# DEVELOPMENT OF SHELLAC-COATED PAPERBOARD WITH IMPROVED PROPERTIES AND ITS VALIDATION AS AN ETHANOL-RELEASING INSERT FOR ANTIMICROBIAL PACKAGING

By

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## ABSTRACT

Fiber-based materials are gaining attention as packaging materials for food and other products. Coatings are required for fiber-based materials to perform as needed. Replacing the petrochemical-based coatings used in fiber-based packaging materials with environmentally friendly alternatives to retain recyclability and biodegradability is desired. These alternatives should offer large-scale manufacturability, low cost, and barrier properties like petrochemical-based coatings for adoption. Shellac is a natural resin with good film-forming properties and hydrophobicity; still, very scarce information on shellac-coated fiber-based materials is available. It opens new possibilities for fiber-based packaging materials based on property improvement (e.g., enhanced barrier) and the capability to carry antimicrobial compounds. This study aims to develop, characterize, and validate multilayer shellac-coated bagasse paperboard with enhanced properties and the ability to release antimicrobial (ethanol). Part 1 of this thesis focuses on the properties improving properties of bagasse paperboard with the effects of shellac layering and concentration. Parts 2 and 3 focus on validating the developed shellac-coated paperboard as an ethanol emitter, its ethanol-releasing capacity, effectiveness against fungal growth (*in-vitro* studies), and a shelf-life study using packaged strawberries.

Bagasse paperboard coated with 2 layers of 40% shellac showed 82% (water vapor permeability), 98.8% (oxygen permeability), and 97% water resistance improvement compared to uncoated paperboard. The bagasse paperboard coated with 40% shellac coating can withstand higher temperatures than cast film. Bagasse paperboard coated with 4 layers of 40% shellac released. 14,200 µL ethanol/L air (ppm) on day 1 and then showed a bi-phasic pattern (80% release within the first 10 days and the rest within 15 days). This ethanol-releasing paperboard reduced *Penicillium* spp. growth by 50% for 4 days and *Botrytis cinerea* growth by > 98% for 7 days in *in-vitro* studies at 23 °C. The developed shellac-coated bagasse paperboard when used as ethanol emitter inside packages with *Botrytis cinerea*-inoculated strawberries reduced mold growth by 30% and strawberry darkening while having no negative effect on fruit respiration, transpiration, and firmness at 23 °C. The developed multilayer shellac-coated paperboard has the potential to be used as a packaging material for food including more sustainable antimicrobial packaging by replacing plastic-based ethanol emitter.

This thesis is dedicated to my Parents, Rajendra and Hemangi Khule, and my brother, Sumeet Khule. Thank you for always supporting and believing in me.

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## TABLE OF CONTENTS

## LIST OF ABBREVIATIONS

| | |
|---|---|
| BP | Bagasse paperboard |
| WVP | Water vapor permeability |
| WVTR | Water vapor transmission rate |
| OP | Oxygen permeability |
| OTR | Oxygen transmission rate |
| EE | Ethanol emitter |
| CFU | Colony forming units |
| MAP | Modified atmosphere packaging |
| CAP | Controlled atmosphere packaging |
| MFC | Microfibrillated cellulose |
| NFC | Nanofibrillated cellulose |
| PS | Polystyrene |
| PE | Polyethylene |
| EVOH | Ethyl vinyl alcohol |
| PLA | Poly(lactic acid) |
| PET | Poly(ethylene terephthalate) |
| PHA | Poly(hydroxyalkanoates) |
| PFAS | Perfluoroalkyl and Polyfluoroalkyl Substances |

# CHAPTER 1: INTRODUCTION

## 1.1. Introduction

Plastic pollution is an escalating environmental problem due to the presence of hundreds of millions of tonnes of plastics disposed of in the environment with single-use plastic packaging being a major contributing component (Phelan et. al. 2022). Consumer awareness, corporate sustainability aims, limited landfill sites and changing regulations have led to a focus on bioplastics and fiber-based packaging materials (Almenar et al., 2023; Kathuria & Zhang, 2022). Fiber-based packaging like cartons, disposable food containers, fiber bottles, and molded fiberboard trays are made from recycled paper, bagasse (waste produced after the sugarcane juice extraction process) pulp, and many other fiber-based materials. The global fiber-based packaging market size was reported to be reached USD 365 billion in 2022 and is expected to hit USD 527 billion by 2032 (Fiber based packaging market forecast, 2023). Fiber-based materials are considered ecologically sustainable materials for packaging applications due to their natural abundance, low cost, renewable origin, sturdiness, compostability, biodegradability, and repulpability (Kathuria and Zhang et al., 2022; Semple et al., 2022). In contrast to these benefits, the major limitations of overall fiber-based packaging is its highly porous structure of fibers making it hydrophilic and thus a poor barrier to vapors, gases, water, and oil (Basak et al., 2024). To overcome these drawbacks, paper coated or laminated with petroleum-based plastics like been widely investigated and commercialized) (Basak et al., 2024; Shankar & Rhim, 2018). The polymer-based coatings can address the aforementioned limitations, but their unfavorable properties like toxicity (e.g., microplastics), leaching into landfills, and jeopardizing paper recyclability and/or biodegradability have created huge awareness to eliminate them (Basak et al., 2024; Khwaldia et al., 2010).

These drawbacks have brought the attention of academia and industry to the development of coatings using natural bio-based materials like polylactic acid, chitosan, starch, and natural waxes as well as synthetic biodegradable materials like polybutylene adipate terephthalate (Kansal et al., 2020; Rastogi & Samyn, 2015; W. Zhang et al., 2014). The above natural bio-based materials have been broadly investigated, but others like shellac have not. Shellac is a naturally occurring polymer refined from a resinous substance excreted by an insect, *Laccifer lacca*, which is parasitic on certain trees, especially in Asia (Yan et al., 2021). It has good functional properties like hydrophobicity, film-forming capability, low molecular weight, great adhesion, biodegradability, oil resistance, nontoxicity, and solubility in many solvents (Yuan et al., 2021). Furthermore, it is

approved for food contact by the U.S. Food and Drug Administration (FDA, 2024). Thus, shellac as a composite, edible film, and coating material for films has widely been studied (Ahuja & Rastogi, 2023; Byun et al., 2012; Saberi et al., 2017; Thombare et al., 2022). However, very little is known about shellac as a paper coating. Hult et al. (2010) and Klayya et al. (2023) reported the improved barrier properties of paper and paperboard when coated with either a layer of shellac blended or as a top layer of microfibrillated or nanofibrillated cellulose. Despite the potential of shellac to enhance paper properties while maintaining the paper environmental benefit of recycling and composting as end of life due to shellac biodegradability (Hult et al., 2010), the potential of shellac by itself as a paper coating is basically unknown.

With the use of shellac as a coating for fiber-based food packaging materials, it also has significant potential in specific food packaging applications i.e. Antimicrobial Packaging. Fresh produce spoils fast because of fungal growth (Davies et al., 2021). Although refrigeration can slow the growth of molds, fresh fruits and vegetables are not always exposed to low temperatures in supply chain and even some of them cannot handle cooling due to chilling injury (Kader, 2002). This has led to the development of different postharvest treatments aiming to reduce microbial growth in fresh produce thereby extending produce shelf life and reducing produce waste (Alves et al., 2023). These include heat treatments, edible coatings, and modified atmosphere packaging (Yang et al., 2010). However, these treatments are not suitable for all types of commodities or simply not feasible for fungal (Yang et al., 2010) reduction for extended periods of time. Thus, there is an urgent need to develop alternative technologies to minimize disease occurrence and extend the shelf-life of fresh produce. Antimicrobial packaging involves the exposure of the food product to a compound that reduces or inhibits microbial growth during the entire commercialization period (Almenar, 2021; Duguma et al., 2023). Many different antimicrobials have been used to create antimicrobial packages including ethanol. Ethanol is highly effective against the growth of bacteria, molds, and yeasts (Mexis and Kontominas, 2014). With post-harvest ethanol treatment, food-grade ethanol has most recently been commercialized in the form of ethanol-emitting sachets, commonly known as ethanol emitters (Ueno, 2024). The latter consists of ethanol vapor being released from a carrier material placed inside a plastic sachet microperforated to control the release. Either commercially available ethanol-emitting sachets or similar ones developed by academia are effective against fungal growth in fresh produce including whole and fresh-cut cherries (Bai et al., 2011), mulberry fruit (Choosung et al., 2019), and grapes (Candir et al., 2012).

Furthermore, these studies have shown that the released ethanol enhanced the aroma profile of mulberry fruit (Choosung et al., 2019), retarded softening, darkening and acid decrease in cherries (Bai et al., 2011), and lowered weight loss and had no adverse effect on stem and grape color, and titratable acidity (Candir et al., 2011).

Due to the risk of accidental sachet consumption (Bodry et al., 2001), industry switch from sachets to active packaging material (Almenar, 2020) and need of plastic free and non-toxic packaging (Almenar et al., 2023), alternative solutions for the conventional ethanol-emitting sachet can contribute to their acceptance and use in produce packaging. The replacement of commercial petrochemical sachet materials like polyvinyl alcohol (PVOH, ethylene vinyl alcohol (EVOH), and polyethylene (PE) with bioplastics (Mugasundari et al, 2022) and carrier materials with paper, and wool is also investigated (Li et al., 2018; Choosung et al., 2019). But this transition from commercial sachet material and carriers to biobased and non-toxic materials is hurdled due to same issues as common plastic sachets and pads while others cannot be scaled up for commercialization due to the lack of feasibility (e.g., controlled release of ethanol for an extended period). Strawberry fruit (*Fragaria × ananassa Dutch.*), the second most popular fruit in the US among many of fresh produce (The Packer, 2023), is highly perishable which leads to a very short shelf life (Ladika et al., 2024) due to fungal decay caused by *Botrytis cinerea*, *Rhizopus stolonifera* and *Penicillium spp*. (Trinetta et al., 2020). Li et al. (2018) reported that ethanol vapor released from filter paper effectively reduced fungal growth, softening, and weight loss in fresh-cut strawberries besides improving their antioxidant capacity. Despite these promising results, the effectiveness of an ethanol emitter on extending the shelf life of intact strawberries has not yet been assessed even if their susceptibility to decay, and contribution to produce waste (economic losses) is significant. To the best of my knowledge, at present, there is no such ethanol emitter with no plastic film (sachet material) involved can generate and control ethanol release rate to delay fungal growth and slow down quality changes in produce during retail storage period.

### 1.2. Goal and objectives

This study aimed to develop, characterize, and validate multilayer shellac-coated bagasse paperboard with enhanced properties and able to release antimicrobial ethanol.

Main objectives:

**Part 1: Development of shellac-coated paperboard**

- To investigate the effects of the concentration of shellac on the water vapor and oxygen barrier and surface properties for shellac-coated paperboard.
- To investigate the effects of the number of layers of shellac coating on the barrier and surface properties of the paperboard.
- To understand and improve the thermal properties of shellac when coated on BP.

**Part 2: *In-vitro* validation of the antifungal capacity of the developed material**

- To understand the ethanol-releasing capacity of shellac-coated BP as affected by the number of layers, time, and temperature.
- To investigate the effectiveness of the developed material against *Penicillium* sp. and *Botrytis cinerea* growth.

**Part 3: *In-vivo* validation of the antifungal capacity of the developed material**

- To investigate the effectiveness of the developed material against *Botrytis cinerea* growth in packaged fruit.
- To understand the effects of ethanol-releasing shellac-coated BP on fruit's weight loss, respiration, texture, and color.

**1.3. Hypotheses**

Part 1: General Hypothesis: The appropriate concentration and number of layers for shellac coating improves the oxygen and water barrier properties and surface properties of the paperboard.

H1: An increase in the concentration of shellac improves barrier and surface properties of shellac-coated BP.

H2: An increase in the number of layers of shellac coating improves the barrier and surface properties of shellac-coated BP.

H3: The thermal resistance of Shellac-coated BP is higher than shellac-cast films of the same composition.

Part 2: General Hypothesis: Shellac-coated BP can release ethanol at a controlled rate resulting in antifungal effect.

H1: The shellac-coated paperboard can release the ethanol in a controlled manner.

H2: There is an effect of number of layers coating, temperature and time on the releasing capacity of the shellac-coated BP.

H3: The shellac-coated BP can release ethanol to reduce and/or inhibit *Penicillium spp*. and *Botrytis cinerea* fungal growth.

Part 3: General Hypothesis: Shellac-coated BP can be used as an ethanol-emitting packaging insert with the ability to reduce or inhibit mold growth and extend the shelf life of the strawberries.

H1: The shellac-coated BP can reduce or inhibit Botrytis cinerea growth in fresh strawberries.

H2: The ethanol released from the shellac-coated BP will not affect the physiochemical attributes of the strawberries.

**1.4. Structure of Thesis**

CHAPTER 1 covers the introduction, goals, objectives, and hypothesis. CHAPTER 2 consists of the literature review conducted. This thesis is divided into three parts where Part 1 involves the development, characterization, and validation of shellac-coated BP with different numbers of layers and shellac concentrations to improve the properties of the paperboard. CHAPTER 3 focuses on the Part 1 of the study. Parts 2 and 3 focus on validating the developed shellac-coated bagasse paperboard as an ethanol emitter by studying its ethanol-releasing capacity and its effectiveness against fungal growth in *in-vitro* studies and a shelf-life study using packaged strawberries. CHAPTER 4 focuses on these two Parts. CHAPTER 5 focuses on the conclusion derived from both the parts and the future work.

# CHAPTER 2: REVIEW OF LITERATURE

## 2.1. Introduction to shellac

Along with different proteins and carbohydrates, lipid components including natural waxes, various fatty acids, surfactants, essential oils, and resinous and non-resinous compounds have been commonly applied as water barrier coating in a hydrocolloid matrix. Shellac, a naturally extracted resinous wax compound has been used as a low-cost, low-toxic barrier coating in the food industry to extend the shelf-life of many products, and in the pharmaceutical industry for moisture protection of drugs, controlled delivery system for various drugs and as an enteric coating for probiotics and drugs (Yuan et al., 2021).

It is a natural polymer refined from a resinous substance excreted by an insect, *Laccifer lacca*, which is parasitic on certain trees in India, Thailand, China, and a few other countries (Yan et al., 2021). The global Shellac market size was estimated to be worth USD 159 million in 2021 and is forecast to a readjusted size of USD 181.7 million by 2028 with a CAGR of 1.9% during the forecast period 2022-2028 (Global Shellac market report, 2022). It has been in demand recently for many applications and has been used in India and neighboring countries in its pure form as a red-colored dye, decorative coating, wood furnish, and fruit wax for centuries now due to its natural, non-toxic, biodegradable and eco-friendly nature (Azouka et al., 1993a; Kumar et al., 2022; Thombare et al., 2022).

### 2.1.1. Chemical structure

The earliest recorded work on the shellac chemistry and composition revealed and confirmed the components i.e. aleuritic acid and the structure is shellolic acid with other acids like butolic acid, jalaric acid, laksholic epilaksholic acid, laccishellolic acid etc (Azouka et al., 1993). This complex biomacromolecule has a special molecular structure consisting of aleuritic acid and cyclic terpene acid behaving as hydrophobic and hydrophilic parts of this amphiphile molecule, respectively. In addition, this oligomeric resin has functional groups like -OH, -COOH, -CHO, by which many other polymers could interact with it by forming an H-bond or electrostatic interaction resulting in the fabrication of functional materials (Luo et al., 2016 and Azouka et al.,1993). These compositions can slightly differ based on the insect species and the host tree from which the shellac in its raw form is obtained (Azouka et al., 1993; Farag & Leopold, 2009a).

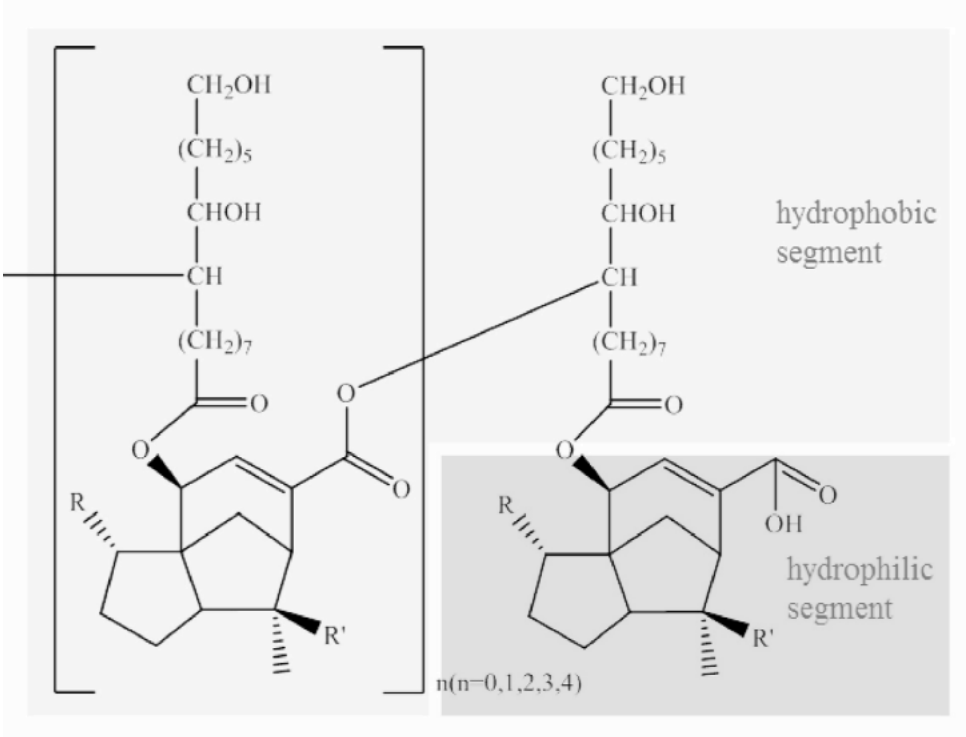


**Figure 2.1.** Chemical structure of shellac (Reused with permission from Yuan et al. (2021)) Copyright Elsevier (2021).

### 2.1.2. Extraction and manufacturing of shellac

When the lac cultivation cycle starts, broodlac i.e. a section of stick from the host tree chopped from encrusted with Laccifer Lacca is collected (Derry, 2012). This broodlac is then tied to an appropriate host tree that is considered for shellac harvesting. When the lac is harvested, the encrusted branches are removed and the lac pieces are scraped off, called sticklac. This sticklac is converted into seedlac by washing and crushing using a millstone or pounding machine into small uniform-shaped pieces with no contaminants (Derry, 2012). Next important step is to convert seedlac to shellac which can be done by three different processes.

The first one, the most traditional one is hot filtration where hot melted seedlac is filtered through a sieve-like cotton hose separating wax containing shellac as a product (Farag & Leopold, 2009). The second most common method used is bleaching where bleached shellac is obtained by treating the dissolved seedlac with sodium hypochlorite or other bleach agent (Farag & Leopold, 2009). Luangtana-anan et al., (2021) stated that sensitive groups like hydroxyl and carboxyl groups are increased due to the bleaching process and thus, bleached shellac has a higher tendency of polymerization and becomes stable. The most suitable refining process is the solvent-extraction

process in which seedlac is dissolved in an alcohol solution, decolorized by activated carbon treatment, filtered, and is then cast into a thick film (Derry, 2012; Farag & Leopold, 2009).

### 2.1.3. Properties of shellac

Shellac as a resinous polymer has good functional properties like hydrophobicity, film-forming capability, great adhesion, pH responsiveness, low molecular weight, solubility in many solvents, among others (Yuan et al., 2021). Its molecular weight is around 1,000 g/mol and has a density of around 1.035-1.21g/mL (Sulthan et al., 2023). The tensile strength of shellac casted film is similar to polyethylene (PE) but has a low % elongation at break (Phan The et al., 2008; Sulthan et al., 2023). Thus, it is not considered a choice as a flexible packaging material (Phan The et al., 2008; Sulthan et al., 2023).

