AGRICULTURAL UNIVERSITY OF ATHENS
DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION
LABORATORY OF FOOD PROCESS ENGINEERING

M.Sc. IN FOOD PROCESSING, PRESERVATION AND BIOTECHNOLOGICAL PROCESSES – BIO-ECONOMY PRODUCTS DEVELOPMENT

Master's thesis

Valorization of sugar industry co-product for the production of pectin-based edible coatings for the shelf-life extension of frozen shrimps

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M.Sc. Food Processing, Preservation & Biotechnological, Processes – Bio-economy Products Development
Department of Food Science & Human Nutrition
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ABSTRACT

In this study was conducted the valorization of sugar industry co-products through the development of a biorefinery for the production of biodegradable and bio-based coatings based on pectin, as well as the production of bacterial cellulose. Pectin coatings were manufactured and subsequently applied to frozen shrimp under freeze-thaw cycles, with ascorbic acid serving as a browning inhibitor. Initially, after free sugar extraction of the initial SBP solids, the impact of time (1h, 2h, 3h & 4h) and temperature (60°C, 70°C & 80°C) on pectin extraction with nitric acid was investigated in both the residual solid and the isolated pectin. The yield of pectins varied from 4.6% to 11.7% (w/w) depending on the temperature and the extraction time and the highest pectin yield was noted at 80°C for 4 h. The optimal process chosen for the scale-up was 80°C for 1 h since they provided high pectin yield and significant galacturonic acid content. Subsequently, the SBP residue without free sugars and pectins was subjected to enzymatic hydrolysis, resulted in a hydrolysate with a sugar concentration of 27.3 g/L, with glucose being the predominant sugar (84.9%). The produced SBP hydrolysate was utilized as a carbon source for the production of BC using *Komagataeibacter sucrofermentans* DSM 15973, achieving a production of 2.1 g/L BC, with a yield 0.06 $g_{BC}/g_{consumed\ sugars}$ and productivity of 0.33 g/L/day. Pectin coatings were subsequently prepared by adding three diverse concentrations of ascorbic acid (0.5%, 1% and 2%). The produced coatings were applied to frozen shrimps subjected to freeze-thaw cycles, and their effect on shelf life was assessed through a series of physicochemical analyses. The average L* value did not show statistically significant differences among the shrimps coated with a combination of pectin and AA, but it differed significantly from both the control group and the shrimps coated with pectin alone. In addition, the color difference between control and pectin alone coated samples after 16-day period remains stable, while the color difference between control and pectin with AA coated samples steadily increases. The ImageJ software analyses did not reveal significant differences in either intensity (weighted) or the sum of R+B+G among the samples or across

different days. Shrimps coated with a combination of pectin and ascorbic acid received higher scores in the sensory analysis after the midpoint of the sensory analysis duration. The presence of ascorbic acid in all concentrations (0.5%, 1% & 2%) resulted in a reduction in PPO activity.

Scientific area: Bioprocess engineering

Keywords: Sugar Beet Pulp, Pectin, Bacterial Cellulose, Biorefinery, Shrimp packaging

Αξιοποίηση παραπροϊόντων βιομηχανίας ζάχαρης για την παραγωγή βρώσιμων επικαλύψεων με βάση την πηκτίνη για την επέκταση του χρόνου ζωής κατεψυγμένων γαρίδων

M.Sc. Επεξεργασία, Συντήρηση & Βιοτεχνολογικές Διεργασίες Τροφίμων – Ανάπτυξη Προϊόντων Βιο-οικονομίας
Τμήμα Επιστήμης Τροφίμων & Διατροφής του Ανθρώπου
Εργαστήριο Μηχανικής & Επεξεργασίας Τροφίμων

