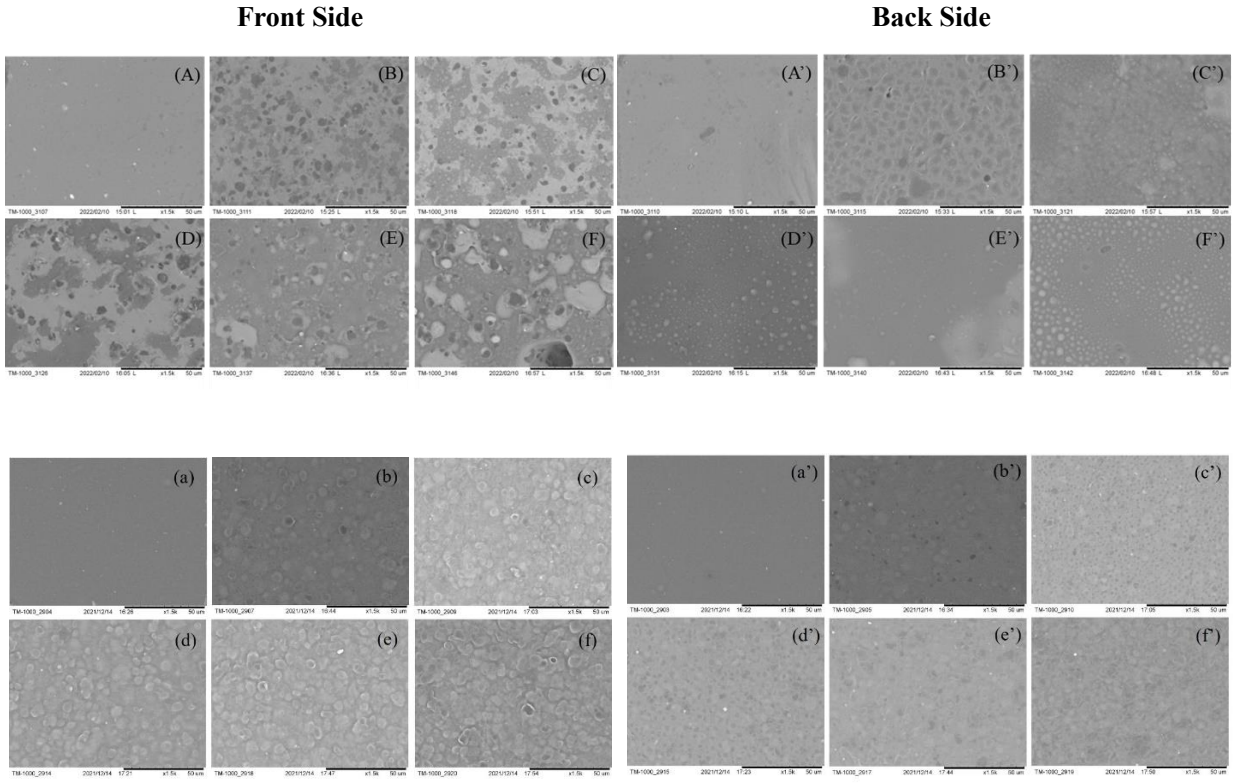

Fig. 3.7: SEM micrographs: Front and back sides of **(A & A')** the plantain peel pectin (PP) film, and **(B & B')-(F & F')** the plantain peel pectin-BSLNs (PP1, PP2, PP3, PP4, PP5) films. Front and back sides of **(a & a')** the citrus peel pectin (CP) film, and **(b & b')-(f & f')** the citrus peel pectin-BSLNs (CP1, CP2, CP3, CP4, CP5) films. BSLNs is beeswax solid lipid nanoparticles.

Table. 3.1 Film formulations of the plantain peel pectin (PP) film, plantain peel pectin-BSLNs (PP1, PP2, PP3, PP4, PP5) films, citrus peel pectin (CP) film, and citrus peel pectin-BSLNs (CP1, CP2, CP3, CP4, CP5) films. BSLNs is beeswax solid lipid nanoparticles.