In terms of thermal properties, shellac's softening point is around 65℃ (Ahuja & Rastogi, 2023; Thombare et al., 2022). The glass transition temperature (Tg) and melting temperature (Tm) of shellac are around 30-50℃ and 60℃ based on the different types of shellac (Ahuja & Rastogi, 2023; Thombare et al., 2022). A natural tendency of shellac, aging can occur when shellac is exposed air (Yan et al., 2021). Long-term heating of shellac past a melting point will lead to hard, horn-like intractable compounds due to inter-esterification at high temperatures (Sulthan et al., 2023; Yuan et al., 2021). After heating shellac until its molten state, it changes into a rubbery condition which is a stage between the fresh and old shellac. Eventually, the rubbery state transforms into a brittle, firm structure making it a thermoset (Yan et al., 2021).

With this structural chemistry, major drawbacks include aging which leads to brittleness (Phan The et al., 2008), self-esterification, insolubility in solvents, low transparency owing to orange and brown color, poor mechanical properties and stability together influencing decrease in barrier (Luangtana-anan et al., 2017a, 2017b; Phan The et al., 2008 over a period of time. Many of these issues may be resolved by physical blending or chemical reactions with other materials or additives or structural modifications (Ahuja and Rastogi 2023). Research investigations has been focusing on the addition of plasticizers, curing of the compound, bilayer coatings with other biopolymers, blending, and modifications of the structure.

### 2.1.4. Recent work and its application

Different forms of wax-containing or wax-free (dewaxed) forms as pure orange shellac, dewaxed orange shellac, bleached shellac, and refined bleached shellac are commonly used for different applications. It has been investigated to produce shellac-based edible films and fruit coatings with

improved thermal, mechanical, and barrier properties with stable film (Kumar et al., 2022, Ahuja & Rastogi, 2023, 2024).

In terms of packaging, shellac films have an elongation at break low as 3.05%, brittleness and aging over time, making it unsuitable for flexible packaging applications. Its application as composite films and coating in terms of packaging material is being obstructed due to its drawbacks caused by aging (self-esterification) which leads to film brittleness, insolubility in solvents, and stiffness over time (Ahuja & Rastogi, 2023; S. Kumar et al., 2022; Thombare et al., 2022). Different plasticizers like Polyethylene glycol (PEG) (Khairuddin et al., 2016; Luangtana-anan et al., 2017; Phan The et al., 2008) stearic acid, lauric acid, Tween20 (Byun et al., 2012; Saberi et al., 2017), and glycerin (Phan The et al., 2008; Zhang, et al., 2021) have been investigated for improved film properties with increased % elongation. Shellac when blended with other polymers or compounds like chitosan (Yuan et al., 2021), agar and cassava (Phan The et al., 2008), CMC (Mohamed et al., 2019), HPMC (Asrar, 2012), pea starch/ guar gum (Saberi et al., 2017), soybean protein isolate (Zhang et al., 2020) results in films with significant improvement properties like Cobb, water vapor barrier and % elongation of shellac films. The use of shellac as a paper-coating packaging material has not been investigated widely so far and is discussed in section 2.2.7 (Hult et al., 2010; Klayya et al., 2023)

## 2.2. Paper-based packaging

Paper as well as other pulp-based products are considered as an option instead of single-use plastic for many applications including food packaging because of its eco-friendly nature, abundant resources, low weight, good mechanical properties like stacking strength, biocompatibility, and recyclability (Coltelli et al., 2016; Dey et al., 2020; Semple et al., 2022). With numerous applications from packaging to Printed media, paper-based products are also used in food packaging in paper bags, composite cans, fiber drums, and rigid boxes. Folding cartons, multilayer structures (e.g. Tetra Pak, Pure-Pak) are used as primary packaging material for cereals as bag -in-the-box, milk, and juice cartons.

### 2.2.1. Manufacturing of paper and paperboard

The basic paper manufacturing processes include pulping, bleaching, and papermaking (Semple et al., 2022). Two major types of pulping processes are mechanical and chemical pulping. Mechanical pulping takes place by cleaning and grinding the pulp to separate fibers (except lignin) which results in a reduced fiber length and strength. The chemical pulping process is the separation

of lignin from cellulosic fibers by heat and chemical treatment (such as alkaline sulfate process, also called as 'kraft process' and acid sulfate process) in a chemical digestor (Dey et al.,2020).

Molded fiber packaging products are manufactured by transfer molding, one-cast, and thermoforming (Semple et al., 2022a). The manufacturing process affects the tensile strength, hydrophobicity, stability, and other products based on the product thickness, network porosity, and evenness of the material thickness. Thermoforming, also called as "thin-wall" or "precision" molding is a commonly used method. Unlike conventional casting and drying method, thermoforming includes hot pressing, curing and drying in one single step (Semple et al., 2022).

### 2.2.2. Molded fiber products

Molded fiber product has been one of the packaging sustainable solutions widely used for disposables, egg cartons, and thermoformed trays for protecting various products during transportation and is derived as a solution to replace plastic thermoformed trays. They contribute to production volume of 30% of total paper packaging when made from recycled paper, Polylactic acid (PLA), straws, bagasse (sugarcane waste), and other sources. Molded paperboard containers have a potential to replace PET/PLA containers and/or clamshells used for packaging fruits and vegetables (like strawberries, raspberries, kiwis, jalapenos, cucumbers, and many others), meat tray package and many other applications. It has been derived as a solution to replace plastic thermoformed trays and many other plastic packaging systems. In contrast, a major limitation of overall paper-based packaging is its highly porous structure of fibers with hydroxyl (-OH) groups making it a poor barrier and hydrophilicity (Basak et al., 2024).

### 2.2.3. Bagasse paperboard (BP)

Paper, paperboard, and molded fiber products from various renewable resources like recycled paper products, and inedible fibrous wastes (stalks, leaves, seedpods) are gaining lot of attention since few years now. There are many examples available in the literature for the use of molded pulp-based products from wheat straw (S. Singh et al., 2011), flax, cotton stalks, banana bunch cellulosic fiber (Arévalo et al., 2019), bamboo (Suhaimi et al., 2022) sugarcane waste called as "bagasse", rice straws, pineapple leaves, and banana stems. Bagasse is a naturally occurring by-product made from sugar cane processing (Hossam & Fahim, 2023). Bagasse would wind up in a landfill if businesses did not utilize it and turn it into valuable things like biodegradable paper-based packaging products with minimum additional processing (Hossam & Fahim, 2023). As per the publications, in 2019, approx. 279 million metric tons of sugarcane waste (including bagasse)

was generated by milling and processing of huge amount (1949 million tons) of sugarcane (R. V. Singh et al., 2022). The highest production was reported by Brazil followed by many other countries such as India, China, Thailand, Pakistan, Mexico, Colombia, Indonesia, Philippines, and the United States (R. V. Singh et al., 2022).

Bagasse-based products are also manufactured similar to the paper products. Following the crushing and juice extraction of sugar cane stalks, a fibrous residue bagasse is converted into wet pulp which is then compressed and turned into a dry pulp board. Feeding through a molding device is the last step used for shaping the board into a product like a plate, bowls, trays, and many others. Sometimes it is combined with additives, fillers, and/or coatings to increase the strength or substance that repels water and oil for food packaging application. Bagasse is found to be one of the good alternatives to wood used for papermaking because of its abundance, long fiber length, low cost, low refining energy requirements and consumption, good sheet formation, and smoothness of the products (Bhardwaj et al., 2023).

### 2.2.4. Properties of paper and paperboard products

Paper and paperboard products are widely used for packaging applications where the generic difference between paper and paperboard is the grammage i.e. anything greater than 250 g/m$^2$ (GSM) is considered as paperboard (Robertson, 2013). Different variants of paper like kraft paper, bleached paper, glassine paper, grease-proof paper, and others are used for various food packaging applications. Kraft paper is generally machine-glazed possessing very course structure and very high strength, while bleached paper is soft, expensive and white (Deshwal et al., 2019). Similarly, different types of paperboards like linerboard, food board, folding boxboard, chipboard, whiteboard, clay-coated paperboards and others are commonly known for their strength, durability, and usability for wide range of applications with direct food contact and secondary package (Deshwal et al., 2019). Bagasse paper pulp material has been emerging as a great substitute to molded trays and disposable containers because can tolerate heat up to 200 ºC as opposed to other paper products like kraft paper, its biodegradable and compostable with no residues after complete degradation (R. V. Singh et al., 2022).

With these properties, paper-based products and their affinity towards moisture leads to restriction to be used as a packaging material. The surface absorbance of the water by the paper is measured by Cobb test wherein typical values for business paper ranges (22-26 g/m$^2$), unsized paper (>50 g/m$^2$) and for bagasse molded pulp tableware containing approx. 5% moisture repellant (15-20

$g/m^2$). In terms of water permeability, the porous cellulose structure of microfibrils, composed of long-chain cellulosic molecules in a crystalline state with amorphous regions disrupting the crystalline structure leads to the fiber network porosity. This limits the water-vapor-barrier properties of paper as it easily absorbs water or moisture from the environment or from food resulting in the migration of permeants (water molecules) by diffusion through void spaces, pinholes as well as in condensed form through the fiber cell wall (Khwaldia et al., 2010). The moisture absorption by the fibers affects its physical and mechanical strength where a 5-10% decrease in strength for every 1% increase in moisture can be observed (Khwaldia et al., 2010).

### 2.2.5. Coating and barrier properties

As mentioned in section 2.2.5 almost all papers are porous and very hydrophilic by nature, they provide issues in various packaging applications owing to destitute intrinsic capabilities for water, oxygen, and oil barriers (Basak et al., 2024b) Petroleum-based polymers (Poly(ethylene terephthalate) (PET), Poly(lactic acid) (PLA), Polyethylene (PE), perfluoroalkyl and poly-fluoroalkyl substances (i.e. PFAS), and waxes are extensively utilized in paper coatings to improve its oil and water resistance. Using different techniques like co-extrusion, extrusion coating (curtain coating), bar (rod) coating, spray coating, and knife coating are used widely in the paper industry to produce coated paper products (Basak et al., 2024b). Base coatings promote surface water and oil resistance and barrier properties, but their unfavorable qualities; loss of recycling and biodegradability have created awareness to eliminate (Basak et al., 2024b; Khwaldia et al., 2010). Separating laminated or coated plastics from paper is a big challenge and thus, plastic-coated paper packaging technology is not recycled on a large scale currently ending up in landfills many times.

### 2.2.6. Need for sustainable coating solutions

Few studies have been done to improve the recyclability of plastic-coated paper by adding a water-soluble layer between the paper and plastic layers that enables their separation (Tyagi et al., 2021). Moreover, the migration of toxic compounds from the coatings are potentially toxic to humans (Begley, 2008). They are commonly used for fast-food paper packaging, microwavable bags, wrappers and others. Stringent regulations have been placed on the use of PFAS with only 8 types of PFAS allowed by the FDA (FDA, 2024). Thus, packaging producers and converters are moving towards different sustainable and non-toxic materials. PFAS and similar hazardous compounds have also been a huge issue in molded fiber products which leads to migration of PFAS in food product and leaching into the soil if landfilled.

Tackling this, new commercial biodegradable polymers have been appealing to the food packaging industry. The candidates like the natural biodegradable polymers including poly amino acids like plant/microorganism proteins (e.g. soy protein and whey protein), polysaccharides (e.g. starch, chitosan, and cellulose) (Amorin-da-Silva et al., 2024; Obradovic et al., 2017; Shankar & Rhim, 2018), lipids and waxes (e.g. beeswax (Santos et al., 2023)), (ii) polymers produced by microorganism through secondary metabolism occurring to store energy in the form of esters and polyesters (e.g., polyhydroxyalkanoates (PHA) and poly(lactic acid) (PLA) (Rhim et al., 2007), and (iii) mix-and-match blend polymers which are based on materials either chemical (e.g., ester/amide copolymers) or physical (polymer/Thermoplastic starch blends). Nevertheless, most biopolymers are not still used in industrial applications because of the challenges in manufacturing them on a large scale due to the material's innate water-holding capacity, crystallinity, and others (Basak et al., 2024b).

Shellac as a natural film-forming resinous compound has been investigated for various applications and is a good candidate for paper coatings. However, there is very limited knowledge about shellac as a coating material for paper. (Hult et al., (2010) investigated shellac coating blended with microfibrillated cellulose (MFC) or as a base coat of MFC proving improved water vapor barrier and tensile properties. Also, it was demonstrated that shellac helped fill up pores left in the MFC base coating resulting in improved oxygen barrier. (Klayya et al., (2023b) investigated bilayer coating with shellac and shellac with cellulose nanofibrills (NFC) and modified cellulose nanofibrils (mNFC) coating showed almost 70% reduced water vapor transmission rate, approx. 50% reduction in oxygen transmission rate and a 90% smaller oil contact angle resulting in improved bagasse paperboard for food packaging applications.

### 2.3. Microbial deterioration and active packaging applications

Extrinsic factors such as temperature, relative humidity, gas composition, microorganisms, and light affect and determine the shelf-life of a food product) (de Abreu et al., 2012a). Microbiological spoilage is a major problem for bakery and fresh produce often limiting their shelf lives and causing economic loss for both manufacturers and consumers. This is attributed to poor packaging, sanitation practices in manufacturing, storage conditions, and low product turnover. Different preservation methods are used during food manufacturing and processing, but still deterioration can occur during the supply chain. Controlling the effect of these factors using various packaging

technologies like modified atmosphere packaging (MAP), controlled atmosphere packaging (CAP), vacuum packaging and active packaging has been investigated since few years now.

Active packaging is a packaging technology that utilizes specific additives, known as "active compounds," to enhance the quality and/or safety of perishable products like fruits, vegetables, bakery, dairy, and others (Almenar, 2021). Active compounds can be grouped as: (1) scavenging compounds, and (2) releasing compounds (Almenar, 2021). Active packaging can be used in different configurations, such as sachets containing volatile substances; direct incorporation of substances in polymers; coating or adsorbing onto polymer surfaces; and immobilization to polymers (by ion or covalent linkages) (Otoni et al., 2016).

### 2.3.1. Antimicrobial packaging

Antimicrobial macromolecules as a direct coating material or encapsulated /blended with a carrier coating material have not been investigated much. Sachets and pouches have been primarily used, commercialized, and investigated for antimicrobial packaging as they prohibit carrier agents (like silica, silicon dioxide, gels, zeolites, and many others) from leading to contamination and toxicity in food products. A popular method of encapsulating active agents involves three steps: antimicrobial agent incorporation into a carrier material, placement of carrier inside the sachet, and sealing the sachet material. Sachet material should have low permeability to the active agent to enable its release. Similarly, carrier material should be able to hold the active agent before releasing it. The type of material used as a carrier material defines the releasing and loading capacity of the antimicrobial. Many carrier agents with antimicrobial properties (ethanol, carbon dioxide, silver ions, chlorine dioxide, antibiotics, organic acids, essential oils, and spices, etc.) have been tested to inhibit the growth of microorganisms that lead to the deterioration of food products.

### 2.3.2. Ethanol as an antimicrobial agent

Ethanol has been used as an antimicrobial agent in bread before the baking process to extend its shelf life (Bodbodak & Rafiee, 2016). The antimicrobial activity of ethanol is proven and well-documented for its effective extension of the shelf-life of bread at concentration levels of 0.5% to 3.5% in the loaf. Ethanol has germicidal properties; it kills molds as well as some yeasts and bacteria by destroying the cell wall structure and cell membrane with coagulation of vital proteins which leads to inhibition of the growth of this spoilage micro-organized (Mugasundari & Anandakumar, 2022). Molds like *Aspergillus and Penicillium*, bacteria like *Staphylococcus,*

*Salmonella, Escherichia coli*, and some of yeasts are proven to be killed or controlled by exposure to ethanol (Brody et al., 2001; Robertson, 2013; Smith et al., 2004). However, there are some difficulties in applying ethanol as a preservative to foodstuffs: off-flavors, rapid volatilization, and consumer resistance. The use of ethanol vapor emitter via ethanol sachet has been gaining popularity and is widely used instead of spraying ethanol because of the overall migration limit and addition limit as a preservative, off-flavors produced, rapid volatilization of ethanol (Bodbodak & Rafiee, 2016; Latou et al., 2010b; Mexis & Kontominas, 2014).

### 2.3.3. Ethanol emitter (EE)

The application of ethanol as a food preservative in a vapor form emitted from a sachet or film has been gaining popularity (Mexis & Kontominas, 2014b). These sachets contain ethanol and water, which are absorbed by a carrier material. Some sachets, in addition to ethanol, may contain trace amounts of flavoring substances, such as vanilla or other flavors, to mask the odor of alcohol (de Abreu et al., 2012b; Mexis & Kontominas, 2014). A slow or immediate and rapid release of ethanol vapors from the carrier material (can be a powdery chemical compound, gels, or absorbent materials) into the headspace is regulated by the permeability of the sachet material to water vapor and ethanol or the chemical structure of the carrier.

### 2.3.4. Sachets as ethanol emitter (EE)

A popular method of encapsulating active agents involves three steps: antimicrobial agent incorporation into a carrier material, placement of carrier inside the sachet, and sealing the sachet material. Sachet material should have low permeability to the active agent to enable its release. Similarly, carrier material should be able to hold the active agent before releasing it. The type of material used as a carrier material defines the releasing and loading capacity of the antimicrobial. In Japan, there are many ethanol-releasing emitters as the use of ethanol at lower concentrations in the headspace (4-12%) is effective in the prevention of microbial growth of microorganisms, bacteria, and molds (de Abreu et al., 2012b). Few of the patented applications of ethanol sachets, like Antimold-Mild® and Negamold® Tender (Freund Industrial Co., Ltd) by Japanese manufacturers, are placed alongside the food products within an enclosed packaging system (Bodbodak & Rafiee, 2016). Antimold 30, 60, and 80 containing 3, 6, and 8 g of alcohol powder (mixture of silicate powder with ethanol), respectively, (Freund Industrial Co., Ltd., Tokyo, Japan) are heat-sealed and made of a laminated polymer sheet (Paper/Ethyl acetate copolymer) designed for the slow release of ethanol vapor into the headspace (Candir et al., 2012). Commercial carrier

substances like silica gel, silicon dioxide, and sachet materials like Polyvinyl alcohol (PVOH), Ethyl vinyl alcohol (EVOH), and Polyethylene (PE) (Bai et al., 2011; Suzuki et al., 2004) are used in the majority ethanol emitter sachets currently.

**Table 2.1.** Different carrier and sachet materials were reported for ethanol emitter development.

| Carrier material | Food Product | Type | External material | Reference |
|---|---|---|---|---|
| Silicon dioxide | NA | Sachet | PE/nylon laminate | Day et al., (2008) |
| Finely divided silica | NA | Sachet | NA | Pereira de Abreu et al., (2012) |
| Silicon dioxide | Bread | Sachet | Bioplastic and PP | Mugasundari & Anandakumar, (2022) |
| Ethanol gel | Ciabatta bread | Sachet and spray | PS/EVOH/PE | Hempel et al.,(2013) |
| Sodium stearate and diatomite | Chinese bayberry | Sachet | Plastic sealed container with snap-on lid | (Mu et al., 2017) |
| Wool ball | Cherry tomatoes | NA | PS container sealed with Saran wrap | Liu et al., (2019) |
| Filter paper | Fresh mulberry | Sachet | Non-perforated PP | Choosung et al., (2011) |

With an increasing focus on sustainability and public health, efforts towards replacing the antimicrobial sachets and coatings with natural, sustainable materials are initiated. Few researchers have investigated the replacement of carrier to paper or other natural compounds and sachet petroleum-based materials with bio-based materials to eliminate the risk of disintegration, contamination, and accidental consumption of the carrier compound, and to reduce the use of petroleum-based polymers (Fadija et al., 2023). To analyze the capacity of ethanol emitters to preserve mulberry fruit, a sachet was made up of commercial polyethylene with filter paper pre-absorbed with absolute ethanol liquid and heat sealed together (Choosung et al., 2019).