ΠΕΡΙΛΗΨΗ

Στην παρούσα μελέτη πραγματοποιήθηκε αξιοποίηση των παραπροϊόντων της βιομηχανίας ζάχαρης μέσω της ανάπτυξης ενός βιοδιυλιστηρίου για την παραγωγή βιοδιασπώμενων επικαλύψεων με βάση την πηκτίνη, καθώς και την παραγωγή βακτηριακής κυτταρίνης. Παρασκευάστηκαν επικαλύψεις με βάση την πηκτίνη, οι οποίες, στη συνέχεια εφαρμόστηκαν σε κατεψυγμένες γαρίδες υπό κύκλους ψύξης-απόψυξης, με τη προσθήκη ασκορβικού οξέος, το οποίο χρησιμεύει ως αναστολέας της αμαύρωσης. Αρχικά, μετά την ανάκτηση των ελεύθερων σακχάρων από το αρχικό στερεό, διερευνήθηκε η επίδραση του χρόνου (1h, 2h, 3h & 4h) και της θερμοκρασίας (60°C, 70°C & 80°C) στην εκχύλιση της πηκτίνης με νιτρικό οξύ τόσο στο υπολειπόμενο στερεό όσο και στην απομονωμένη πηκτίνη. Η απόδοση σε πηκτίνη κυμαινόταν από 4.6% έως 11.7% (w/w) ανάλογα με τη θερμοκρασία και το χρόνο εκχύλισης και η υψηλότερη απόδοση πηκτίνης σημειώθηκε στους 80°C για 4 h. Η βέλτιστη διαδικασία που επιλέχθηκε για το scale-up ήταν οι 80°C για 1 h, δεδομένου ότι παρείχαν υψηλή απόδοση πηκτίνης και υψηλή περιεκτικότητα σε γαλακτουρονικό οξύ. Στη συνέχεια, το υπόλειμμα SBP χωρίς ελεύθερα σάκχαρα και πηκτίνες υποβλήθηκε σε ενζυμική υδρόλυση, με αποτέλεσμα την παραγωγή υδρολύματος με συγκέντρωση σακχάρων 27.3 g/L, με κυρίαρχο σάκχαρο τη γλυκόζη (84.9%). Το παραγόμενο υδρόλυμα SBP χρησιμοποιήθηκε ως πηγή άνθρακα για την παραγωγή BC χρησιμοποιώντας το βακτήριο *Komagataeibacter sucrofermentans* DSM 15973, επιτυγχάνοντας παραγωγή 2.1 g/L BC, με απόδοση 0.06 $g_{BC}/g_{καταναλωθέντων\ σακχάρων}$ και παραγωγικότητα 0.33 g/L/day. Στη συνέχεια παρασκευάστηκαν επικαλύψεις πηκτίνης με την προσθήκη τριών διαφορετικών συγκεντρώσεων ασκορβικού οξέος (0,5%, 1% και 2%). Οι παραχθείσες επικαλύψεις εφαρμόστηκαν σε κατεψυγμένες γαρίδες που υποβλήθηκαν σε κύκλους κατάψυξης-απόψυξης και η επίδρασή τους στη διάρκεια ζωής αξιολογήθηκε μέσω μιας σειράς φυσικοχημικών αναλύσεων. Η μέση τιμή L* δεν παρουσίασε στατιστικά σημαντικές διαφορές μεταξύ των γαρίδων που είχαν επικαλυφθεί με συνδυασμό πηκτίνης και AA, αλλά διέφερε σημαντικά τόσο από το control όσο και από τις γαρίδες που είχαν επικαλυφθεί μόνο με πηκτίνη. Επιπλέον, μετά τη μέρα 16 η διαφορά χρώματος μεταξύ των δειγμάτων control και των δειγμάτων που έχουν επικαλυφθεί μόνο με

πηκτίνη παραμένει σταθερή, ενώ η διαφορά χρώματος μεταξύ των δειγμάτων ελέγχου και των δειγμάτων που έχουν επικαλυφθεί με πηκτίνη σε συνδυασμό με ΑΑ αυξάνεται. Οι αναλύσεις του λογισμικού ImageJ δεν έδωσαν σημαντικές διαφορές ούτε όσον αφορά στην ένταση της φωτεινότητας (Intensity (Weighted)), ούτε όσον αφορά στο άθροισμα R+B+G μεταξύ των δειγμάτων ή μεταξύ των διαφορετικών ημερών. Οι γαρίδες που ήταν επικαλυμμένες με συνδυασμό πηκτίνης και ασκορβικού οξέος έλαβαν υψηλότερες βαθμολογίες στην οργανοληπτική ανάλυση μετά το μέσο της διάρκειας της οργανοληπτικής ανάλυσης. Τέλος, η παρουσία του ΑΑ σε όλες τις συγκεντρώσεις (0,5%, 1% & 2%) είχε ως αποτέλεσμα τη μείωση της ενεργότητας του ενζύμου PPO.

Επιστημονική περιοχή: Μηχανική βιοδιεργασιών

Λέξεις κλειδιά: Πούλπα ζαχαρότευτλου, Πηκτίνη, Βακτηριακή Κυτταρίνη, Βιοδιυλιστήριο, Συσκευασία Γαρίδας

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1. Introduction

1.1. Plastics

Plastics are recognized as the most extensively utilized materials, particularly in packaging roles. The distribution of global packaging demand for the year 2019 by material type is shown in **Fig. 1**. The demand for plastics is continuously rising due to their versatile applications in various fields (Asgher et al., 2020). In 2021, worldwide plastic production amounted to approximately 390.7 million metric tons, an annual growth rate of four percent. Globally, the largest producer of plastics is Asia, with China alone contributing 32 percent to global production in 2021. Second in terms of plastic production worldwide ranks North America, with a share of 18 percent in 2021 (Statista, 2022a).