Films	Ingredient amount in film forming solution (g/100 mL)		
	Pectin	Glycerol	BSLNs
CP	4	1.5	---
CP1	4	1.5	0.5
CP2	4	1.5	1
CP3	4	1.5	1.5
CP4	4	1.5	2
CP5	4	1.5	2.5
PP	4	1.5	---
PB1	4	1.5	0.5
PB2	4	1.5	1
PB3	4	1.5	1.5
PB4	4	1.5	2
PB5	4	1.5	2.5

Table. 3.2: Zeta potential (mV) of the stock BSLNs and the film forming dispersions for PP1, PP2, PP3, PP4, PP5, CP1, CP1, CP2, CP3, CP4, CP5 films. BSLNs is beeswax solid lipid nanoparticles

	Zeta Potential (mV)
BSLNs	− 35.31 ± 2.32 [a]
PP1	− 43.61 ± 2.93 [b]
PP2	− 55.88 ± 2.07 [c]
PP3	− 51.69 ± 1.50 [c]
PP4	− 51.35 ± 0.81 [c]
PP5	− 51.87 ± 6.78 [c]
CP1	− 43.39 ± 0.76 [ab]
CP2	− 49.84 ± 2.19 [b]
CP3	− 47.59 ± 1.85 [b]
CP4	− 46.37 ± 0.94 [b]
CP5	− 49.89 ± 2.14 [b]

Table. 3.3: Calculated area under each light transmission curve at 200–800 nm, and the corresponding light barrier property sequence (a larger sequence number means a stronger light barrier ability).

Films	Area under the curve (nm^2)	Light barrier property sequence
PP	20125.8	2
PP1	11570.6	4
PP2	5555.0	5
PP3	2025.5	8
PP4	1542.4	10
PP5	813.1	12
CP	32216.9	1
CP1	12274.7	3
CP2	5305.7	6
CP3	2964.6	7
CP4	1642.6	9
CP5	1191.8	11

CHAPTER IV

GENERAL CONCLUSIONS, CONTRIBUTION TO KNOWLEDGE AND RECOMMENDATIONS FOR FUTURE WORK

4.1. General conclusions

A simple and efficient green process to recover pectin from plantain peels was developed, which has high possibility to be scaled-up and industrialized in the country of origin of the waste (e.g., African countries). Enzyme cellulase successfully extracted pectin from plantain peels and 50 U was found to produce the most quantity of pure pectin while the increment of enzyme concentration was found to have little effect on the pectin yield. The extraction process recovered the used alcohol solvent at a high recovery rate (93.94%) for recycling to achieve sustainability. The recovered plantain peel pectin was characterized as low methoxyl pectin while the commercially available citrus peel pectin was high methoxyl pectin. Besides, cellulase hydrolytic reaction had no significant effect on the degree of esterification, reflecting the mild nature of this green method.

To study the value addition of recovered plantain peel pectin, it was transformed into composite films successfully of which the reinforcement was achieved by adding different concentrations of hydrophobic beeswax solid lipid nanoparticle (BSLNs). The prepared BSLNs suspension could be stored at room temperature for a long time with high stability. The feasibility and simplicity of the hot-high stirring shearing method used for BSLNs preparation make it suitable for industrial production. Citrus peel pectin was used as a comparative group for the study. It was found that all the pectin-BSLNs films forming dispersions had high absolute zeta potentials which indicated that the formulations were all stable. Both plantain

peel pectin film and citrus peel pectin film were reinforced in water and light barrier properties by adding BSLNs. Apart from that, incorporation of BSLNs into plantain peel pectin film formed a stronger film by increasing its tensile strength while impaired the mechanical properties of citrus peel pectin film by decreasing its tensile strength and elongation at break. Generally, films made with plantain peel pectin showed a much higher elasticity than films made with citrus peel pectin, which could give the package a flexibility to more effectively cover the whole food products, thus are more suitable candidates for edible films.

4.2. Contribution to knowledge

1. A green approach using enzyme cellulase to extract pectin from plantain peels was conducted for the first time. The design of the experiment could also be applied to other agro-waste that are still underutilized.
2. Plantain peel pectin was characterized for its type via degree of esterification testing and FTIR analysis, to furnish this information for the first time in the literature.
3. Reinforcement of pectin film with different concentrations of beeswax solid lipid nanoparticles was conducted for the first time. The enhanced films were assessed comprehensively based on their water and light barrier properties, mechanical properties, chemical composition, crystallinity, and microstructure.
4. Plantain peel pectin was made into pectin composite films and evaluated based on its various properties of which such complete evaluation had not been done in the literature before.

4.3. Recommendations for future work

1. In our experiment, free cellulases were used for pectin extraction. Further studies on utilization of immobilized cellulase for pectin extraction from plantain peels are required in order to complete the green approach design. The catalytic efficiency, recyclability, reusability of the immobilized cellulase and its effect on the pectin yield compared with free cellulase should be investigated.
2. Plantain peels are also rich in other biopolymers like starch or active ingredients like phenolic compounds which are useful ingredients. Future research is justified on residue reutilization by recovering useful compounds as mentioned.
3. The pectin films made in this research were only assessed based on their properties. Future studies on their real application in food products are required to evaluate their potential to become a true alternative to the conventional plastics.

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