### 2.3.5. Carrier materials used for ethanol emitters (EE)

The volatile antimicrobial compound ethanol is commonly pre-absorbed in a carrier material and this carrier material is then sealed in a plastic pouch. Examples of carriers used for ethanol include newspaper (Chervin et al., 2005), filter paper (Choosung et al., 2019), paper towels, Japanese paper (Bai et al., 2011; Ji et al., 2019; Wang et al., 2011), cotton absorbent clothes, wool (Daifas et al., 2000; Kapetanakou et al., 2015; Liu et al., 2019), silica gel powders (Bai et al., 2011; Suzuki et al., 2004). The main challenge that hurdles the commercialization of the above carriers is their capability to hold and release ethanol at high concentration, efficiency, and lower cost (Westlake et al., 2022). With active coatings, the carrier material can be a coating material with an active agent loaded in it or the active agent can be directly coated on the substrate. Currently, there is no literature available that investigated or proved ethanol-releasing coating with controlled release

### 2.3.6. Shellac as an active packaging element

In terms of shellac as a carrier material for active compounds, Ariyanto et al. (2019) showed the use of shellac-coated paper to release 1-methacyclopropene when exposed to stepwise humidity changes to inhibit ethylene production of fruits like strawberries. Another study proved the usage of PVP/shellac film as a core-sheath base structure for eugenol loading by co-axial electro-spun technique which proved an extension of the shelf life of strawberries (Li et al., 2020). In other studies, it was reported that shellac has a relatively high pKa (acid dissociation constant (Ka) of a solution) value i.e. 6.9-7.5 results in a slow drug release in intestinal fluids and thus it can be used as a coating for tablets in the pharmaceutical industry (Qussi & Suess, 2006a). Other than the above, there is no study that has investigated shellac as an active packaging material.

### 2.3.7. Antimicrobial packaging for strawberries

Fungal pathogens are one of the main causes of strawberries' postharvest losses leading to economic loss and food waste. The physiological characteristics of strawberries, like low pH, high water activity, and high sugar concentration provide an ideal environment for infestation and fungal growth. The most common fungal pathogens that affect strawberries: *Botrytis cinerea, Rhizopus stolonifer* and *Penicillium* spp can cause deterioration of the postharvest fruit quality. *B. cinerea*, also called "grey mold", is a plant disease that affects many fruits and vegetables. Antimicrobial packaging approaches have been assessed in fresh produce over many years (Almenar, 2021)Numerous methods such as cool storage at 6 °C, controlled atmosphere at an intermediate $CO_2$ and $O_2$ composition (3-10% $CO_2$, 18–-11% $O_2$), relatively high $CO_2$ content

(10%), oxygen scavenging, which could deplete oxygen dramatically, and gamma irradiation are used to extend the shelf-life of strawberry, leading to better sensory quality and higher nutritional value. However, the technical guidance required for these approaches is not always easy, cheap, or convenient for farmers and retailers in developing countries. Furthermore, post-harvest treatment by spraying or dipping of strawberry fruit with an antimicrobial or sanitizing agent may also cause further physical damage (Li et al., 2017). Antimicrobial packaging with a system releasing antimicrobial agents at a controlled rate has been investigated as a promising option (Sethi & Gupta, 2016).

# CHAPTER 3: EFFECTS OF SHELLAC CONCENTRATION AND NUMBER OF COATING LAYERS ON IMPROVEMENT OF SHELLAC COATED BAGASSE PAPERBOARD PROPERTIES

This chapter focuses on Part 1 of the studies focusing on understanding the effect of shellac concentration and number of coating layers on the properties of the bagasse paperboard for different food packaging applications.

## 3.1. Materials and methods

### 3.1.1. Materials

Bleached dewaxed shellac powder was obtained from Saraogi Shellac Overseas Corporation (Kolkata, India). Food-grade ethanol (190% proof) was purchased from Decon Labs, Inc. (King of Prussia, PA, USA). Citric acid was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Bagasse paperboard was obtained from Pactiv Evergreen (Lake Forest, IL, USA).

### 3.1.2. Shellac-coated paperboard preparation

Bleached dewaxed shellac (24, 40 and 60%) and citric acid (0.5% w citric acid/w shellac) were dissolved in ethanol. The solution was stirred using a magnetic stirrer (Scilogex, Rocky Hill, CT, USA) at 650 rpm for an hour at 32 ºC and then two more hours with no heat. The homogenous, sticky solution was then filtered using a mesh of 45 microns size (standard copper mesh) (Dual MFG Co. Inc., Franklin Park, IL, USA). Bagasse paperboard was cut into pieces of approx. 14 cm in diameter and placed inside an oven (Thermo Fisher Scientific, Waltham, MA, USA) at 32 ºC overnight. The pre-conditioned paperboards were then coated with the above shellac solutions using a bar coater K303 Multicoater (RK Print Coat Instruments Ltd., Royston, UK). The bar size 8 was used to deposit a thin layer of the coating on the paperboard substrate at the speed setting 02. The coated paperboards were allowed to dry at 42 ºC in a common oven after each layer was added. The drying time was dependent on the number of layers (10 mins for L1 and 15 mins for L2, L3, and L4). Once the coated paperboards were ready, they were stored inside plastic Ziploc bags in a walk-in environmental chamber (Environmental Growth Chamber, Chagrin Falls, OH, USA) with controlled temperature and humidity set at 23 ºC and 50% RH until used. At least four paperboards per shellac concentration and layer number were produced. Figure 1 shows some of the resulting coated bagasse paperboards.

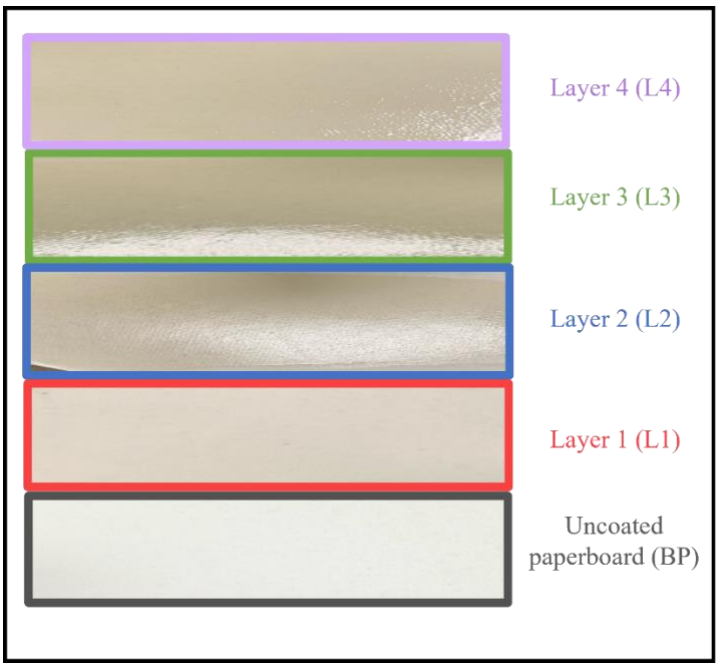


**Figure 3.1.** Visuals (changes in color and glossiness) of uncoated bagasse paperboard (BP) and coated BP with 40% shellac concentration and a different number of layers (L1-L4).

### 3.1.3. Shellac film preparation

A 40% shellac film was obtained by pouring the solution described in section 2.2. on a Teflon plate. The cast film was left to dry in an environmental chamber with controlled conditions (23 °C and 50% RH) for 48 hours. Then, it was peeled off from the plate and stored until use.

### 3.1.4. Coated paperboard characterization

#### 3.1.4.1. Barrier properties

##### 3.1.4.1.1. Water vapor permeability

A gravimetric method as per ASTM E96 (ASTM,1980) was used to determine the water vapor permeability coefficients (WVP) of the uncoated and coated bagasse paperboards. One piece from each coated paperboard was cut and masked with aluminum foil to obtain a test area of 0.00049 $m^2$. Each masked piece was placed on top of the bottom part of a permeation cell containing water, and high barrier grease (Dow corning, Midland, MI, USA) was applied on the rubber grip to seal the top part of the cell well enough. The assembled permeation cells were placed in the environmental chamber previously mentioned (23°C and 50% RH). The weight of each permeation cell was measured every hour for about 6-9 hours. The linear portion of weight loss (grams) versus time (day) curves were used to calculate the water vapor transmission rate (WVTR) (Joo et al., 2012). Water vapor permeability (WVP) was calculated as reported by Joo et al. (2012).

$WVP = (WVTR \times t)/\Delta p$

Where t is the thickness of the coated paperboard in meters and $\Delta p$ is the partial water vapor difference between inside and outside the permeation cells. One piece from each replication (3 to 5) per treatment was used. WVP is reported in kg m/m$^2$ s Pa.

**3.1.4.1.2. Oxygen permeability**

Oxygen permeation rate was measured using a paramagnetic oxygen transducer (Series 1100, Servomex Co., Sussex, U.K.). The system assay setup for the testing consisted of 1-liter glass jars (Ball Corporation, OH, United states) with two circular 11 mm drill holes in their lids. One of the holes was covered with a coated paperboard piece masked with aluminum foil tape to obtain a testing area of 0.00049 m$^2$ and the other hole was covered with a septum used to withdraw 10 µL of headspace gas from each jar that was injected into the injection port of the transducer. The chart response recorder peaks were used to calculate oxygen permeability. Standard gas with 2% oxygen was used to calibrate the machine before every use. The height of each peak was used to calculate % oxygen. By plotting milligrams of oxygen in the 0.92 liters volume jar vs time interval, rate of O2 transmission was calculated. The modeled pressure gradient created between the jar system and the environment by plotting pressure gradient vs time interval. Using the following equation, Oxygen permeability (OP) was calculated.

$OP = (OTR \times L)/\Delta P$

Where L is the thickness (m) of the coated BP and $\Delta P$ is the partial oxygen pressure difference (Pa). The glass jars were stored in the lab (23 ℃ and 50% RH) and readings were taken for 8-12 hours after every setup. One piece from each replication (3 to 5) per treatment was used. Oxygen permeability (OP) is reported in kg m/m$^2$ s Pa.

**3.1.4.2. Water absorptiveness**

The water absorptiveness of the coated paperboards was obtained following ISO 535 (ISO 535:2023). Briefly, 100 mL of deionized water was poured over the coated paperboard (circular shape, diameter 12 cm) after this was placed in a Cobb tester (Gurley Precision Instruments, Troy, NY). After one minute, the weight was recorded. The difference in weight between the coated paperboard before and after the test was used to calculate the Cobb 60 value, which represents the weight of the water absorbed (g) per paperboard area (m$^2$) over one minute. The Cobb test was performed for 60 seconds referring to ISO standard Cobb60 test method. The equation used for calculating Cobb value is as follows:

$$Cobb60 = (M2 - M1) \times 100$$

where M2 is the mass of the test piece after water absorption and M1 is the mass of the test piece before water absorption. One piece from each replication (3-5) per treatment was used.

#### 3.1.4.3. Thermal properties

##### 3.1.4.3.1. Thermogravimetric analyses

The thermal decomposition of 40% shellac cast film (produced as mentioned in section 2.3.7.1), uncoated and coated bagasse paperboard with 40% shellac two layers (L2) were determined using a thermogravimetric analyzer (TGA Q50; TA Instruments, Newcastle, DE, USA). An amount between 6-10 mg of each type of coated paperboard piece was placed in an aluminum pan (TA Instruments, Newcastle, DE, USA) and then heated from 25 to 600 ℃ at a rate of 10 ℃/min. The percent weight loss of paperboard as a function of temperature under a nitrogen air (40-60%) atmosphere was analyzed using TA analysis software. Pieces from three replicates per treatment were evaluated.

#### 3.1.4.4. Morphological characterization

Uncoated and shellac-coated bagasse paperboards were observed using a JEOL 6610LV (tungsten hairpin emitter) Scanning electron microscope (SEM) (JEOL 6610LV, JEOL, MA, USA). Before conducting SEM analysis, uncoated and coated bagasse paperboard pieces were coated with a 2.7-nm iridium layer using a Quorum Technologies/Electron Microscopy Sciences Q150T turbo-pumped sputter coater (Quorum Technologies, Laughton, East Sussex, England BN8 6BN) purged with argon gas. The coated pieces were mounted on aluminum stubs using adhesive tabs (M.E. Taylor Engineering, Brookville, MD, USA). The parameters set for the examination were a working distance of 3 mm and an accelerating voltage of 10 kV. Magnifications 100x were used for the surface images and 500x for the cross-section images. Three replicates per type of paperboard were evaluated. The images produced by SEM were analyzed with a plug-in software Image J (National Institutes of Health, MD, USA; global scale settings 1 px = 100 μm) to determine the thickness of the coated paperboard and the shellac coating. Results are presented as number of layers vs thickness (mm).

#### 3.1.4.5. Thickness

The thicknesses of the uncoated and shellac-coated BP were measured using an electronic digital micrometer (Fowler® 0-1 Digital Counter Micrometer, Port Washington, NY, USA). 3-4 readings were taken at random points on the paperboard samples. The mean values from the readings were

calculated. Three replicates per type of paperboard were evaluated. The results are presented as the thickness of the coated paperboard (mm) or the coating layer (mm) vs. the number of layers.

**3.1.4.6. Coating load increase ( wt.%)**

With the addition of each coating layer, the same coated paperboard was weighed again after the complete drying of the coating for an hour at 50 ℃. Four replicates per paperboard with 1 to 4 layers were evaluated. The % increase in coating load was calculated by measuring coating load by wt.% using a paper sample of size 15.6 × 10.4 cm using the equation mentioned below (Hamdani et al., 2024). The difference between the weights of coated (after each layer was added) and uncoated paper was recorded in g, and then this difference was divided by the weight of uncoated paper. Subsequently, the obtained value was multiplied by 100 to report the values in (wt.%).

Coating Load increase (wt.%) = (weight after coating − weight before coating/weight of uncoated paper) × 100

**3.1.4.7. Chemical structure**

Fourier Transform Infrared spectroscopy (FTIR) spectra were obtained for the coated bagasse paperboards, which differed in a number of layers and shellac concentration). The FTIR spectrophotometer (model Shimadzu IR-Prestige 21, Shimadzu Scientific Instruments Columbia, MD, USA) with the mid-infrared region (4000-650 $cm^{-1}$) with 60 scans was used. The spectra obtained from the instrument were then analyzed using Origin Pro software (OriginLab. Corporation, Northampton, MA, USA).

**3.1.4.8. Statistical analysis**

Linear regression was used to analyze WVP, COPP, OP, and thickness with ‘stats’ package in R. The first model was applied to compare control (Layer 0, concentration 0) versus the other layer & concentration combinations. The second model (exclude control) was applied to analyze the layer and concentration effect, including the two-way interaction between layer and concentration. To analyze the effect of layer for SEM thickness (multiple measurements for the same location) and %weight (4 replicates), linear mixed model from the package ‘lmerTest' in R was used. The replicates were included as random effect. To compare the thickness and layer thickness between SEM and Micrometer method under 40% concentration, linear regression was applied, including the two-way interaction between layer and method. Mean separation and letter differentiation was performed using the "emmeans" and “multcomp” packages in R. The significance level for all

analyses was 0.05, and the p-value for multiple comparisons was corrected by Bonferroni correction.

### 3.2. Results and discussion

#### 3.2.1. Barrier Properties

##### 3.2.1.1.Water Vapor Permeability

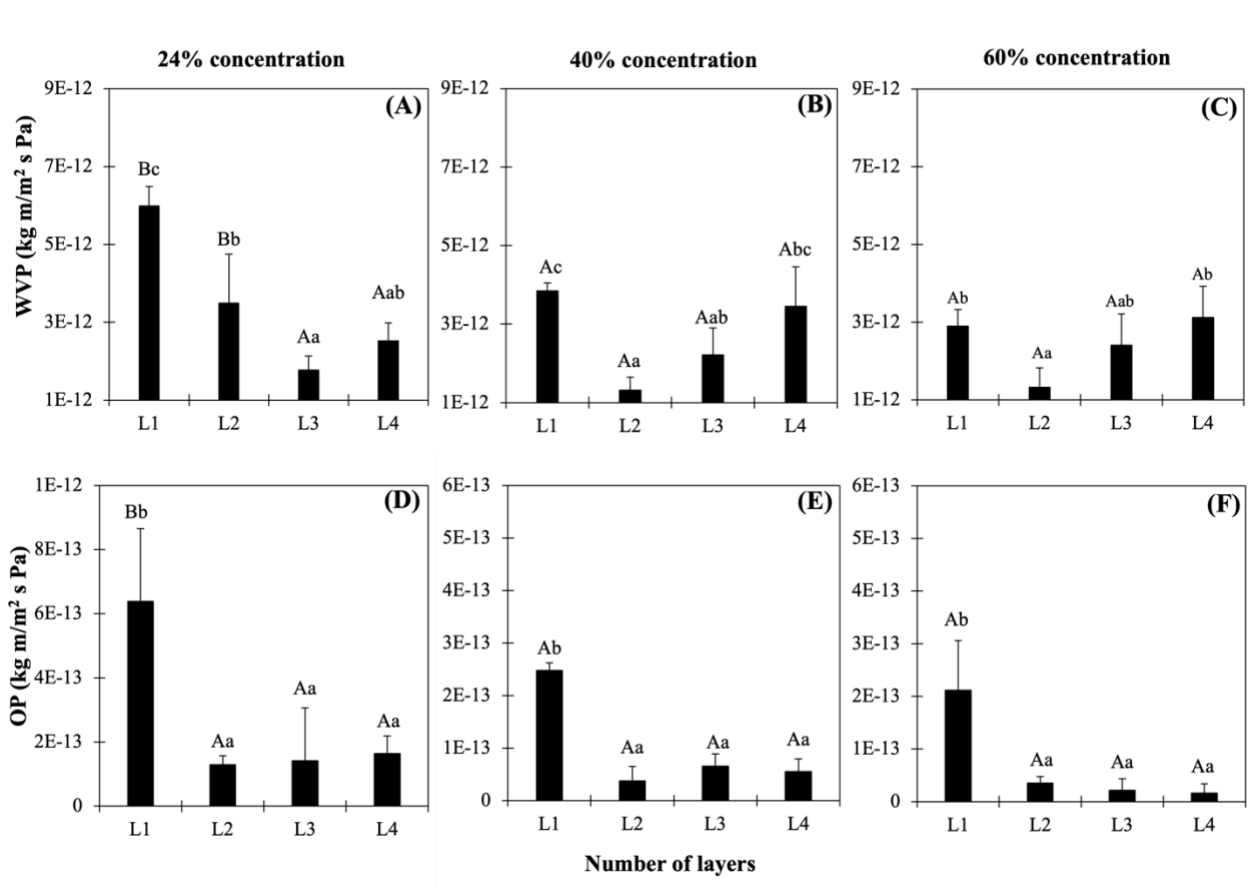


**Figure 3.2.** Water vapor permeability (WVP) coefficients (A-C) and Oxygen permeability (OP) coefficients (D-F) for shellac-coated BPs differing in number of coating layers with shellac concentrations of 24%, 40%, and 60%. Different upper-case letters indicate a significant difference among shellac concentrations for the same layer. Different lower-case letters indicate a significant difference among layers for the same shellac concentration.

Figures 3.2A-C show the WVP coefficients of shellac-coated BPs differing in the number of coating layers with shellac concentrations of 24, 40, and 60%. The WVP coefficient of the uncoated BP was $7.30 * 10^{-12}$ kg m/m$^2$ s Pa while those of the shellac-coated BPs ranged from $1.31 * 10^{-12}$ to $5.98 * 10^{-12}$ kg m/m$^2$ s Pa. Therefore, the coating of BP with shellac can decrease its WVP up to 6-folds. There was an effect of the concentration of shellac present in the

coating formulation on the WVP of the shellac-coated paperboard ($p = 0.0017$). The increase in shellac concentration decreased WVP or had no effect depending on the number of layers deposited on BP. Shellac concentrations of 40 % and 60% decreased the WVP of BP compared to 24% shellac only for layers 1 and 2. However, there was no significant difference between the shellac concentrations 40% to 60%. In contrast, Saberi et al. (2017) reported for a casted shellac film that 40% shellac resulted in better WV barrier properties compared with lower (24%) and higher (60%) concentrations of shellac.