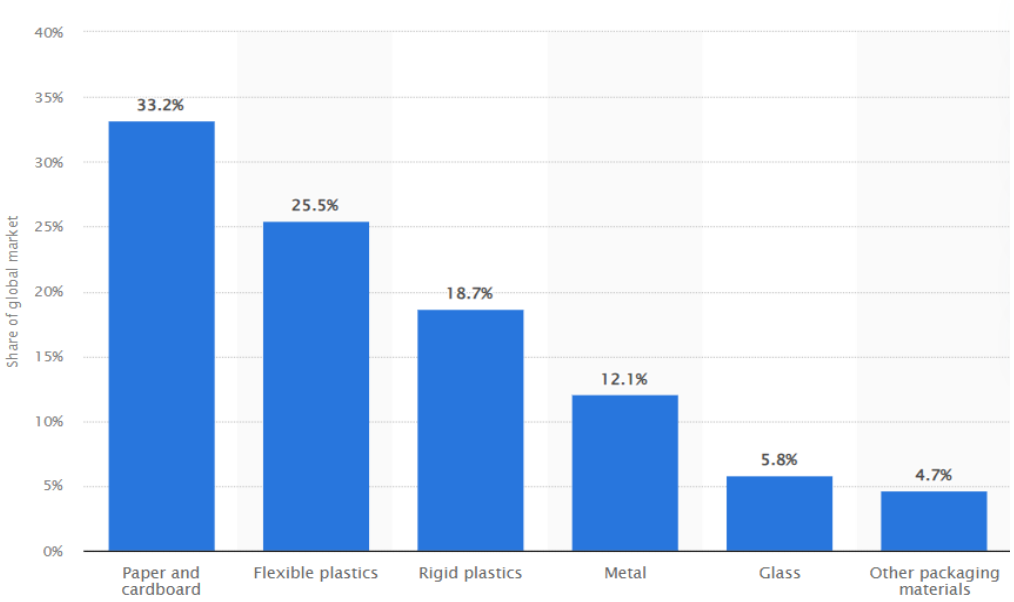

Figure 1. Global packaging material type distribution demand in 2019 (Statista, 2022a).

The majority of the plastic products commonly used, such as polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), and polyethylene terephthalate (PET), are derived from petrochemical resources. To produce these petroleum-based polymers, fossil fuels and natural gases serve as the primary raw materials. Due to their excellent mechanical characteristics, good thermal stability, as well as their chemical and biological inertness, they are suitable for diverse array of uses packaging industry (Gumede et a1., 2018). Nevertheless, the widespread usage of synthetic and non-biodegradable petroleum-based materials has led to significant environmental impacts (Qasim et al., 2021). For this reason, the need for conventional plastic materials replacement has become a subject of major interest.

1.1.1. Food Packaging

The main purpose of food packaging is to adequately shield food from external factors and potential dangers (Otto et al., 2021). Food packaging serves four primary roles: safeguarding and prolonging freshness, containment, conveying information and marketing, and enhancing convenience. Packaging is employed to shield the product against spoilage and damage or contamination caused by external factors, like bacteria, pests, air exposure, humidity, odors, contaminants, and particulate matter, among others. Another function of packaging is to communicate with the consumer before purchase, through the use of written texts (the list of ingredients, nutritional information, etc.) and the brand logo. Furthermore, these packages are customized to suit the consumer's lifestyle. For instance, they can save time, as seen in their suitability for ready meals, or they can be user-friendly, featuring easy opening, reclosable options, or compatibility with microwave use (Salgado et a1., 2021). Consequently, both the design of packaging and the materials choice must be meticulously considered. Different types of packaging materials are used for direct contact with food items. These materials include glass, metal, paper and cardboard (which may contain wood components), as well as various types of plastics (Otto et al., 2021).

Plastics encompass a diverse range and are the most prevalent materials employed in food packaging, e.g., bottles, foils and a multitude of other containers (Geueke et al., 2018). More precisely, petrochemical-based plastics find widespread application in the food packaging sector due to their affordability, excellent tensile characteristics, and their effectiveness as a barrier against oxygen, carbon dioxide, and water vapor. The above-mentioned plastics have been utilized in both flexible and rigid packaging and they are divided into thermoplastics or thermosets plastics. Thermoplastics can be treated and re-treated by the application of heat. Their reprocessability renders them well-suited for recycling, as they can be readily molded into diverse forms, making them highly appropriate for food packaging. Low-density polyethylene (LDPE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), high-density polyethylene (HDPE), polystyrene (PS), and expanded polystyrene are the thermoplastics extensively utilized in the production of food packaging materials. Conversely, once thermosets have taken shape, they no longer have the ability to be reprocessed with heat. This makes them non-recyclable and therefore not frequently utilized in food packaging (Ncube et a1., 2020).

1.1.2. Packaging Waste

Plastic pollution is a global environmental threat, the effects of which affect almost every marine and freshwater ecosystem worldwide (Borrelle et a1., 2020). Nonetheless, the repercussions of marine plastic pollution extend beyond harm to marine ecosystems; they also jeopardize the oceans, wildlife, public health, and economic activities that depend on the well-being of marine ecosystems (Beaumont et a1., 2019). Almost half of the world's plastic waste comes from plastic packaging and single-use plastic packaging constitutes a substantial portion of the millions of tons of plastic waste that escapes collection systems and enters waterways annually (Phelan et a1., 2022). In the European Union, the production of plastic packaging waste has been on a continuous annual increase since the end of the 2010s decade. As depicted in **Fig. 2**, in 2019, the quantity of plastic packaging waste generated within the European Union reached roughly 15.4 million metric tons, marking a growth of over 25 percent when compared to the figures from 2005 (Statista, 2022b).

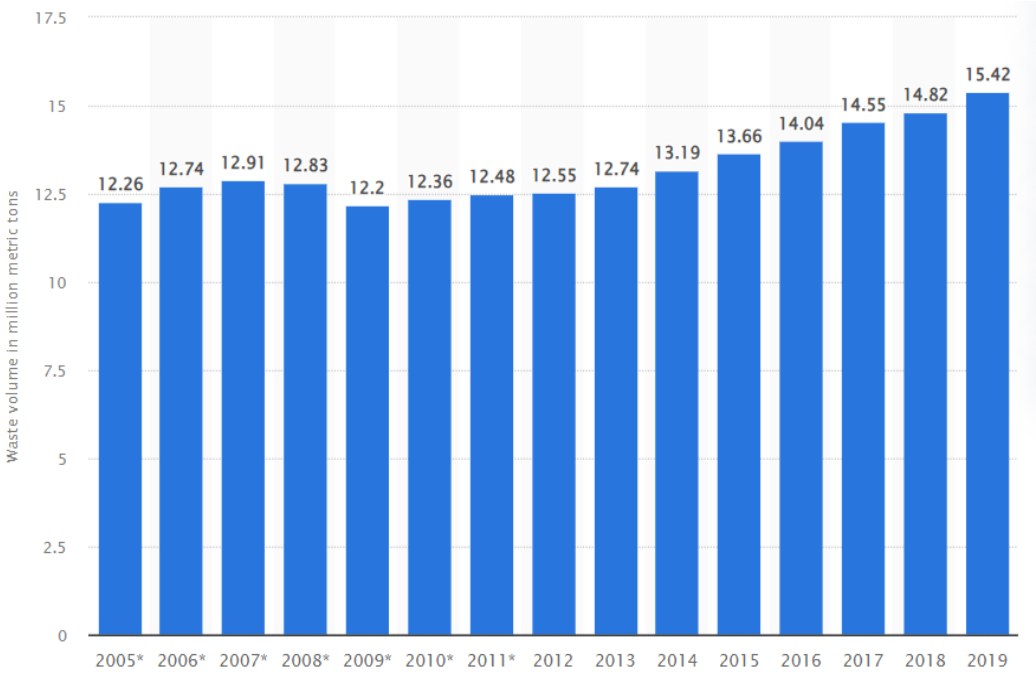

Figure 2. Accumulation of plastic packaging waste in the European Union from 2005 to 2019 (in million metric tons) (Statista, 2022b).

In 2019, the global population was estimated to be approximately 7.7 billion people, and projections suggest that it will reach approximately 9.7 billion by the year 2050. Concurrently, there is an anticipated 50% increase in global food demand. This rising requirement for food production is leading to a growth in the utilization of food packaging materials. The majority of food packaging is made from single-use plastics, which are disposed of relatively quickly. Consequently, there is a strong call for the food industry to prioritize

reducing, reusing, and recycling packaging materials. A comprehensive waste management strategy should involve collaboration among all stakeholders to attain a circular economy (Ncube et al., 2021).

1.2. Biopolymers, bioplastics and edible packaging

As mentioned above, the majority of plastic products are derived from petrochemical resources and they find a broad spectrum of uses in the packaging industry thanks to their excellent characteristics (Gumede et a1., 2018). However, because of their adverse environmental effects, there is a constantly growing necessity to replace them with other materials that have similar properties while being more environmentally friendly.