The addition of a single layer of either 40% or 60% shellac to the uncoated BP reduced its WVP from 7.30* $10^{-12}$ kg m/m$^2$ s Pa to 2.90-3.90* $10^{-12}$ kg m/m$^2$ s Pa (50-60% reduction). Regardless of the concentration of shellac, there was a significant decrease in the WVP of the shellac-coated BP when this was coated with 2 layers rather than 1 ($p < 0.0001$). This decrease was also observed when the shellac-coated BP was coated with 3 layers rather than 1, but only when the shellac concentration of the layers was either 24% or 40%. The addition of layer 4 resulted in WVP similar to those of layers 2 and 3 depending on the shellac concentration. The best combination layering and concentration for WVP reduction in BP was found to be 2 layers of 40% shellac (1.31 $10^{-12}$ kg m/m$^2$ s Pa which reduced the WVP of the uncoated BP by 82%. These results are most likely related to the thickness of the coated BP. While the thickness of the whole shellac-coated BP is approximately the same when this having 2 to 4 layers (~ 0.65 mm; Figure 3.2 B), the thickness of the coatings varied significantly from 0.03 to 0.1 (Figure 3.2 C) for layers 2 to 4. This shows that the shellac-coated BP with a thicker coating layer had a thinner BP. This reduction in BP thickness could have resulted from the absorbed shellac that interacted with the paper fibers to create a more porous structure, the more compressed bagasse paperboard., or a combination thereof. Klayya et al. (2023) found that bagasse molded pulp coated with a layer of shellac with microfibrillar cellulose had improved barrier properties due to less shellac absorption. It is known that paperboard has a poor barrier to water vapor with complex permeance behavior. Due to the porous structure and hydrophilicity of the cellulose fibers, the water vapor or moisture permeance has non-fickian behavior meaning the permeation rate changes over time (Naitzel et al., 2023). This makes it very difficult to reduce WVP significantly to attain barrier-coated paperboard.

**3.2.1.2. Oxygen Permeability**

Figure 3.2 D-F show the OP coefficients of shellac-coated BPs differing in the number of coating layers with shellac concentrations of 24, 40, and 60%. The OP coefficient of the uncoated BP was

$3.37 * 10^{-12}$ kg m/m$^2$ s Pa while, those of the shellac-coated BPs ranged from $1.64 * 10^{-14}$ to $6.38 * 10^{-13}$ kg m/m$^2$ s Pa. Therefore, the OP of BP was decreased by two orders of magnitude with the shellac coating. The concentration of shellac had an effect on the OP of the shellac-coated BP ($p < 0.0001$). When comparing different concentrations irrespective of the number of layers, a similar effect as WVP is observed. The increase in shellac concentration decreased OP or had no effect depending on the number of layers deposited on BP. Shellac concentrations of 40 % and 60% decreased the OP of BP compared to 24% shellac, but only for layers 1 and 3. However, there was no significant difference between the shellac concentrations 40% to 60%.

The oxygen barrier of the BP increased with the addition of shellac layers ($p < 0.0001$). Regardless of the shellac concentration, with the addition of layer 1, the oxygen barrier of BP improved one order of magnitude (from $10^{-12}$ to $10^{-13}$) ($p < 0.0001$). In agreement with these results, the literature reports one order of magnitude reduction for paperboard coated with one layer of shellac although the studies report such decrease when shellac was mixed with micro-fibrillated cellulose (Hult et al., 2010; Klayya et al., 2023). With further addition of one layer, OP decreased another order of magnitude (from $10^{-13}$ to $10^{-14}$) if the shellac concentration of the layer was either 40% or 60% ($p < 0.0001$). There was no further improvement in oxygen barrier with the addition of layers 3 ($p=0.4519$) and 4 ($p=0.5806$). As mentioned in section 3.2.4., the paper structure is mainly composed of a three-dimensional cellulose fiber matrix with a porous structure. The addition of a coating layer fills up these small voids resulting in decreased gas permeability (Zhang et al., 2014). In alignment with the above OP results, the SEM (Figure 3.5) and FTIR (Figure 3.8B) results showed significant improvement in filling pores and increasing intensity of the peaks, respectively, until layer 2. Thus, 2 layers of 40% shellac was considered to be the optimum layering ($3.76^* 10^{-14}$ kg m/m$^2$ s Pa) for reduction of OP in BP with minimum shellac coating used. The no change of OP with layers 3 and 4 might be due to the maximum filling of the paperboard pores being achieved with layer 2. with the maximum improvement attained with the coated paperboard structure.

### 3.2.2. Water Absorptiveness

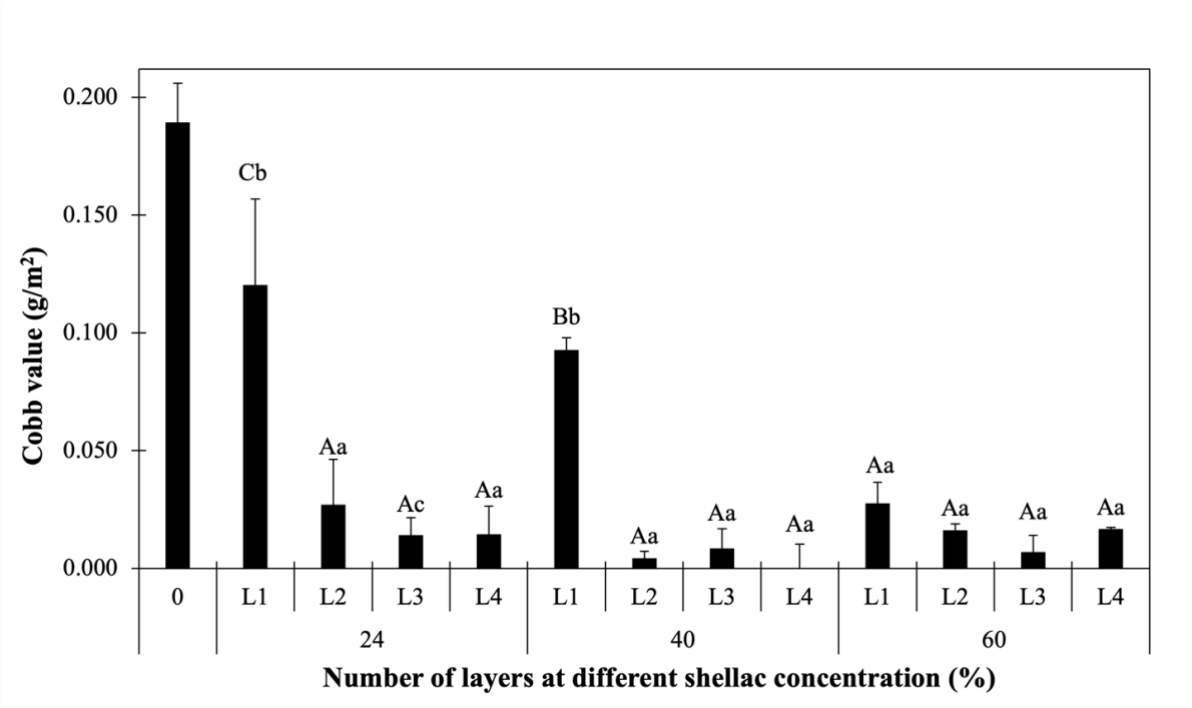


**Figure 3.3.** Cobb60 (g/m²) of uncoated BP and shellac-coated BPs differing in layering (L1 to L4) and shellac concentration (24, 40 and 60%).

A low Cobb value indicates low amount of water absorbed by the paper surface and vice-versa for a high Cobb value (Kunam et al., 2022). The Cobb60 value of the uncoated BP was 0.18 g/m$^2$. Nassar et al. (2014) reported a much higher Cobb value of 63 g/m$^2$ for uncoated BP, but the authors tested BP for Cobb for longer periods (120 or 180 seconds). The Cobb60 values of the shellac-coated BPs ranged from 0.0001 to 0.12 g/m$^2$. Thus, shellac coating can decrease water absorptiveness in BP by 33% to 99.9% (Nassar et al., 2014) Figure 2.3 shows the effect of the addition of shellac layers on reducing water absorbency in shellac-coated BP ($p < 0.0001$). Even with the addition of a single layer of 24% shellac, the Cobb60 value of uncoated BP decreased by 33% (from 0.18 g/m$^2$ to 0.12 g/m$^2$). Comparing the number of layers, shellac-coated BP decreased significantly its water absorptiveness when the shellac layering changed from 1 to 2 layers ($p < 0.0001$) with a shellac concentration of either 24 or 40%. More than 2 layers in the shellac coating did not reduce water absorptiveness further regardless of the shellac concentration.

There was a significant decrease in water absorbency for the shellac-coated BP with different shellac concentrations ($p < 0.0001$). Comparing the three concentrations, shellac-coated BP decreased significantly its water absorptiveness when the shellac concentration of the coating

increased from 24% to 40% (p = 0.0075), but not from 40% to 60% (p = 0.0823). Thus, 2 layers of 40% shellac was considered to be the optimum layering (0.0044 g/m2) for reduction of OP in BP with minimum shellac coating used. This implied a water absorptiveness reduction of 97.5% compared to the uncoated BP. As mentioned in section 3.2.4., the addition of layer 1 and 2 resulted in filling up of the paperboard's porous surface and also penetrating the paperboard. Moreover, smooth film formation of shellac on the to surface of the paperboard resulted in hydrophobicity (Hult et al., 201).

### 3.2.3. Thermal Properties

#### 3.2.3.1.Thermal degradation

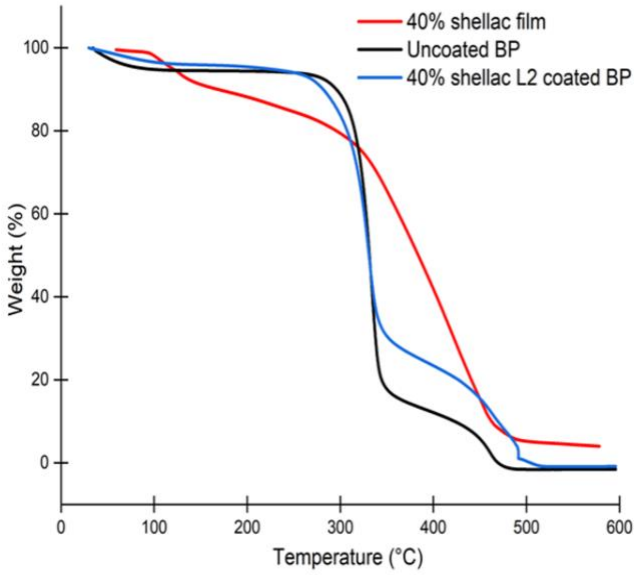


**Figure 3.4.** TGA thermograms of 40% shellac film (no BP), uncoated BP, and BP coated with 2 layers of 40% shellac.

**Table 3.1.** Thermogravimetric analysis of 40% shellac film (no BP), uncoated BP, and BP coated with 2 layers of 40% shellac. The weight of the sample at temperatures of three stages of degradation and residue is reported.

| Sample details | | First stage degradation | | Second stage degradation | | Third stage degradation | | Residue | |
|---|---|---|---|---|---|---|---|---|---|
| Sample name | W*i* (mg) | Temperature (°C)-T1 | Weight loss in (mg) | Temperature (°C)-T2 | Weight loss in (mg) | Temperature (°C)-T3 | Weight loss in (mg) | Temperature (°C) | Residue weight |
| Shellac | 5.9±0.4 | 101±0 | 0.12±0.01 | 120±0 | 0.31±0.02 | 164±5 | 0.59±0.03 | 500 | 0.35±0.16 |
| Uncoated BP | 5.0±2.5 | 205±33 | 0.10±0.01 | 284±2 | 0.25±0.10 | 303±1 | 0.50±0.03 | 500 | 0.54±0.04 |
| 40% shellac Coated L2 | 5.6±0.6 | 84±9 | 0.11±0.04 | 255±6 | 0.34±0.08 | 282±5 | 0.56±0.06 | 500 | 0.7±0.10 |

Figure 3.4B shows the TGA thermograms of 40% shellac film (no BP), uncoated BP, and BP coated with 2 layers of 40% shellac. Table 3.1 compiles the weight loss (mg) and temperature (°C) of the three stages of thermal degradation of the above-mentioned samples. The first weight loss step of the shellac film happened at around 101℃ and can be attributed to the evaporation of all the solvent present in the shellac casted film. The second and third weight loss steps happened at 120℃ and 164℃ and can be attributed to the degradation of acid components (-COOH) present in shellac. The last degradation step happened at around 500℃, representing the complete decomposition of the hydrocarbon chains remaining in the shellac. This last step started at 300 ℃ and it was not a sharp decrease like the ones observed for other synthetic polymers and bio-based polymers. Ahuja & Rastogi, (2024) reported a similar thermal behavior for casted shellac film without citric acid and attributed this to the complex and heterogeneous chemical structure of shellac that resulted in first the degradation of shellac structure due to chain scission and secondly to the thermal aging of shellac at elevated temperature occurring during the TGA (Ahuja & Rastogi, 2024).

For uncoated BP, the first weight loss step happened at around 200℃ and can be attributed to the evaporation of moisture absorbed or present in the hydrophilic pulp fibers and pores in the pulp board. The second and third weight loss steps happened at 284℃ and 303℃. A similar thermograph has been reported by Klayya et al., (2023) for uncoated BP. There was a significant difference in the degradation temperatures between coated and uncoated BP. Uncoated BP had better stability when compared with coated BP. Therefore, the shellac coating results in lower degradation

temperatures in agreement with the DSC results (Figure 4A). However, when comparing the shellac-coated BP with the shellac film, the former had better heat stability as the second-step degradation (T2) and third-step degradation (T3) increased from 119 ℃ to 218 ℃ and 163 ℃ to 279℃. This makes shellac-coated BP a good candidate for many applications that might involve exposure to heat.

### 3.2.4. Morphological characterization

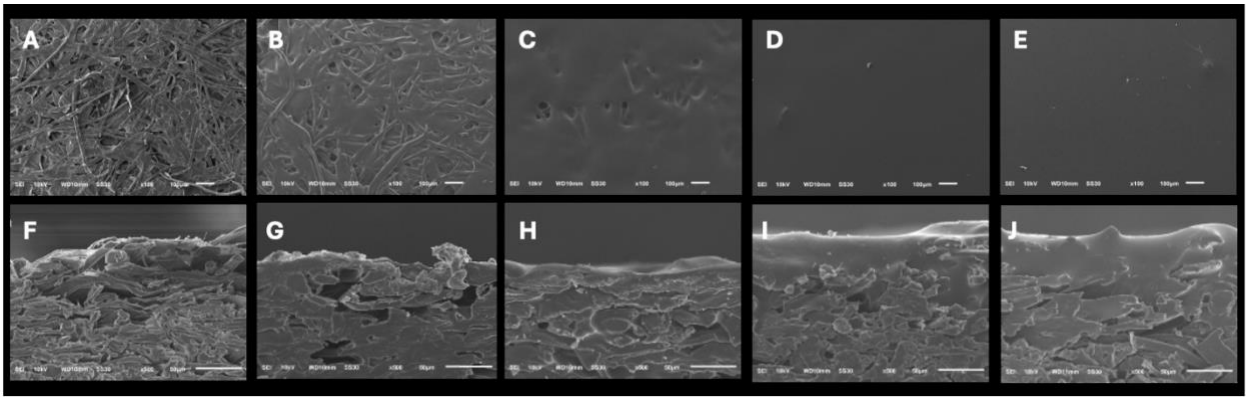


**Figure 3.5**. Scanning electron micrographs of uncoated paperboard and 40% shellac-coated paperboards with 1, 2, 3, and 4 layers (left to right). Surface images (A-E; 100X magnification) and cross-section images (F-J; 500X magnification).

The porous structure of the uncoated paperboard (Figure 5A) was less visible as each shellac layer was added (Figures 3.5B-D). Three shellac layers were enough to fill all the pores of the bagasse paperboard and form a smooth coating surface on it (Figure 3.5D). This reduction in porosity of the paperboard is supported by the FTIR results (section 3.7). Figures 2F-J show the formation of a thicker coating with the addition of each layer. The thickness of each layer is provided in section 3.5. Figures 5I-J also show that part of the coating was absorbed into the fibrous structure of the bagasse paper, which resulted in good adhesion between the shellac coating and the bagasse paperboard.

### 3.2.5. Thickness

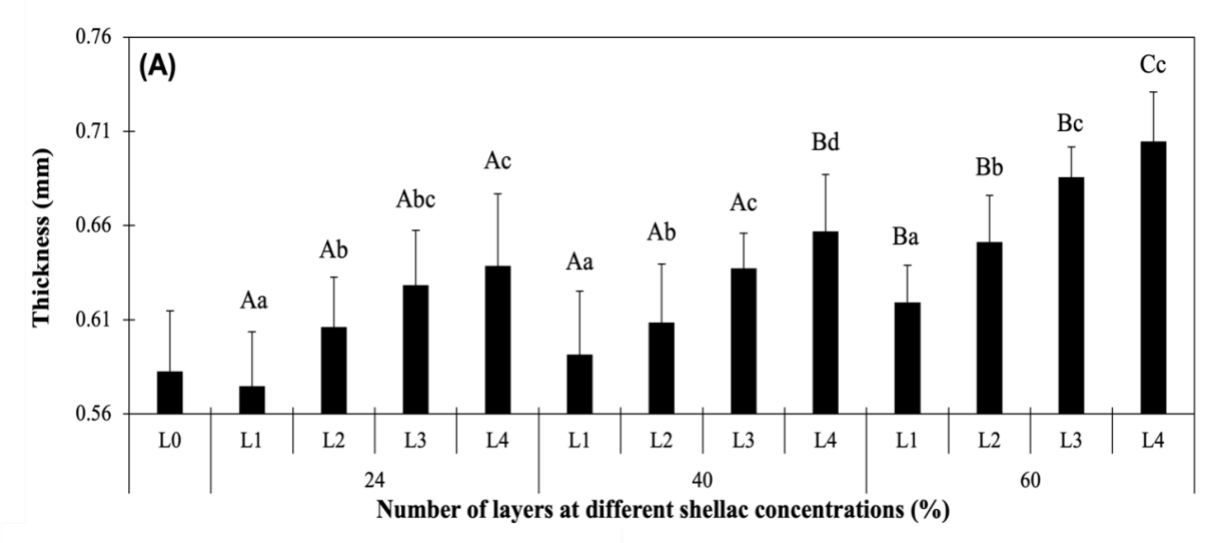


**Figure 3.6**. Thicknesses of the uncoated bagasse paperboard and coated bagasse paperboards differing in shellac concentration and number of layers as given by a micrometer. Different upper-case letters indicate a significant difference among shellac concentrations for the same layer. Different lower-case letters indicate a significant difference among layers for the same shellac concentration.

The uncoated bagasse paperboard had a thickness of 0.580 ± 0.002 mm. This thickness increased up to 0.705 mm with the addition of shellac. Both shellac concentration and shellac number of layers had an impact on this increase ($p < 0.0001$). For all three shellac concentrations, there was an increase in paperboard thickness ($p < 0.05$) as each layer of shellac was added to it except for the addition of the last layer if this made of either 24 or 60% shellac. In agreement, the SEM results (Figure 3.5) show an increase in paperboard thickness as each of the four 40% shellac layers was added to it. Paperboard thickness was the same if a layer was made of 24 or 40% shellac, however, it increased ($p < 0.05$) if the layer was made of 60%. This applied to layers 1 to 3. In the case of layer 4, its increase in shellac concentration resulted in an increased paperboard thickness. The lower average thickness of the paperboard with one layer of 24% shellac compared to the uncoated paperboard can be justified by the pressure applied during the coating process by the bar coater reducing the thickness of the base uncoated paperboard.