For this purpose, in 2019, the EU Directives on the reduction of the environmental effects of certain plastic materials were announced, making it imperative to gradually replace conventional plastics with alternative materials that are environmentally and health-friendly. More specifically, according to Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment, steps are taken to prevent and minimize the environmental effects of specific plastic items, especially in aquatic settings and human well-being. Furthermore, initiatives are promoted to advance a circular economy by adopting innovative and sustainable practices for plastic products, business models, and materials. This approach aims to foster economic growth and enhance the internal market's efficiency. A summary of the main measures follows: 1. Prohibition of single-use plastics: The Directive prohibits the utilization and sale of specific single-use plastic items, including cotton swabs, utensils, plates, straws, beverage stirrers, balloon sticks, and any products constructed from oxo-degradable plastics. 2. Plastic bottle collection targets: The Directive sets out objectives for separating plastics for recycling. By 2029, the aim is to attain a 90% collection rate specifically for plastic bottles (with a target of 77% by 2025). Achieving these targets can be facilitated through approaches like deposit return systems or by implementing goals within extended producer responsibility schemes. 3. Producer liability: Manufacturers of plastic products are considered responsible for their environmental consequences. They have to design products that are more easily recyclable and handle the waste that is generated. 4. Awareness raising and labelling: The Directive encourages awareness-raising to inform the public about the harmful consequences of single-use plastics. It also promotes clear labelling of specific products to highlight their environmental impact. 5.

Management of fishing gear: Measures are inserted to deal with the impact of lost or abandoned fishing gear on marine ecosystems. 6. Promotion of sustainable alternatives: The Directive encourages the development and promotion of more environmentally friendly alternatives to single-use plastics.

Biopolymers are a category of polymers, which are large molecules made up of repetitive subunits. They are generated by living organisms and can be obtained from microbial processes, extracted from plants, or synthesized chemically using fundamental biological components (Rebelo et a1., 2017). In recent times, the food industry has increasingly adopted biopolymers as packaging materials, as they degrade relatively quickly through natural microbial processes in suitable environment conditions such as oxygen, moisture and temperature, without causing ecological problems (Othman et a1, 2014). Biopolymers can be classified into three main groups depending on their source and how they are synthesized (**Fig. 3**). The initial group consists of natural polymers, the second group consists of synthetic polymers generated through various condensation or ring-opening polymerization processes and finally the third group includes biopolymers made by several types of microorganisms in a specific media fed with suitable nutritional ingredients (Taherimehr et a1., 2021).

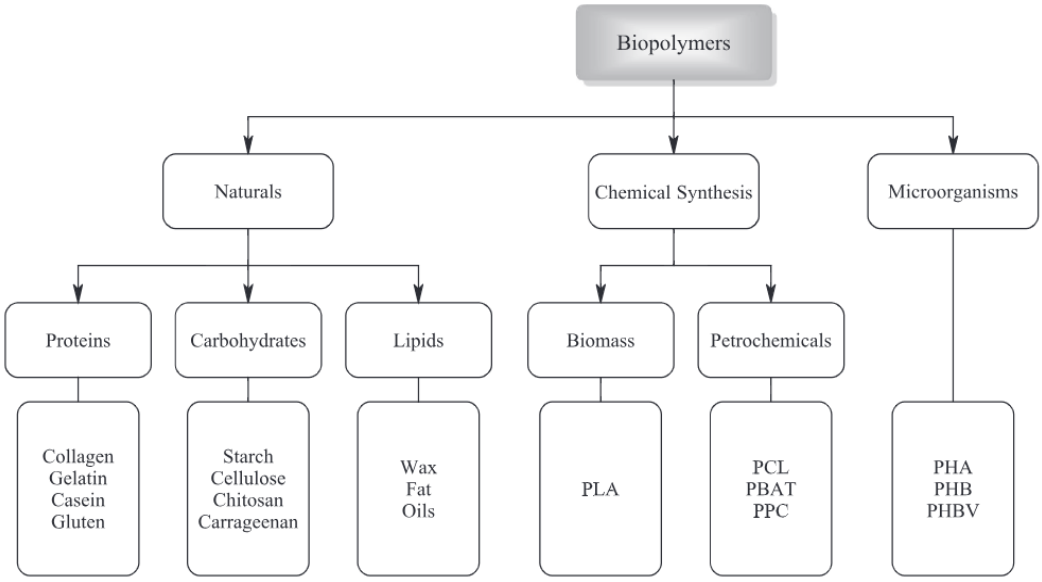

Figure 3. Classification of biopolymers based on their sources of origin (Taherimehr et al., 2021).

Bioplastics, or biopolymer plastic, are a subset of biopolymers. According to European Bioplastics, a plastic material is defined as a bioplastic if it is either biobased, biodegradable, or features both properties (**Fig. 4**). Based on the above definition there are the following

fundamental groups of bioplastics/biopolymers: 1. Biodegradable petrochemical-based bioplastics, 2. Biodegradable (mainly) biobased bioplastics, 3. Non-biodegradable biobased bioplastics (Endres, 2019). Bio-based means that the material is either wholly or partly obtained from biomass. Biodegradable signifies that the material can break down into natural substances like carbon dioxide, water, and biomass as a result of microorganism activity (Di Bartolo et al., 2021). Compostable plastics are a subset of biodegradable plastics, the decomposition of which occurs through biological procedures comprising adapted mixtures of microorganisms. Compostable plastics degrade under controlled conditions (e.g., temperature, moisture level, pH, oxygen) to carbon dioxide, water and inorganic compounds without leaving traces of visually discernible or toxic residues (Kjeldsen et al., 2018).

Biodegradable plastics can be produced from either petrochemical raw materials or renewable raw materials. The degradability of biopolymers depends solely on their chemical and physical microstructure and not on the source of the raw materials used or the manufacturing process used to produce them. This means that biopolymers do not have to be made exclusively from renewable materials. On the contrary, not all biopolymers based on renewable raw materials need to be degradable(Endres, 2019). Plastics degrade due to changes in their surface properties caused by interactions with various factors. The mechanisms of degradation of plastics in the environment are the following four: photo-, thermo-oxidative, hydrolytic and bio- degradation by microorganisms to ultimately resulting in the complete breakdown into CO_2 and H_2O. **Fig. 5** illustrates the categorization of plastics based on their degradation type.

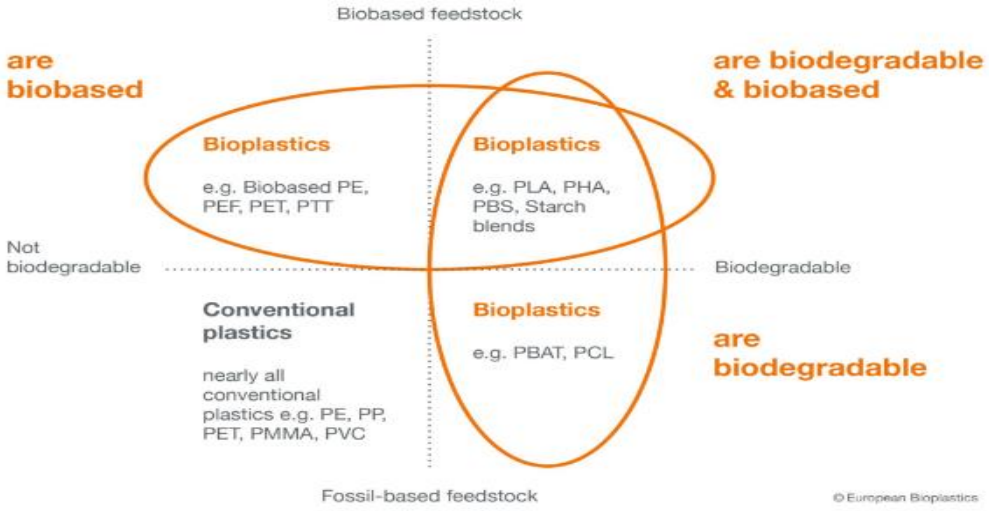

Figure 4. Material coordinate system for bioplastics (European Bioplastics, 2020).

The worldwide production ability for bioplastics is projected to rise from approximately 2.1 million tonnes in 2020 to about 2.8 million tonnes by 2025. In year 2020, Asia produced 46 percent of bioplastics while a quarter of capacity was in Europe. Europe's share is projected to increase up to 28 percent by 2025. Packaging represents the most extensive application area for bioplastics, accounting for nearly 47 percent of the overall bioplastics market in 2020 (European Bioplastics, 2020).

Some of the advantages that bioplastics have over conventional plastics include the following: they are environmentally friendly, take less time to degrade, are non-toxic and the production of many of them (e.g., polylactic acid) requires less energy consumption (Pathak et al., 2014). However, their comparatively higher cost in comparison to conventional plastics, limited mechanical properties and high vapor permeability prevent their widespread application in food packaging (Taherimehr et al., 2021).