### 3.2.6. Coating load increase (%)

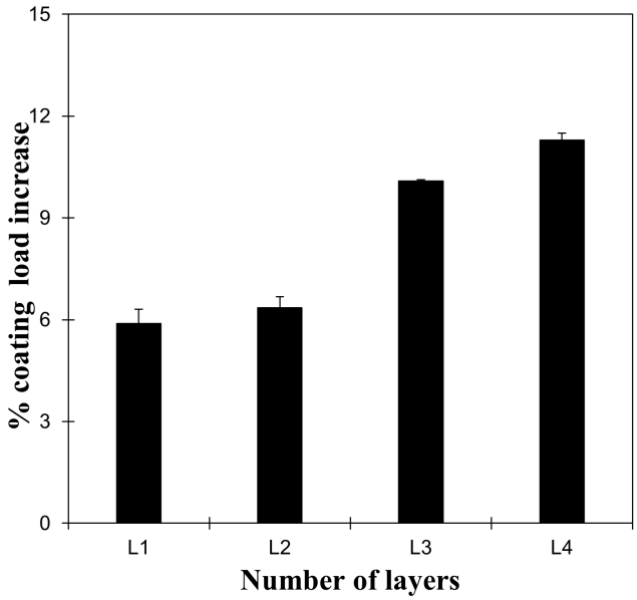


**Figure 3.7.** % Coating load increase for 40% shellac concentration coating with different number of layers.

The effect of adding additional layers of shellac on the basis weight of the uncoated bagasse paperboard is reported in Figure 3.7. The % coating load increase was in the range of 5.9% ± 0.4 to 11.3% ± 0.2. The addition of shellac layer to the BP shows 5.9% weight increase. There is no significant increase with layer 2 but with the % weight is increased further 1.5 folds with addition of layer 3 and 4.

### 3.2.7. Chemical characterization

The chemical structures of uncoated BP and coated BPs differing in % shellac concentration and number of coating layers were obtained using FTIR spectrophotometry (Figure 3.8). These structures were compared to confirm when the full coverage (filling of pores) of the uncoated BP took place and the chemical changes in the shellac coating as a function of both the number of shellac layers (Figure 3.8A) and shellac concentration (Figure 3.8B).

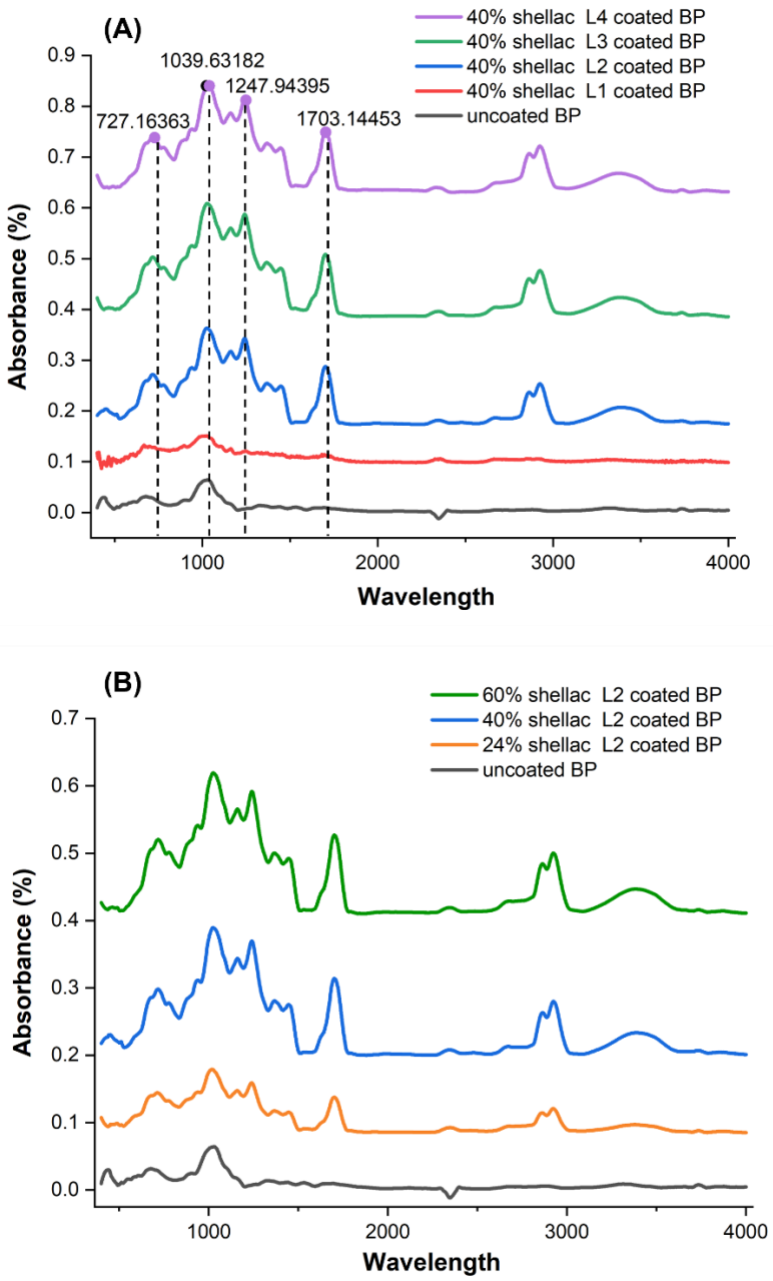


**Figure 3.8.** FTIR spectra of uncoated BP and BPs coated with shellac differing in (A) number of layers (L1 to L4) and (B) concentration (24, 40 and 60%).

The FTIR spectrum of the uncoated BP exhibited a peak at 1037 $cm^{-1}$ attributing to C-C stretching and a drop at 2400 $cm^{-1}$, which might be relevant to the filler used in paperboard manufacturing (Figures 3. 8 A and 8B). A similar FTIR spectrum was obtained by Jain et al., (2023). As shown in Figures 3.8 A and 8B, all the FTIR spectra of the shellac-coated BP except for the one with only 1 layer of shellac showed the same absorption peaks as those reported in the literature for shellac

films and flakes including 3400-3500 $cm^{-1}$ (bonded hydroxyl groups present in the long chain fatty acid), 2850 $cm^{-1}$ and 2920 $cm^{-1}$ (due to methyl ($CH_3$) and methylene groups ($CH_2$)), 1700 $cm^{-1}$ (carbonyl groups of carboxylic acid and aldehydes and esters overlapping), 1247 $cm^{-1}$ (C-O stretching due to aromatic esters), 1465 $cm^{-1}$ ($CH_2$ bending), 1379 $cm^{-1}$ ($CH_3$ bending), 1039 $cm^{-1}$ (C-C stretching), 1166 $cm^{-1}$ (C-O stretching of a secondary alcohol), 700 $cm^{-1}$ (C-H rocking of the long chain alkanes and ring structures) (Ahuja & Rastogi, 2024; Nurhayati et al., 2020).

As shown in Figure 3.8A, the peaks of the FTIR spectrum of the monolayer shellac-coated BP had lower intensities than those of the FTIR spectra of the uncoated BP and multilayer shellac-coated BPs. Only the shellac-characteristic peak at 1700 $cm^{-1}$ was perceptible. The above indicates the almost full absorption of the first layer of shellac in agreement with the SEM (Figure 3.5) and thickness (Figure 3.6) results. Klayya et al. (2020) observed similar absorption when one layer of shellac was added to BP and concluded no layer formation. While the addition of a 2$^{nd}$ layer of shellac increased the intensity of the peaks, there was no significant increase in peak intensity with the addition of the 3$^{rd}$ and 4$^{th}$ layer of shellac. Two shellac layers were enough to cover the BP and considered a good candidate for developing coated BP as a food packaging material. The FTIR spectra of bilayer shellac-coated BPs varying in shellac concentration showed the characteristic peaks of shellac mentioned above (Figure 3.8A). While the increase in shellac concentration from 20% to 40% in the coating increased intensity in the peaks, the further increase to 60% resulted in the same peak intensity indicating no further improvement. Therefore, shellac-coated BP consisting of 2 layers of 40% shellac was confirmed to be the optimum combination as concluded in barrier and water absorptiveness test.

# CHAPTER 4: *IN-VITRO* AND *IN-VIVO* VALIDATION OF ETHANOL-RELEASING SHELLAC-COATED PAPERBOARD

In this chapter, Part 2 and Part 3 of the studies are presented. After development and characterization of the shellac-coated paperboard for wider range of food packaging application in the previous chapter, this chapter emphasis its use as an ethanol-emitting inserts for extension of the shelf-life of fresh produce. Parts 2 and 3 focus on validating the developed shellac-coated bagasse paperboard as an ethanol emitter by studying its ethanol-releasing capacity and its effectiveness against fungal growth firstly in *in-vitro* studies and then shelf-life study using packaged strawberries. Specifically, Part 2 focuses on in-vitro validation using an assay system (glass jar of 0.236 L), and part focuses on in-vivo validation in packaged strawberries.

## 4.1. Materials and methods

### 4.1.1. Materials

Bleached dewaxed shellac powder was obtained from Saraogi Shellac Overseas Corporation (Kolkata, India). Food-grade ethanol (190% proof) was purchased from Decon Labs, Inc. (King of Prussia, PA, USA). Citric acid and potato dextrose agar were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Bagasse paper pulp board was obtained from Pactiv Evergreen (Lake Forest, Illinois). Strawberries were purchased from Whole Foods Market, Inc. (Okemos, MI, USA). Petri dishes of 60 mm diameter and 15 mm height from Corning Inc. (Corning, NY, USA) were used for *in-vitro* tests.

### 4.1.2. Preparation of the ethanol-releasing bagasse paperboard

Bagasse paperboards with one to four 40%-shellac layers were produced following the procedure described in Chapter 3, section 3.1.2. At least four replications per layer were produced from a different shellac solution batch.

### 4.1.3. Kinetics of the ethanol-releasing bagasse paperboard

#### 4.1.3.1. Assay system

236-mL glass jars (Ball$^{@}$ Corporation, CO, USA) closed with modified screw lids were used as assay systems. Each lid was modified by drilling a central 11-mm hole and plugging a rubber septum (size-20 mm) (Sigma Aldrich Inc, St. Louis, Mo, USA) into it. High-barrier grease (Dow Corning, Midland, MI USA) was applied to the contact area between the lid and the septum using a toothpick to ensure attachment.

#### 4.1.3.2. Ethanol quantification

An amount of 100 µL sample from the bioassay system was drawn through the rubber septum to its lid to measure ethanol released from the shellac-coated bagasse paperboard and accumulated in the headspace of the assay system using a100-µL gas-tight syringe (Gastight-1710; Hamilton Co., Reno, Nev.). The collected vapor was injected in the split injection port of a gas chromatograph flame ionization detector (GC-FID) Hewlett-Packard 6890 series (Agilent Technology, Palo Alto, CA) equipped with a flame ionization detector and a HP-5 column (30 m × 0.32 mm × 0.25 µm, Hewlett-Packard, Agilent Technology, Palo Alto, CA, USA). The temperatures of the oven, injector, and detector were set at 40, 225, and 225 °C, respectively. The injections were analyzed and recorded using Empower Pro 2 software (Version 2005-2008 Waters Corporation).

#### 4.1.3.3. Digital microscopy

Images of bagasse paperboards with one to four 40%-shellac layers and uncoated bagasse paperboard were obtained using a Keyence VHX-6000 microscope with CMOS digital cameras (Keyence, Osaka, Japan). The results are presented as cross-section micrographs of bagasse paperboards with one to four 40%-shellac layers at 200X magnification.

#### 4.1.3.4. Ethanol-releasing bagasse paperboard selection

Bagasse paperboards with one to four 40% shellac layers of dimensions 12.3 cm x 6.3 cm were placed inside the assay systems (236 mL) glass jars with lids with septum inserted; mentioned in section 4.1.3.1) and these were stored in a walking-in chamber (Thermo Fisher Scientific, Waltham, MA, USA) set at 23 ℃. The ethanol in the jar headspace was drawn out and injected in the GC as reported in section 4.1.3.2. every 24 hours for three consecutive days. Four replicates per paperboard type were used. Each replicate was produced from a different shellac solution batch. The results are presented as µL ethanol/ L air (ppm) vs number of layers. The paperboard with higher ethanol-releasing capacity was selected for further experiments. The ethanol content present in the headspace of the assay systems was quantified using a calibration curve. The calibration curve (Figure 4.2.1) was plotted by injecting specific amount of liquid ethanol (0.5-6 µL) inside the empty assay system (glass jar of 0.236 L). The jar was left sealed for 20 mins and then the headspace gas sample was drawn. The graph plotted (Figure 4.2 A) shows the GC peak for the headspace gas sample injected and the equation was used to calculate ethanol in µL in all further studies conducted.

### 4.1.4. Ethanol-releasing bagasse paperboard characterization

#### 4.1.4.1. Effects of temperature, relative humidity, and time on ethanol release

Four assay systems containing the paperboard (of approx. 12.3* 6.3 cm size) selected in 4.1.3.4 were tightly closed at room conditions (23 ℃ and 50% RH) and stored at 23℃ for 1 day. Then, these assay systems were moved at 3℃ for 3 days. The ethanol in the assay systems at the different temperatures was recorded by gas chromatography (4.1.3.2). Another set of four assay systems also containing the paperboard selected in 4.1.3.4 and closed at ambient conditions were stored at 23 ℃. After the ethanol reached equilibrium inside the assay systems, this was recorded by gas chromatography (4.1.3.2) and the lids of the assay systems were screwed out and back screwed on for the ethanol to escape. The same procedure was continued until the release of ethanol from the bagasse paper was minimal. Another set of four assay systems also containing the paperboard selected in 2.3.4 along with a 60-mm petri dish with PDA media with or without mold were closed at ambient conditions and were then stored at 23 ℃. The ethanol in the assay systems containing petri dishes was recorded by gas chromatography (4.1.3.2). Each assay system contained a paperboard produced using a different shellac batch. The results are presented as ethanol in µL ethanol/L air (ppm) in the 0.236 L assay jar.

#### 4.1.4.2. Ethanol release kinetics and curve fitting

Four assay systems containing the paperboard selected in 4.1.3.4 and closed at ambient conditions were stored at 23 ℃ for 80 days. The jars were opened for 1 minute and closed again. Ethanol was withdrawn 24 hours after the closing of the jars. The Cumulative fractional release (%) of ethanol was calculated using the equation below.

Cumulative fractional release (%) = (Release at time (t)/ Cumulative release) *100

The cumulative fractional release was plotted versus time and the resulting curve was analyzed for fitting into five kinetic models (zero order, first order, Korsmeyer-Peppas, Higuchi, and Hixson-Cromwell). The model plot, best fit, and model parameters were calculated using the Excel add-in DDsolver (Zhang et al., 2010). The best-fit model was determined by comparing the regression coefficients ($R^2$) of the 5 models mentioned below with reference to (Sibaja et al., 2015, Zhang et al., 2010).

A. Zero order model

$$Q_t = Q_0 + k_0 \times t \qquad \text{……...……Equation 2}$$

where $Q_0$ and $Q_t$ are the amounts of ethanol released initially and at time t and $K_0$ is the zero-order release constant.

B. First order model

$$\log Q_t = \log Q_0 - \frac{k_1 \times t}{2.303} \quad \text{……...……Equation 3}$$

where $Q_0$ and $Q_t$ are the amounts of ethanol released initially and at time t and $K_1$ is the first order release constant.

C. Korsmeyer-Peppas model

$$\frac{Q_t}{Q_0} = k_{KP} \times t^n \quad \text{……...……Equation 4}$$

Where $Q_t$ / $Q_0$ is the fraction of ethanol released at time t, $K_{KP}$ is the release constant for this model and n is the release exponent indicating the mechanism of the release.

D. Higuchi Model.

$$Q_t = K_h \times t^{1/2} \quad \text{……...……Equation5}$$

Where Qt is the amount of ethanol released in time t and $K_h$ is the release constant.

E. Hixson-Cromwell model

$$W_0 - W_t = K_0 \times t \quad \text{……...……Equation6}$$

Where $W_0$ and $W_t$ is the initial amount and the amount released at time t and, $K_s$ is the release constant.

**4.1.5. Antimicrobial properties**

**4.1.5.1. Inoculum preparation**

*Botrytis cinerea* and *Penicillium* spp. were isolated from decayed strawberries. Fungal spores were confirmed using an optical microscope and transferred to 60-mm*15-mm petri dishes (Corning containing potato dextrose agar (PDA) (Sigma-Aldrich Corp., MO, USA). After 2 weeks, fungal mycelia were removed from the PDA with a sterile spatula and transferred to sterile tubes containing 1 mL of sterile distilled water each. The tubes were vigorously shaken, and the solutions filtered through pieces of cheese cloth placed on the top of sterile tubes to collect water containing only spores. These tubes were shaken and 50 µL of water with spores was pipetted and placed on a hemocytometer (Bright-Line, Hasser Scientific, PA, USA). The spore count was adjusted to $1\times10^6$ CFU/mL via the Neubauer Hemocytometry method.

**4.1.5.2. In-vitro study**

An amount of 3.5 µL of each conidial suspension was placed on the center of 60-mm petri dishes containing PDA that were then transferred to the assay systems described in section 4.1.3.1., which had previously been sterilized. The above occurred inside a biological safety cabinet (Esco, Changi, Singapore) with the ventilation fan on. Before screwing the lids on the bioassay jars, 12

cm × 6.5 cm of the paperboard selected in section 4.1.3.4. and of uncoated paperboard were placed on the jar walls without touching the petri dishes resulting in the treatment bioassay systems and control bioassay systems, respectively. Bioassay systems without paperboard were used as negative controls. Three bioassay systems of each type per mold type were stored at in the environmental chamber mentioned previously for 7 days. Every 24 hours, fungal colony growth was measured as reported by McKay et al. (2021). The ethanol level in the jar headspace was drawn out of the jar headspace through the septum and injected in the GC reported in section 2.3.2. On day 7, the bioassay systems containing the ethanol-releasing bagasse paperboard were opened and the petri dishes were retrieved. The central piece of the PDA media of each petri dish was cut in the form of a 1 cm x 1 cm square and transferred to a new bioassay system containing no paperboard to determine the fungicidal or fungistatic activity of ethanol on the molds by measuring fungal growth as described above. The sub-cultures were prepared inside the biological safety cabinet mentioned previously. Results are presented as (1) fungal growth ($cm^2$) over storage time (days) for cultures and sub-cultures, (2) images of the colonies of the cultures and sub-cultures on days 1,3, and 6, and (3) µL of ethanol/L of air inside the bioassay systems.

**4.1.5.3. In-vivo study**

**4.1.5.3.1. Preparation of the packages (with control and ethanol releasing BP)**

Approx. 145 grams of strawberries uniform in color, size, and free of defects were weighed and kept on the weight boats inside the biological safety cabinet mentioned previously. The strawberries were wounded using a sterile needle and then inoculated with 3.5 µL of $10^6$ CFU/mL conidial suspension of *B. cinerea*. The inoculated strawberries were then placed inside rigid plastic trays made of polyethylene terephthalate (720 ml, Clear Lam Packaging, Elk Grove, IL, USA) with bottom inserts (approx. 135 $cm^2$; Figure 1) consisting of the ethanol-releasing bagasse paperboard selected in section 4.1.3.4 (treatment) or uncoated bagasse paperboard (control). The trays were sealed with peelable microperforated polylactic acid film (8 micro-perforations, 1.6 mil, Evlon PLA, Bi-Ax International, Wingham, Ontario, Canada) at 180°C with a sealing time of 10 seconds using a T200 semi-automatic tray sealer (MultiVac Wolfertschwenden, Germany). Preliminary results showed that this packaging design without the bottom insert provided a headspace composition of 12.9% carbon dioxide and 5.4% oxygen for 250 grams of strawberries. The packaged strawberries were stored at 23 ℃ and 50% RH. This temperature was selected

because it is optimal for the germination of the spores of both molds (Ji et al., 2021; Plaza et al., 2003). Four replications per package type were tested.