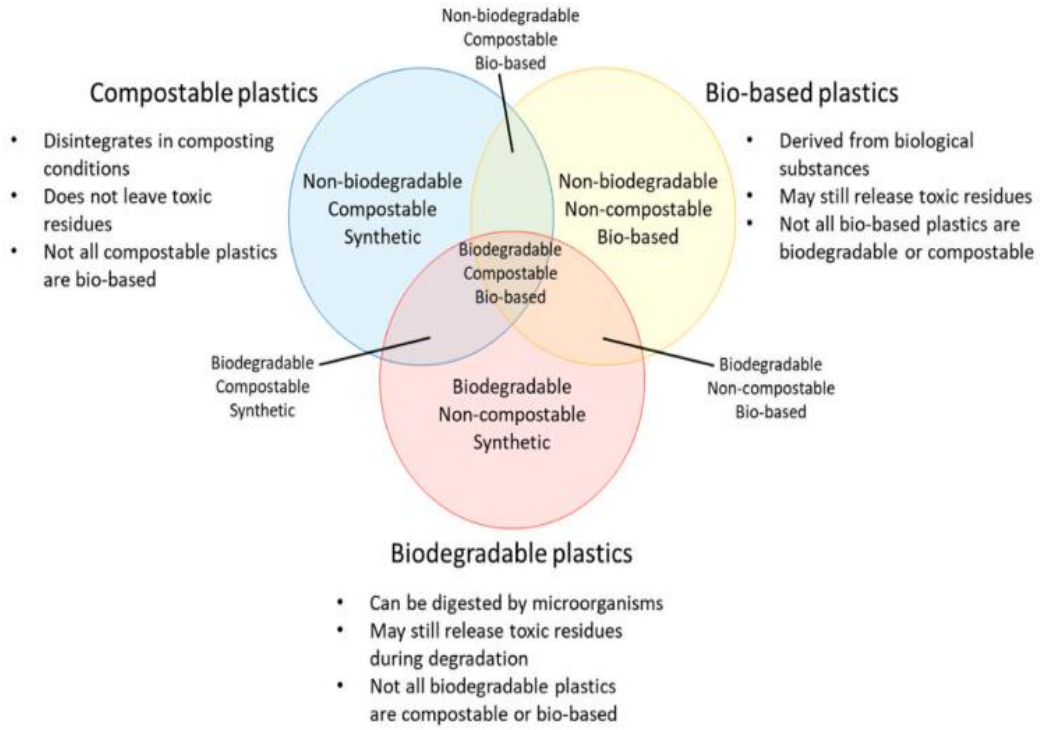

Figure 5. Categorization of plastics based on their degradation type (Idris et al., 2023).

Modern consumers have an increased demand for natural, high quality and safe food globally. They also require packaging that does not lead to environmental pollution and is manufactured using sustainable methods. The growing need to replace traditional food packaging has prompted a shift toward environmentally friendly and edible packaging solutions. Edible packaging typically involves the use of sustainable and biodegradable

materials as a disposable covering or coating for food, thus minimizing waste (Trajkovska Petkoska et a1., 2021).

An edible film or coating is described as any substance employed to cover (coat, wrap) various food items, aiming to prolong their shelf life. This substance is able to be ingested along with the food, either with or without removal. Edible films serve the purpose of safeguarding and sealing food, preventing moisture loss, and enabling the regulated exchange of gases, which are integral to respiration processes. Additionally, they can maintain sterility and prevent the loss of essential food components. The film or coating used is usually extremely thin, less than 0.3 mm thick (Pavlath & Orts, 2009).

Edible films and coatings consist mainly of polysaccharides, proteins, and lipids. These components can be used individually or together, forming mixtures or layers with different ratios of structural elements. Additionally, they often incorporate supplementary substances that serve to adjust the physicochemical characteristics, such as membrane integrity, structural stability, barrier properties, mechanical strength, and adhesion behavior (Lazaridou & Biliaderis, 2020). Edible films are produced through casting or extrusion processes, and the edible solution is applied to food items by dipping or spraying. The key distinction lies in how they are used: edible films involve wrapping solid edible laminate around the food, while edible coatings consist of applying the edible solution directly onto the food product. Both materials must be edible and possess the capacity to create films or coatings around the food items (Kumar et al., 2022). Edible films and coatings are sometimes used interchangeably, but they are applied in distinct ways **(Fig. 6)** (Otoni et a1., 2017). Edible films and coatings have diverse applications in various food products, including cheese, meat, fish, poultry, as well as fruits and vegetables (Kumar et a1., 2022).

The main advantages of edible packaging are (Trajkovska Petkoska et al., 2021):

1. Preservation of moisture and aroma: Edible packaging can be used as a protective layer, preventing the leakage of humidity, flavors and ingredients from and between foods, thereby keeping them fresher for longer.
2. Gas exchange control: edible packaging enables the controlled exchange of basic gases such as carbon dioxide, oxygen and ethylene associated with food respiration, contributing to the preservation of optimal food quality.