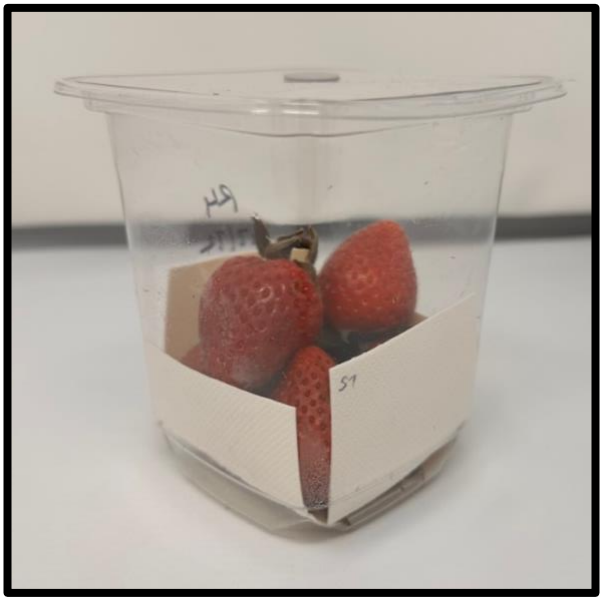

**Figure 4.1.** Developed antimicrobial package.

#### 4.1.5.4. Shelf-life study

##### 4.1.5.4.1 Fungal growth

The strawberries of each package were visually checked for *B. cinerea* growth every 24 hours for 5 consecutive days. Four packages per treatment were used. Results are expressed as a percentage of disease incidence vs time (Almenar et al. 2007).

##### 4.1.5.4.2. Package headspace composition

The ethanol released in the headspace of the two packaging designs was measured every 24 hours using gas chromatography as described in section 4.1.3.2. Changes in oxygen ($O_2$) and carbon dioxide ($CO_2$) contents were monitored in all packages using a paramagnetic oxygen transducer (Series 1100, Servomex Co., Sussex, U.K.) and carbon dioxide analyzer (Series 1100, Servomex Co., Sussex, U.K.) on days 0 and 5. Four packages per treatment were used. The results are presented as % $O_2$ and % $CO_2$.

##### 4.1.5.4.3. Weight loss

The weights of every package were measured everyday including day 0 using a precision analytical balance (Ohaus, Suiza). The weights of the empty packages were also collected on day 0. Four packages per treatment were used. Results are presented as a percentage of weight loss (%).

#### 4.1.5.4.4. Color

The color of the strawberries was determined on days 0 and 5 using a LabScan XE colorimeter (HunterLab, Reston, VA, USA) with a 13-mm opening after this being calibrated with white, black, and green plates. Readings were taken from the apex (tallest point) of the side of the strawberry opposite to the wound. Four packages per treatment were used. The results are presented as brightness (L*) and redness (a*).

#### 4.1.5.4.5. Texture

The texture of the strawberries was evaluated on days 0 and 5 using a texture analyzer (TA.XTPlus, Texture technologies, Hamilton, MA, USA) using a TA-53 3-mm diameter puncture probe. The texture analyzer was set at compression, 2.0 mm/sec test speed, 5.0 mm target distance and 7.0 *g* of Auto-Trigger. Every strawberry was cut by half vertically along the broad hemisphere and the two halves were analyzed. The readings were taken from the apex (tallest point) of the side of the strawberry. Four packages per treatment were used. The results are presented as Firmness in Newtons (N).

### 4.1.6. Statistical analysis

Linear regression was applied to assess the difference of (1) ethanol released from the ethanol-releasing paperboard as a function of the number of layers, temperature, and media, (2) mold growth as a function of treatment (glass jar with and without ethanol-releasing paperboard or uncoated paperboard), day, and the two-way interaction between treatment and day, (3) each quality parameter (color, texture, weight loss, package headspace, disease incidence) as a function of treatment (package with and without ethanol-releasing paperboard) and day, and the two-way interaction between packaging treatment and day, and (4) ethanol released from the ethanol-releasing paperboard inside packages with and without strawberries. Mean separation and letter differentiation was performed using the ‘emmeans’ and “multcomp” packages in R. The significance level for all analyses was 0.05, and the p-value for multiple comparisons was corrected by Bonferroni correction.

## 4.2. Results and Discussion

### 4.2.1. Factors affecting ethanol release from the ethanol-releasing bagasse paperboard

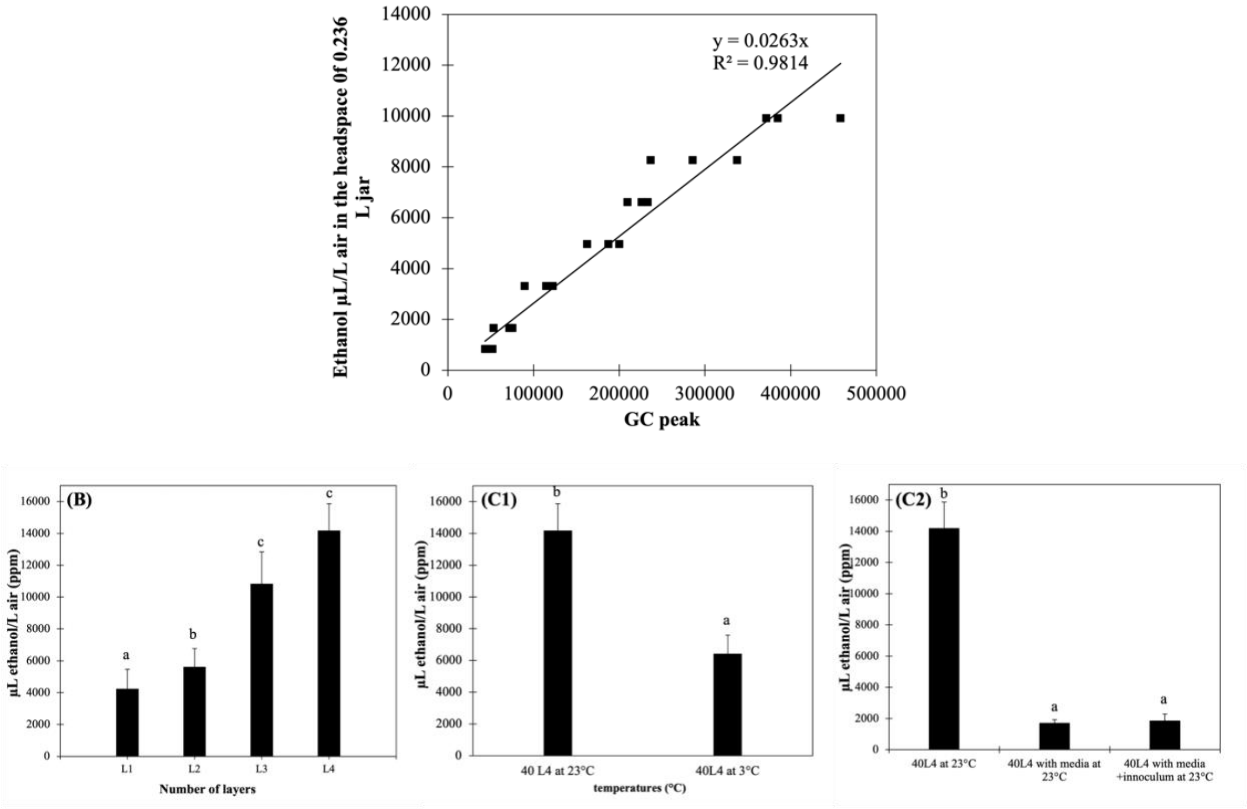


**Figure 4.2.** (A) GC Calibration curve for Ethanol vapor in the assay headspace (0.236 L jar) Ethanol released in µL ethanol /L air (ppm) from bagasse paperboard (size, 12.3*6.3 cm) coated with: (B) one to four layers of shellac and exposed to 23°C and 50%RH; (C) four layers of shellac exposed to (C.1) different temperatures (23°C and 3°C). (C.2) culture media (with and without mold) and Different letters indicate significant differences ($p \leq 0.05$) among treatments.

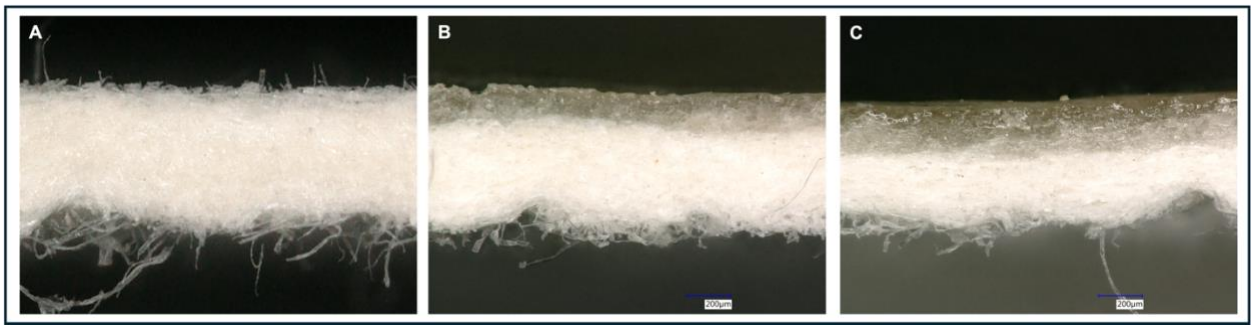


**Figure 4.3.** Cross-section micrographs of uncoated (A) and 40% shellac-coated bagasse paper with two shellac layers (B), and four shellac layers (C) taken using a Keyence microscope at 200X magnification.

#### 4.2.1.2. Effect of layering on ethanol release

The release of ethanol from the ethanol-releasing bagasse paperboard at 23°C and 50%RH increased with the addition of layers of shellac ($p < 0.0001$). Specifically, it changed from 4200 to 14,200 µL ethanol/L air (ppm) (4 layers) (Figure 4.2.B). After the first shellac layer applied, the further addition of shellac layers resulted in 38, 18, and 25% higher release of ethanol compared to the previous layer. Therefore, the least change in ethanol release was from layers 2 and 3. Based on the above results, an ethanol-releasing bagasse paperboard coated with four layers of shellac was selected for future testing. The increasing ethanol-releasing capacity of the paperboard with layering can be explained by the thickening of the shellac layer (Figure 4.3). More shellac means more ethanol trapped into it that can be released. The greater change in ethanol release between layers 1 and 2 compared with layers 2 to 3 and 3 to 4 can be attributed to layer 2 being an external shellac layer (shellac being deposited on the paperboard) instead of an internal layer. Most of the shellac of the first layer was absorbed because the porous structure of the paperboard, which resulted in a mixture of paperboard and shellac that allowed the ethanol to be released faster and thus to lessen its amount when the paperboard was introduced in the assay system. Two layers of the shellac coating resulted in the formation of a layer on the top surface of the paperboard although there was some absorption of shellac by the paperboard (Figure 4.3B). An increased coating thickness and a higher shellac absorption by the paperboard were obtained with four layers of the shellac coating (Figure 4.3C). Klayya et al. (2023) also reported a high degree of shellac penetration into the bagasse paperboard that reduced porosity due to the low viscosity of the shellac solution.

#### 4.2.1.3. Effect of time on ethanol release

As observed in the figure, the coated paperboard presented two different release patterns. The first one was a fast-release pattern that lasted 10 days and resulted in the release of ~80% of the ethanol i.e. from 14,200 µL ethanol/ L air (ppm) to 1500 µL ethanol/ L (ppm) followed by a slow-release pattern that lasted about 2 weeks and resulted in the release of the remaining 20% of ethanol (from 1500 µL ethanol/ L air (ppm) to 340 µL ethanol/ L air (ppm)). This bi-phasic pattern was also reported by Mu et al., (2017) for ethanol-releasing gels prepared by gelatinization reaction between ethanol and sodium stearate. The change in the ethanol-releasing capacity of the shellac-coated paperboard could be attributed to a chemical change in the polymer structure after 10 days. Most likely the ester groups present in shellac started to cross link with each other (interesterification) making more difficult for the remining ethanol to leave the polymer matrix. It is well-known that shellac aging results in chain crosslinking and this is caused by the loss of entrapped ethanol from the shellac over time (Ahuja & Rastogi, 2023; Coelho et al.,2024) Compared with our shellac-coated bagasse paperboard, Mu et al. (2017) reported a much faster ethanol releasing capacity (90% after 8 hours) for a gel made of ethanol and sodium stearate. however, this was exposed to air rather than enclosed in a glass jar.

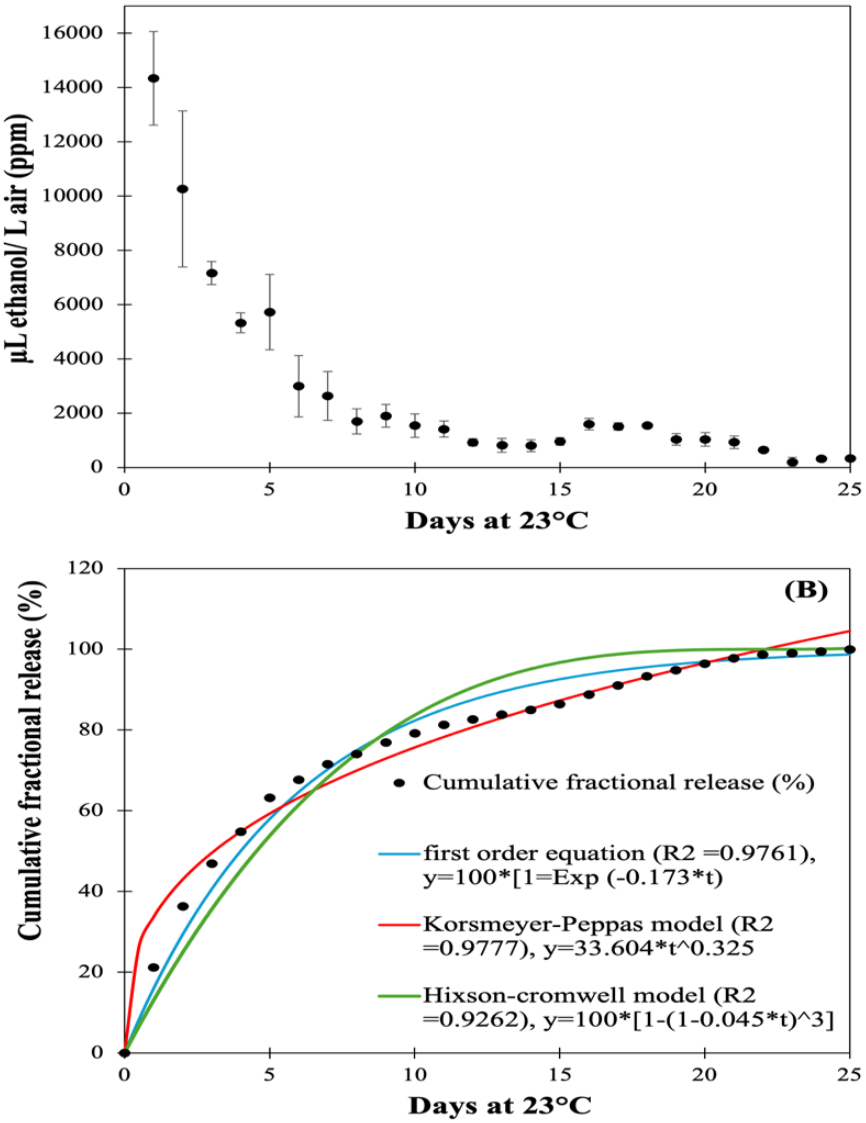


**Figure 4.4.** (A) Amounts of ethanol released (µL ethanol/ L air (ppm)) from bagasse paperboard (size 12.1* 6.3 cm) coated with 4 layers of shellac over time and (B) Cumulative fractional release of ethanol (%) over time and data-fitted curves for first-order, Korsmeyer-Peppas (KP) and Hixson-Cromwell models.

**Table 4.1.** Equations resulting from the data fitting of % Fractional ethanol release from the ethanol-releasing bagasse paperboard into different kinetic models and their corresponding parameters (k (release constant), 'n' (release exponent), $t^{1/2}$ (amount released in half time of the maximum), and '$R^2$' (regression coefficient)).

| Kinetic model | k | n | t50 | Model plotted equation | R2 |
|---|---|---|---|---|---|
| Zero order | 5.246 | NA | 9.53 | y=5.246*t | 0.1661 |
| First order | 0.173 | NA | 4.01 | y=100*[1=Exp (-0.173*t) | 0.9761 |
| KP | 33.604 | 0.352 | 3.09 | y=33.604*t^0.325 | 0.9777 |
| Higuchi | 22.481 | NA | 4.95 | y=22.481*t^0.5 | 0.9113 |
| Hixson-Cromwell | 0.045 | NA | 4.55 | y=100*[1-(1-0.045*t)^3] | 0.9262 |

Table 4.1 shows the kinetic model parameters obtained when the fractional release of ethanol data (Figure 4.4B) was fitted to different equations using five different drug release kinetic models named the Zero order, First order, Hixson-Cromwell, Higuchi, and Korsmeyer-Peppas models. These kinetic model parameters were the release constant (k), release exponent (n), time at which the amount of adsorption reached a half maximum ($t^{1/2}$), regression equations, and correlation coefficient ($R^2$). The resulting regression equations for each model are also presented in Table 4.1 and three of them (Korsmeyer-Peppas, first order, Hixson-Cromwell models) are plotted in Figure 4.4B. The kinetic model parameter correlation coefficient ($R^2$) was used to determine the best kinetic curve that fits the experimental data, which was the Korsmeyer-Peppas and First order model with an $R^2$ of 0.9777 and 0.9761. Mu et al. (2017) reported the First order model to be the best fit for ethanol release from an ethanol emitter consisting of diatomite charged with a stearate gel containing ethanol. The Hixson-Cromwell model showed to be the third best fit ($R^2$ = 0.9262), however, the equation predicts a higher release by the end of the shelf life of the emitter. The third best-fitting model for the fractional release of ethanol data was the Korsmeyer-Peppas model. This is a semi-empirical model that exponentially relates release to the elapsed time. 'n' can be used to characterize several release mechanisms and to interpret diffusion mechanism in general cases (Sibaja et al., 2015). 'n' was calculated as 0.352, which is less than 0.5 and an n less than 0.5 determines the release mechanism as a Pseudo-fickian diffusion mechanism (Sibaja et al., 2015). Thus, the release of ethanol was strongly dependent on polymeric factors like chain mobility and functional groups present in the polymer structure, which affects interaction between ethanol and the polymer matrix. Shellac aging due to loss of ethanol causes shellac to be a more crosslinked

structure, creating a more hindered path of diffusion remaining release for leading to prolonged time of release.

#### 4.2.1.3. Effect of temperature on ethanol release

The fluctuations of ethanol shown in Figure 4.2. C1 (e.g., days 4 and 5) could be attributed to changes in temperature over storage. The assay systems were not stored in a controlled environment and a decrease in temperature is expected to reduce ethanol vapor and vice-versa. The authors did not record the temperature fluctuations occurring over storage, so a change in temperature from 23ºC to 3ºC was used to verify changes in ethanol vapor in the bioassay systems with temperature. As shown in Figure 2C, the amount of ethanol inside the assay systems dropped by 54% (from 14,200 µL ethanol/ L air (ppm) to 6600 µL ethanol/ L air (ppm) with $p < 0.0001$. when these were moved from 23ºC to 3ºC. The Clausius-Clapeyron equation was used to calculate the decrease in ethanol vapor pressure with a decrease in temperature from 23ºC to 3ºC for comparison purposes. The vapor pressure of ethanol at 23ºC was calculated to be 8,433 Pascals while at 3ºC it was 3,052 Pascals, which means a drop of approximately 60%, which is a little higher than the one obtained in this study, but it supports the claim of the fluctuations of ethanol shown in Figure 4.2 C1 and B being caused by changes in temperature over storage. These results also demonstrate that the amount of ethanol present in the environment surrounding the ethanol-emitting bagasse paper depends on the temperature at which the film is exposed to.

### 4.2.2. *In-vitro* antimicrobial properties of ethanol-releasing bagasse paperboard

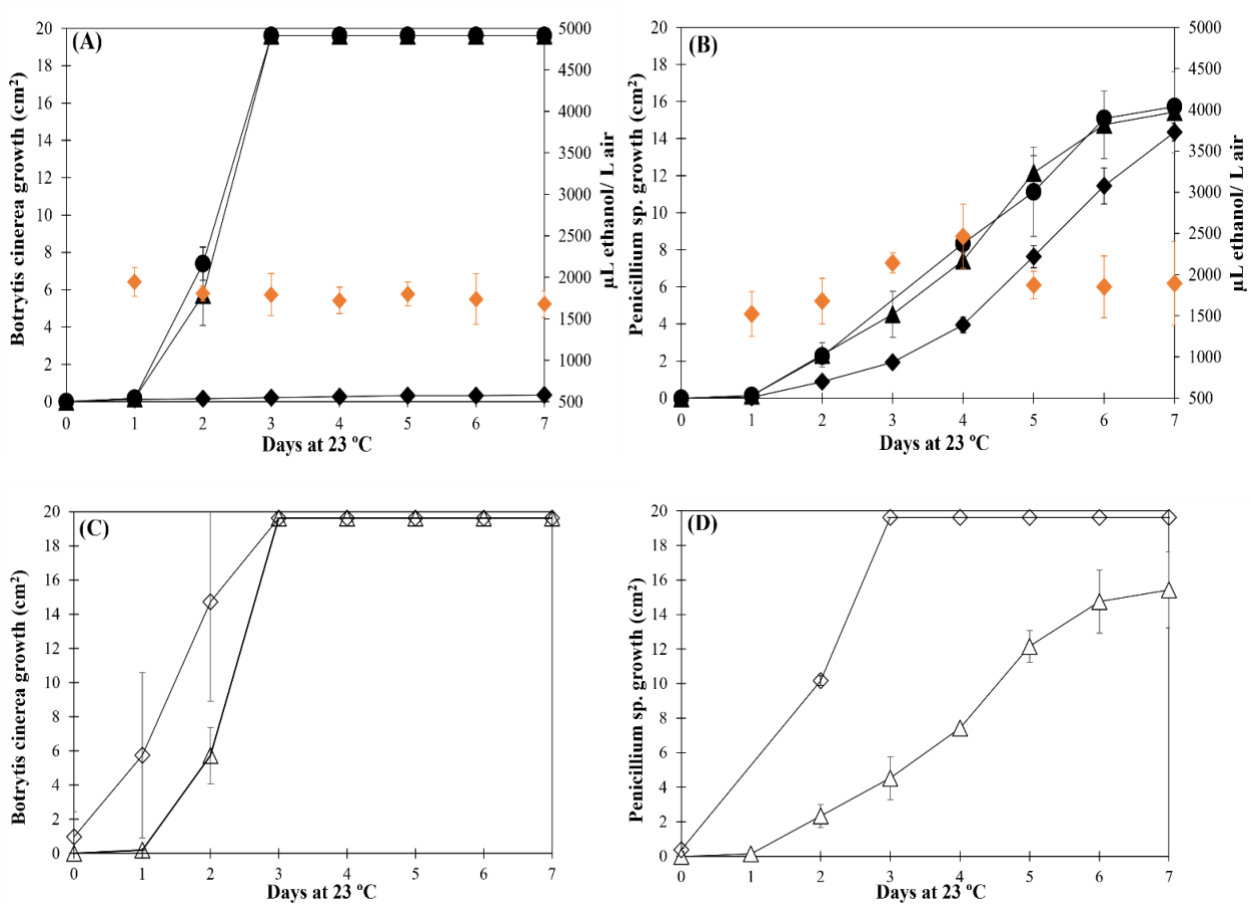


**Figure 4.5.** Growth of *Botrytis cinerea* (A) and *Penicillium* spp. (B) exposed to no bagasse paperboard (-▲-), uncoated bagasse paperboard (-●-), and shellac-coated bagasse paperboard (-◆-) for 7 days at 23ºC. The graphs include the amount of ethanol (µL ethanol /L air (ppm)) released in the bioassay system by the coated paperboard (size, 12*6.5 cm) (-◆-). (C) Comparison of the growth of *Botrytis cinerea* with (-◇-) and without (-△-) previous exposure to the coated paperboard. (D) Comparison of the grow of *Penicillium* spp. with (-◇-) and without (-△-) previous exposure to the coated paperboard. Previous exposure to the coated paperboard indicates sub-cultures.

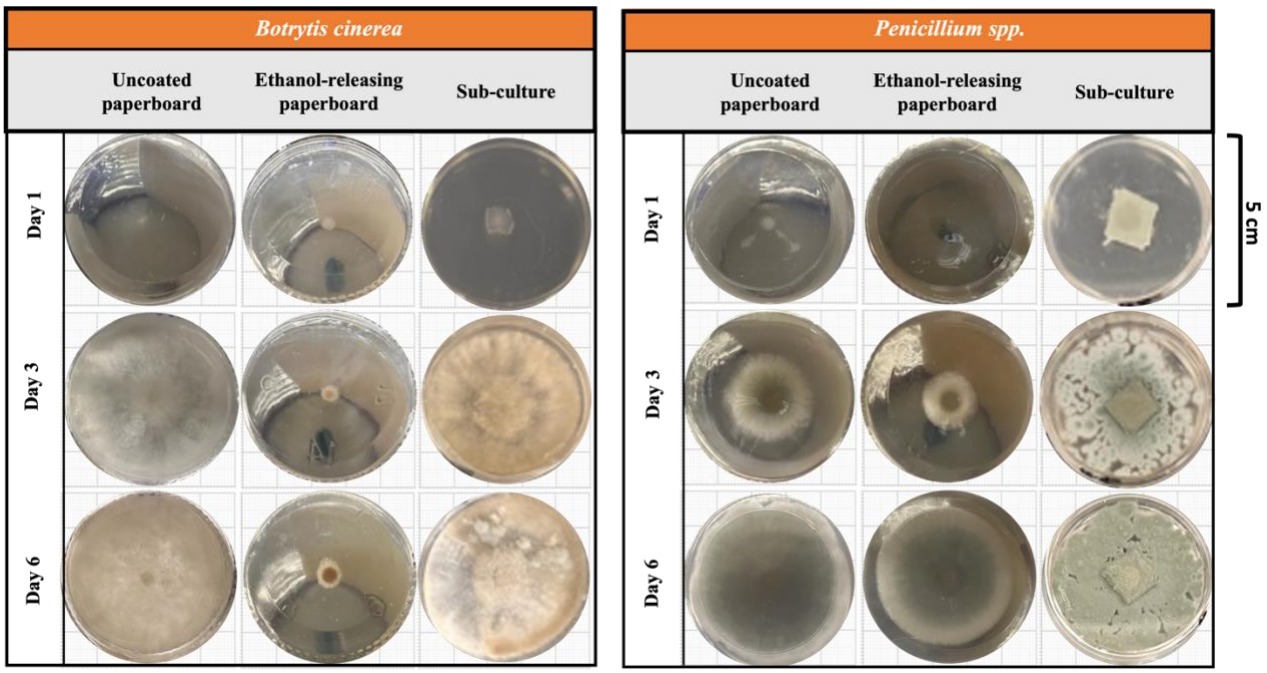


**Figure 4.6.** Visuals of the growth of *Botrytis cinerea* and *Penicillium* spp. in petri dishes with PDA media at the bottom of bioassay systems on days 1, 3, and 6. Comparisons are shown between systems with uncoated or shellac-coated paperboard, as well as the sub-cultures of the molds after exposure to shellac-coated bagasse paperboard.

The effectiveness of the developed ethanol-releasing bagasse paperboard against fungal growth was validated *in-vitro* using two main post-harvest diseases. The growth of *Botrytis cinerea* and *Penicillium* spp. on PDA media exposed to no bagasse paperboard, uncoated bagasse paperboard, and shellac-coated bagasse paperboard inside the bioassay system (section 4.1.3.1.) for 7 days at 23 ºC is presented in Figure 4.5 and visualized in Figure 4.6 for days 1, 3, and 6. The type of paperboard had a significant effect on the growth of *B. cinerea* throughout the whole storage period ($p < 0.0001$). The same significant difference in *B. cinerea* growth was observed when no paperboard was compared with ethanol-releasing bagasse paperboard ($p < 0.0001$). On day 7, *B. cinerea* exposed to the ethanol-releasing bagasse paperboard had a colony area of 0.37 $cm^2$ rather than the 19.63 $cm^2$ obtained when the mold was exposed to either no paperboard or uncoated paperboard ($p < 0.05$). Therefore, the growth of *B. cinerea* was reduced by >98% for 7 days at 23ºC in the presence of the ethanol-releasing bagasse paperboard (size, 12*6.5 cm) (Figure 4.5A). This reduction was achieved by ~ 1,900 µL ethanol/L air (ppm); Figure 4.5A). Similarly, the effectiveness of 1,000 µL ethanol/L air (ppm) against *B. cinerea* growth on PDA media was reported to be 76.6% at 28ºC when tested in a sealed petri dish with filter paper with ethanol was

attached to the inner side of the cover of the petri dish and the lid then sealed with preservative film (Ji et al., 2021).

*Penicillium* spp. grew differently if exposed to the ethanol-releasing paperboard compared with either no paperboard or uncoated paperboard ($p < 0.0001$). Specifically, on day 4, the colony area of *Penicillium* exposed to the ethanol-releasing bagasse paperboard was 3.95 $cm^2$ compared with the 7.90 $cm^2$ colony area if exposed to either no paperboard or uncoated paperboard ($p < 0.05$). As time passed, this difference was less, and on day 6, the colony area of *Penicillium* spp. exposed to the ethanol-releasing bagasse paperboard was 11.45 $cm^2$ while it was 14.75 $cm^2$ for both the uncoated paperboard and no paperboard ($p < 0.05$; Figure 5B). On day 7, *Penicillium* spp. colony area was the same with and without the ethanol-releasing bagasse paperboard (two-way interaction; $p < 0.0001$). Therefore, the exposure of *Penicillium* to the ethanol-releasing bagasse paperboard reduced the growth of the mold by half for 4 days and by 25% afterward until day 6 at 23ºC ($p < 0.0001$; Figures 4.5B). These reductions were achieved by ~ 1,719 µL ethanol/L air (ppm) (Figure 4.5B). According to Lichter et al. (2002), ethanol is more effective against the spores of *Botrytis* than its mycelia, which could apply to *Penicillium* as well and be the reason for the lower effectiveness of the volatile as the amount of *Penicillium* mycelia was greater. Other studies have also reported the effectiveness of ethanol vapor against *Penicillium spp.* growth (Wang et al., 2011). Based on the above results, *B. cinerea* and *Penicillium* spp. differed in their sensitivity to ethanol, with *B. cinerea* being more sensitive to the volatile than *Penicillium*. Thus, *B. cinerea* was the target mold for the *in-vivo* validation of the ethanol-releasing bagasse paperboard against fungal growth. The different sensitivity to ethanol of the two molds may be explained based on the affinity of the volatile for the cell membranes of the spores. Almenar et al. (2007b) also reported that toxic volatile molecules have different affinities to different fungal cell membranes. According to Lichter et al. (2002), ethanol's main target is the lipid membrane of the fungal cells, although it has many other effects on them.

The reduction of the two molds was achieved by ~ 1,719 µL ethanol/L air (ppm). As observed in Figure 4.2. C2, the ethanol present in the bioassay system when this contained a petri dish with and without mold was 1,719 µL ethanol/L air (ppm), while 14,200 µL ethanol/L air (ppm) was obtained with no exposure to PDA media ($p < 0.0001$). This difference in concentration can be attributed to the absorption of ethanol by the culture media as the latter is a hydrogel and ethanol

is soluble in water (Pranata et al., 2019). The solubility of other antimicrobials like acetaldehyde and hexanal in water has also been reported (Almenar et al., 2007a).

The growth of the sub-cultures of the two molds after exposure to the ethanol-releasing paperboard showed that ethanol is a fungistatic but not a fungicidal compound (Figures 4.5C, 4.5D and 4.6). The faster growth of the sub-cultured molds was because they were at the hypha state rather than the spore state when placed on the PDA media. However, the sub-cultures grew weaker (Figure 4.6), which may be the result of a sick mold due to the ethanol capacity of damaging and disintegrating mold cells and cell walls. This causes protein denaturation, inhibition of glucose uptake and water activity, unbalanced cytoplasmic permeability and cytosol leakage (Dao & Dantigny, 2011).

### 4.2.3. *In-vivo* antimicrobial properties of ethanol-releasing bagasse paperboard

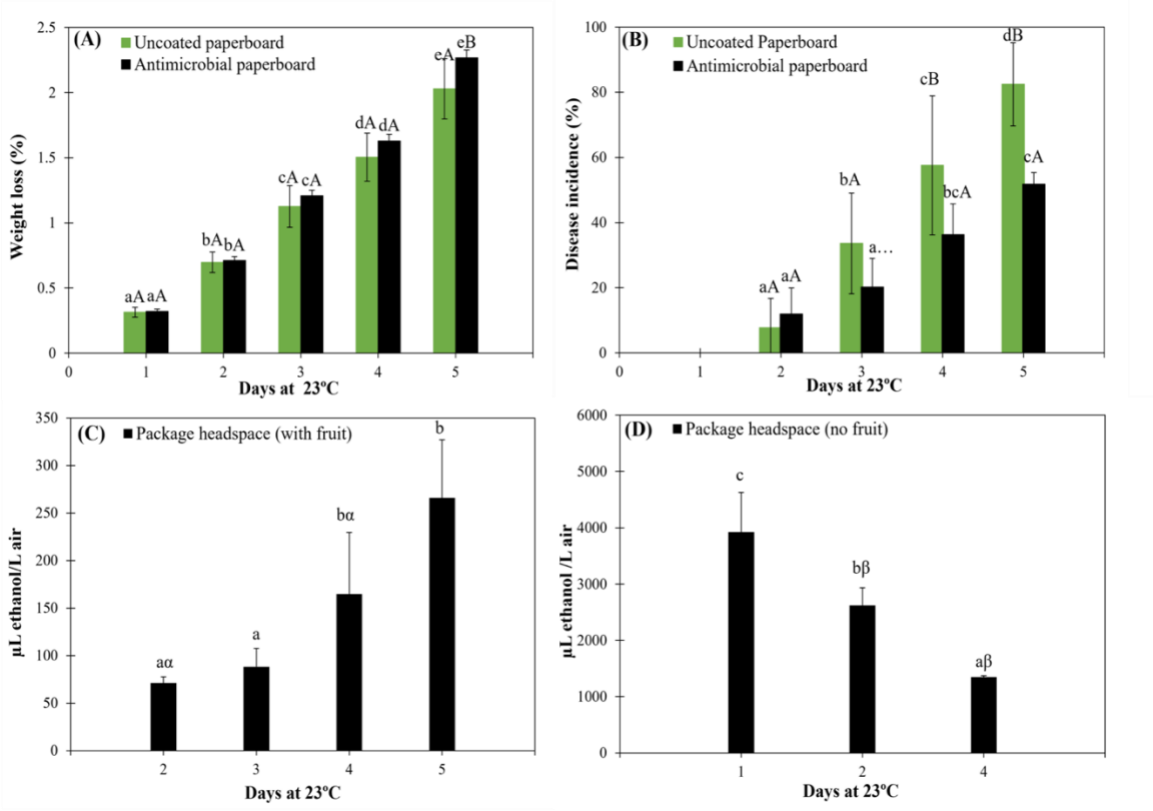


**Figure 4.7.** (A) Weight loss (%) and (B) disease incidence (%) of strawberries in packages with uncoated paperboard or ethanol-releasing paperboard stored for 5 days at 23°C and 50% RH. Ethanol content (µL ethanol/L air (ppm)) in the headspace of packages with ethanol-releasing paperboard (size, 12 cm × 6.5 cm) (C) strawberries or (D) without strawberries. Different lowercase letters indicate differences among days for each package. Different upper-case letters indicate differences between packages each day. Different Greek letters indicate differences between the package containing the ethanol-releasing paperboard with strawberries and without strawberries.

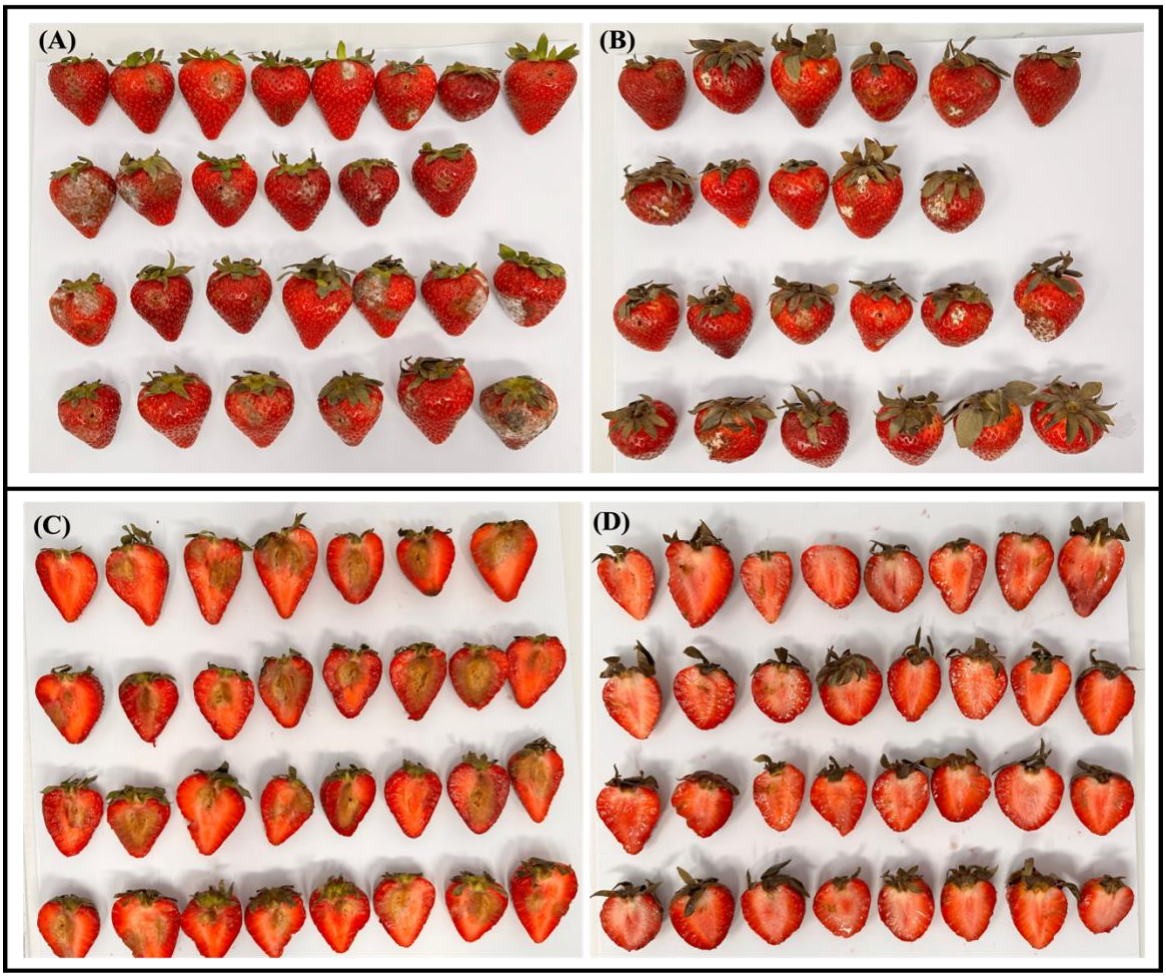


**Figure 4.8.** Images of the exterior and interior of strawberries after 5 days in packages with uncoated bagasse paperboard (A and C) and ethanol-releasing bagasse paperboard (B and D) stored at 23°C and 50% RH.

**Table 4.2.** Package headspace gas composition and color and firmness of strawberries packaged with uncoated bagasse paperboard and ethanol-releasing paperboard on days 0 and 5. Different lowercase letters indicate differences between days for each package and different uppercase letters indicate differences between packages for each day.

| Day | Package | Headspace Gas composition | | Color | | Texture |
|---|---|---|---|---|---|---|
| | | $O_2$ (%) | $CO_2$ (%) | L* | a* | Firmness (N) |
| 0 | Uncoated paperboard | 20.9 ± 0.0aA | 0.04 ± 0.0aA | 35.0 ± 4.0aA | 34.4 ± 3.3aA | 2.3 ± 0.7aA |
| | Ethanol-releasing paperboard | 20.9 ± 0.0aA | 0.04 ± 0.0aA | 35.0 ± 4.0aA | 34.4 ± 3.3aA | 2.3 ± 0.7aA |
| 5 | Uncoated bagasse paperboard | 6.7 ± 0.9bA | 8.6 ± 0.6bA | 30.9 ± 1.9bA | 26.6 ± 3.9bA | 1.6 ± 0.4bA |
| | Ethanol-releasing paperboard | 6.6 ± 0.2bA | 9.1 ± 0.3bA | 32.9 ± 2.9bB | 28.7 ± 3.6bA | 1.7 ± 0.8bA |

#### 4.2.3.1. Weight loss

Figure 4.7A shows the weight loss of strawberries in packages with uncoated paperboard and ethanol-emitting paperboard during 5 days at 23 ºC and 50% RH. The strawberries lost weight over time regardless of the type of package (with uncoated and ethanol-releasing coated bagasse paperboard) ($p < 0.0001$). At the end of the storage, the weight of the strawberries was 2.03% and 2.26% less in the packages with uncoated paperboard and ethanol-emitting paperboard, respectively. The type of package also had an effect on strawberry weight loss ($p = 0.0155$). The weight loss of the strawberries in the different packages was not significantly different until day 5 ($p < 0.05$), although the difference was of only 0.2%. Based on the above, ethanol did not affect strawberry transpiration until day 5 and the transpiration increase resulting from the presence of ethanol was minimum. Similarly, a weight loss of less than 3% has been reported for mulberry

fruit and sweet cherries after the fruits were stored in packages containing ethanol emitters consisting of plastic sachets (Bai et al., 2011; Choosung et al., 2019). In contrast, Li et al. (2023) reported a lower weight loss for fresh-cut strawberries when these were exposed to ethanol vapor before being processed. This is most likely due to the use of polypropylene containers to store the strawberry wedges, which has higher water barrier than microperforated polylactic acid (Selke et al., 2022). The authors also reported that fresh-cut strawberry weight loss was dependent on the concentration of ethanol. Strawberry weight loss in both packages was less than the 10% water loss considered as a limit for fruit acceptance (Gonzalez-Buesa et al., 2014). This may be the reason why the strawberries looked like fresh.

**4.2.3.2. Disease incidence**

The effectiveness of the developed ethanol-releasing bagasse paperboard as a packaging insert against *B. cinerea* was validated *in-vivo* on strawberries for 5 days at 23C and 50%RH. Figure 7B shows the percentage of disease incidence (DI) for strawberries in packages with uncoated bagasse paperboard and ethanol-releasing bagasse paperboard over time. DI in strawberries increased overtime regardless of the type of package ($p < 0.0001$). At the end of storage, DI was as high as 82% and 52% for strawberries in packages with uncoated bagasse paperboard and ethanol-releasing bagasse paperboard, respectively. The type of package also had an effect on DI ($p = 0.0155$). A significantly higher DI was observed for the strawberries of the packages with uncoated bagasse paperboard compared with those with ethanol-releasing bagasse paperboard after day 3 ($p < 0.05$). This can be explained by the effectiveness of ethanol vapor on delaying *B. cinerea* spore germination (Lichter et al., 2002). The strawberries in the two packaging designs differed not only in ID but in how much mold spread on strawberry surface (Figures 4.8A and 4.8B) as well as mold penetration in strawberry flesh (Figures 4.8C and 4.8D). The strawberries exposed to the ethanol-releasing bagasse paperboard (Figure 4.8B) showed external mold growth only around the inoculated wound that looked like a small, white patch as opposed to those exposed to the uncoated bagasse paperboard (Figure 4.8A) which showed a larger, white, furry mold. The delayed spore germination caused by ethanol vapor is further supported by the strawberry cross sections shown in Figures 4.8C and 4.8D. There was little to no mold growth inside the flesh of the strawberries near the wound for the strawberries exposed to the ethanol-releasing bagasse paperboard while there was significant internal rotting for almost all the strawberries exposed to uncoated paperboard. The above differences can be attributed to the ethanol vapor released inside the

package (71 - 266 µL ethanol/L air (ppm) (section 4.2.3.3)). Similar amounts of ethanol released from plastic pouches have been shown effective in reducing fungal growth in fruit. Choosung et al. (2017) reported that 150 µL ethanol/L air generated by a plastic pad containing a filter paper as an ethanol carrier delayed microbial growth by 80% in packaged mulberries after 168 h. Candir et al. (2012) reported that 198 µL ethanol/L air released from a commercial plastic-based ethanol-generating sachet (Antimold 80®) decreased the growth of *B. cinerea* by 45% in bagged grapes after 1 month at 0 ºC. Similar amounts of ethanol vapor (220 ethanol/L) generated from newspaper soaked in ethanol were able to avoid gray mold on grapes for 4 weeks at 0 ºC (Chervin et al., 2005). Also using a commercial plastic sachet (Antimold mild®), Bai et al. (2011) reported that 26 µL ethanol/L air decreased the growth of *Monilinia fructicola* by 1/3 in fresh-cut cherries in clamshells after 7 days at 20 ºC.

#### 4.2.3.3. Ethanol concentration in package headspace

Figures 4.7C and 4.7D show the amount of ethanol present in the headspace of the packages with the ethanol-releasing bagasse paperboard when containing strawberries and without strawberries, respectively. The amount of ethanol vapor was higher in the packages without strawberries regardless of the storage day ($p < 0.0001$), which indicates ethanol absorption by the fruit. This absorption can be explained by strawberries being made up of >90% water and this is because ethanol is a polar molecule which means it is highly soluble in water. This agrees with Figure 4.2 C1 which shows the high absorption of ethanol by the PDA media, which has a high-water content, in the case of the assay systems. Choosung et al. (2019) reported similar reduction of ethanol vapor released from an ethanol-releasing sachet in the headspace of packages with mulberry fruit compared with packages without fruit.

The amount of ethanol vapor in the packages with strawberries increased as a function of time ($p < 0.0001$) while the opposite happened in the empty packages ($p < 0.0001$). The decrease from 3,900 µL ethanol/L air (ppm) to 1,350 µL ethanol/L air (ppm) in 3 days in the empty packages (Figures 4.7C) can be attributed to the loss of the vapor through the polymer matrix and micro-perforations being more than the vapor released from the ethanol-releasing bagasse paperboard necessary to make up the loss. This happened because of the significant reduction in ethanol-releasing capacity of the insert over time (e.g., 60% reduction on day 4; Figure 4.4). The increased ethanol content in the packages with strawberries from 71 µL ethanol/L air (ppm) to 266 µL ethanol/L air (ppm) (Figures 4.7C) in 4 days must have resulted from the interaction between the

ethanol vapor and the fruit that led to the release of the trapped volatile if this not metabolized or the production of ethanol by the fruit. Although the increase in ethanol vapor was similar to the increase in fungal growth, it seems unlikely that they correlate since there was little to no ethanol in the packages with the uncoated bagasse paperboard (data not shown). However, further studies would be necessary to discard the above assumption since the small white spot created by *B. cinerea* in the packages with the ethanol-releasing bagasse paperboard could have generated ethanol in contrast to the furry *B. cinerea* present in the packages with the uncoated bagasse paperboard.

#### 4.2.3.4. Carbon dioxide and oxygen concentrations in package headspace

Table 4.2. shows the headspace gas composition of the packages with uncoated bagasse paperboard and ethanol-releasing bagasse paperboard. $O_2$ concentration decreased in both packaging designs ($p < 0.05$) and reached levels of ~7% on day 5. $CO_2$ concentration increased ($p < 0.05$) up to ~9% in both packaging designs on day 5. This change in headspace gas composition resulted from the respiration of the strawberries and perhaps the growth of *B. cinerea*. Although strawberries can have a significant respiration rate at 23ºC, the $CO_2$ concentration in all packages was < 10% after 5 days at 23ºC due to the micro-perforations in the PLA film that provided adequate gas exchange to avoid too high $CO_2$ levels or too low $O_2$ levels. Excessive $CO_2$ concentrations can cause undesirable changes in strawberries (Choosung et al., 2019; Li et al., 2022) while too low oxygen contents can cause anaerobiosis. The headspace gas composition in both packaging designs was not significantly different ($p = 0.800$ for $O_2$ and $p = 0.426$ for $CO_2$) which indicates that the ethanol concentration generated in the package headspace by the ethanol emitter had no effect on the respiration rates of the strawberries. Therefore, the ethanol absorbed by the fruit (section 3.3.3) did not affect the respiration rate of the strawberries. In contrast, Saltveit and Sharaf (1992) reported an increased respiration rate for tomatoes when these exposed to ethanol vapor.

#### 4.2.3.5. Firmness

The changes in firmness of the strawberries in packages with uncoated bagasse paperboard and ethanol-releasing bagasse paperboard are presented in Table 4.2. The initial firmness of the strawberries was 2.3±0.7 N. This firmness significantly decreased to 1.6±0.4 N ($p = 0.017$) and 1.7±0.8 N ($p = 0.003$) after 5 days for the strawberries from the packages with the uncoated bagasse paperboard and the packages with the ethanol-releasing bagasse paperboard, respectively. No significant differences in strawberry firmness were observed between the different packaging

designs ($p = 0.502$). Thus, the ethanol released from the ethanol-releasing bagasse paperboard had no effect on the texture of the fruit. In contrast, the literature reports less softening for fruit in packages with ethanol-emitting sachets (Bai et al., 2011; Mu et al., 2017).

**4.2.3.6. Color**

The changes in brightness (L*) and color (a*) of the strawberries in packages with uncoated bagasse paperboard and ethanol-releasing bagasse paperboard are presented in Table 4.2. The L* and a* values of the strawberries on day 0 were 35.0 and 34.4, respectively. On day 5, the strawberries from both packages were darker and less reddish as shown by the decreased L* and a* values. However, the strawberries exposed to the ethanol-releasing bagasse paperboard were significantly ($p = 0.0072$) less dark than the ones exposed to the uncoated bagasse paperboard. Similarly, the effectiveness of ethanol released from an ethanol-releasing sachet on retarding the darkening of packaged fruit have been reported for sweet cherry fruits (Bai et al., 2011) and grapes (Candir et al., 2011). No significant differences in a* between the strawberries exposed to uncoated bagasse paperboard and ethanol-releasing bagasse paperboard were obtained ($p = 0.0751$) although the redness of the strawberries from the packages with ethanol-releasing bagasse paperboard was closer to the initial strawberry redness. This shows that the ethanol vapors released from the insert did not result in any negative color changes in strawberries. The strawberries did not show any discoloration resulting from the exposure of the fruit to high ethanol vapor concentrations. In contrast, Choosung et al. (2019) reported red liquid spots on mulberry skin when the fruit was exposed to a stream of 95% ethanol inside a chamber for several days (~1,125 µL ethanol/L air). Exposure to high concentrations of ethanol have been related to cell structure damage and anthocyanin release from the plant tissue (Dao and Dantigny, 2011), which the strawberries did not experienced.

# CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

## 5.1. Conclusion

The first part of the thesis (chapter 3) shows that both shellac concentration and number of shellac layers can be used to improve BP properties. The increase of shellac concentration from 24% to 40% enhanced BP properties, however, a further increase of the shellac concentration to 60% did not. The change from 0 to 1 to 2 layers of shellac coating enhanced BP properties, however, layers 3 and 4 did not. The appropriate combination between these two factors for improvement BP properties was determined to be 40% shellac concentration in combination with two layers of coating. This was found to be the optimum one as it showed a decrease in water vapor (by half) and oxygen permeability (2 orders of magnitude) and water absorption (97%). The thermal results showed that coating shellac on paperboard improves the ability of shellac to withstand high temperatures increasing the number of applications of shellac related to heat exposure in food packaging. The developed shellac-coated paperboard opens new applications in food packaging for BP. 40% shellac-coated paperboard with two layers could be used for to-go containers, trays, and containers for fresh-produce and as coated paperboard multilayer structure for on-the-shelf products.

Based on part 2 and part 3 of this thesis (Chapter 4), it can be concluded that prototypes of ethanol-releasing shellac bagasse paperboard (BP) with a controlled release rate of the vapor have been successfully developed. The ethanol-releasing capacity of the prototypes increased with the addition of the shellac layers. The prototype with four layers shellac coating had a bi-phasic release behavior with a faster ethanol release rate with 80% of the ethanol released within 10 days followed by a much slower ethanol release rate for two weeks. Furthermore, the ethanol vapor after being released can easily decrease in amount if exposed to low temperatures or water-rich products, both very common in produce packaging. The developed ethanol-releasing shellac-coated bagasse paperboard showed effectiveness against the growth of two main postharvest diseases: *B. cinerea and Penicillium* spp. in in-vitro studies and when used as a packaging insert, a.k.a. ethanol emitter, was able to reduce grey mold in *B. cinerea*-infected strawberries and their darkening while no changing the fruit respiration, transpiration, firmness, and redness (in-vivo studies). The experimental findings confirm the antifungal activity of the ethanol-releasing shellac-coated paperboard, subsequently achieving the extended shelf life of the strawberry fruit. As a proof of concept, it can be concluded that ethanol-releasing shellac-coated bagasse paperboard can be a

sustainable, effective, and useful tool for controlling/reducing grey mold disease in strawberries and most likely other fruits. Furthermore, it could be a more environmentally friendly alternative and can potentially replace commercial plastic-based ethanol emitter sachets.

### 5.2. Future work

The future work can be focused on investigating coated paperboard with modified shellac chemical structure. Esterification of shellac under controlled conditions or with another biopolymer can result in a synergetic effect and can lead to even further improvement in the properties. In the case of *in-vivo* testing, the amount of ethanol absorbed by the strawberries should be investigated to understand the effect of ethanol on the strawberries and the slight increase in the ethanol in the headspace of the package with strawberries. The ethanol can lead to the accelerated release of different aromatic volatile compounds specifically ethyl acetate. Further investigation on understanding the increase or decrease in the aroma compounds can support the results obtained in this study.

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# APPENDIX

## CHAPTER 2:

PRODUCTION OF FILMS:

The film formed was not thick enough to be peeled off when formed using thinner and thicker bar types. Thus, the solution with a higher % of shellac was also considered for trying out the ability of the film to be formed. Later, the solutions were directly poured onto the Teflon plates. Also, the coating was bar coated on the calendared and rough sides of the bagasse paper-pulp sheet.

**Table A.** Preliminary Trials for Possible cast film and coated paperboard production.

| Serial Number | Sample ID | Composition | Substrate | Coating | Drying Time (Oven) | Storage time | Comments |
|---|---|---|---|---|---|---|---|
| 1 | 24_SD 1 | 24% Shellac in | No Substrate | On glass with bar number 3,6,8 | No oven drying | 5-6 hours | Very thin film, cannot be peeled |
| 2 | 24_SD 2 | | | | 2 hours | | Very brittle, non-peelable |
| 3 | 24_SD 3 | | On paperboard | | No oven drying | | Testable |
| 4 | 24_SD 4 | | | | 1-2 hours | | Testable, drying over 2 hours lead to burning |
| 5 | 24_SD 5 | | Teflon | pour coating | 2 hours | 5-6 hours | very brittle, non-peelable films |

Table A (cont'd)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | 24_SD 5 | 24% Shellac and 1% Citric acid | No substrate | On glass with bar number 3,6,8 | No oven drying | | Very thin, not peelable, <0.2 mm films |
| 7 | 24_SD 6 | | | Teflon plate | 2-3 hours | 6-7 hours | Thin peelable films, |
| 9 | 40_SD 1 | 40% Shellac in 100ml of ethanol | No substrate | On glass | No oven drying | 8-12 hours | Still thin, non-peelable |
| 10 | 40_SD 2 | | | Teflon | 6-8 hours | | Peelable, testable film |
| 11 | 40_SD 3 | | | Cast coating | 6-8 hours | | |
| 12 | 40_SD 4 | | Paperboard | Bar coating (number 8) | 1 hour | 6-8 hours | Testable samples |
| 13 | 40_SD 5 | | | Dip coated | 1 hour | 6-8 hours | Testable samples |
| 14 | 40_SD 6 | 40% Shellac and 1% citric acid | No Substrate | Teflon | 6-8 hours | 8-12 hours | Peelable, but thin films |
| 17 | 40_SD 8 | | Paperboard | Bar coating (number 8) | 1 hour | 6-8 hours | Testable samples but viscous solution |
| 18 | 40_SD 9 | | | Dip coated | | 6-8 hours | |

Table A (cont'd)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 19 | 40_SD 10 | 40% Shellac and 0.5% citric acid | No substrate | Teflon base | 6-8 hours | 8-12 hours | Peelable testable samples |
| 20 | 40_SD 11 | | | Cast coating | | 8-12 hours | Peelable |
| 21 | 40_SD 12 | | Paperboard | Bar coating (number 8) | 1 hour | 6-8 hours | Testable samples |
| 22 | 40_SD 13 | | | Dip coated | | 6-8 hours | Testable samples |
| 23 | 40_SD 14 | 40% Shellac and 0.25% citric acid | No Substrate | Teflon | 6-8 hours | 8-12 hours | Peelable/testable samples |
| 24 | 40_SD 15 | | | Cast coating | | 8-12 hours | Peelable |
| 25 | 40_SD 16 | | Paperboard | Bar coating (number 8) | 1 hour | 6-8 hours | Testable samples |
| 26 | 40_SD 17 | | | Dip coated | | 6-8 hours | Testable samples |
| 27 | 40_SD 17 | 40% Shellac and 0.25% citric acid | No Substrate | Cast coated on Teflon plates | | | When drying, film burning |
| 28 | 40_SD 17 | | Paperboard | Bar coating (number 8) | | | When heat-cured, **testable samples.** |

Initially, different methods were attempted to produce shellac cast film and conduct preliminary work. The data from this part was supposed to be for comparison of properties with coated paperboard as performed in Chapter 2 for thermal properties. Since none of the trials without

substrate performed well enough as a film, the bar coating method for producing coated paperboard was selected. And solvent cast on the Teflon plate was only used to produce small pieces of film for thermal analysis.

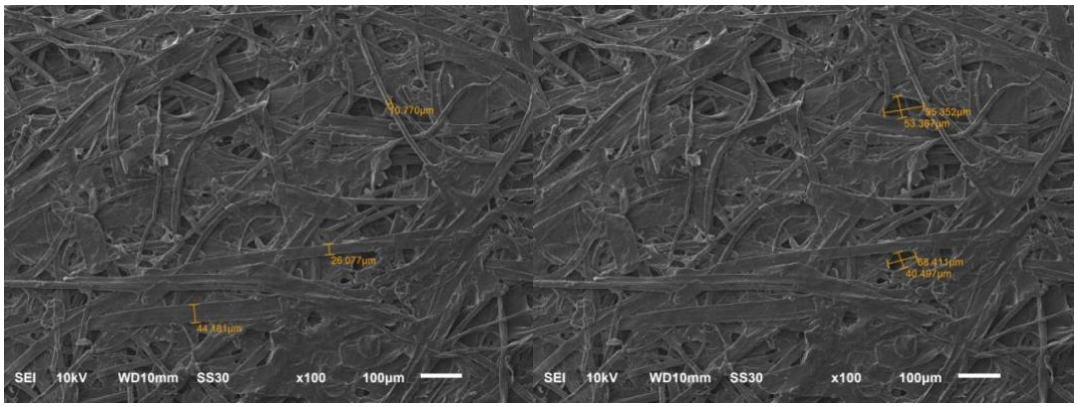


**Figure A**: Micrographs of uncoated paperboard showing pore size and the fiber size of the cellulose surface.

Figure A shows scanning electron micrographs of uncoated paperboard with emphasis on the pore size and the fiber size of the cellulosic structure supporting poor water and oxygen barrier of uncoated paperboard as the results show in Chapter 3.