3. Improved organoleptic properties: Edible packaging can enhance the taste, color, and flavor of packaged foods by providing a range of flavors and pigments, tailoring surface properties and improving the overall eating experience.
4. Functional component carrier: edible materials are able to carry functional components with potential health or wellness benefits, making the packaging not only protective but also beneficial to consumers.
5. Specific material properties: Polysaccharides provide good oxygen barrier properties, proteins offer mechanical strength to protect fruits and vegetables during transport, while lipids have low vapor permeability and effectively preserve flavors and colors.
6. Sustainability: Edible packaging is mainly derived from renewable sources, making it a more sustainable option compared to traditional synthetic materials.

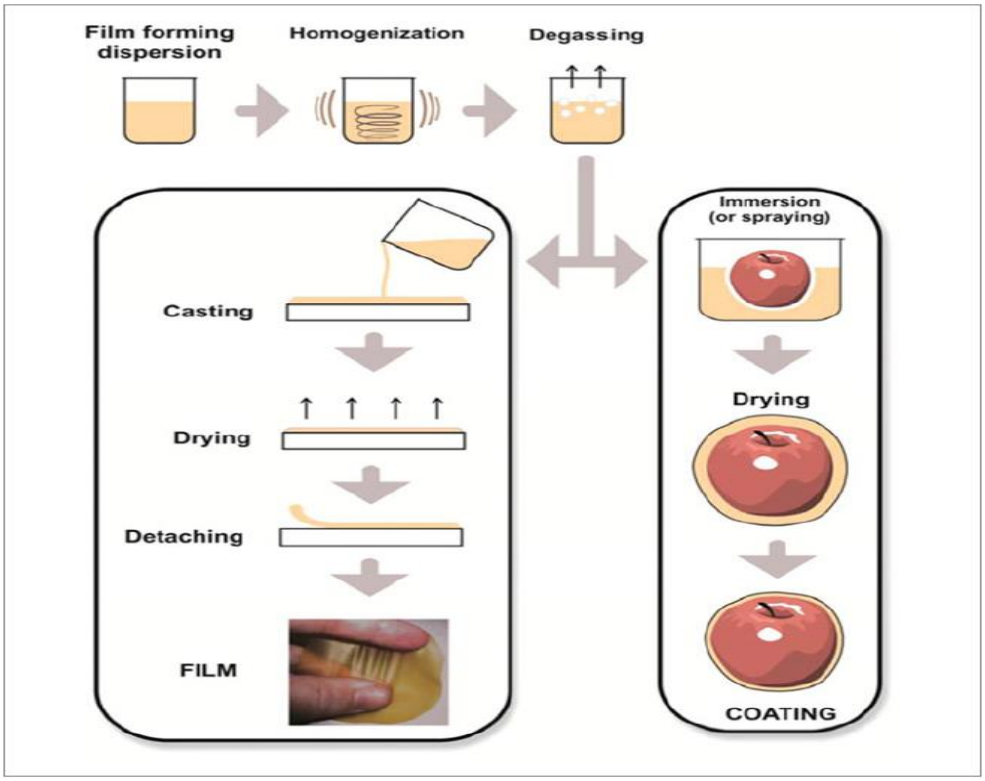

Figure 6. Diagram illustrating the process of creating edible films and coatings (Otoni et al., 2017).

An increasingly significant role of edible packaging materials, as previously mentioned as a benefit, includes their capability to serve as a matrix and carrier for various functional additives. More specifically, edible packaging can carry various functional bioactive compounds, including: 1. Nutraceuticals: Compounds obtained from food sources that offer health advantages, such as vitamins, polyphenols, and omega fatty acids. 2. Antioxidants:

Substances that delay oxidation reactions in food, preventing color changes, flavor alterations, and nutritional losses. 3. Antimicrobials: Agents that hinder the proliferation of microorganisms in food, enhancing food safety and extending shelf life. Examples include essential oils and nanoparticles. 4. Probiotics: Live microorganisms such as lactic acid bacteria or yeasts can be integrated into edible films to impart beneficial effects during food processing and storage. 5. Plasticizers: These additives make edible films more flexible and resilient by enhancing their mechanical properties. 6. Emulsifiers: Surface-active compounds used to stabilize lipid particles in composite emulsion films and improve surface coverage and adhesion. 7. Texture Enhancers: Additives like calcium salts that interact with polymers to increase the firmness of the product. 8. Flavors and Aromas: Natural or synthetic compounds added to edible packaging to preserve or enhance the food's flavor and aroma (Kumar et al., 2022; Lazaridou & Biliaderis, 2020; Trajkovska Petkoska et al., 2021).

1.3. Biorefineries

Reducing our reliance on finite fossil fuels is of utmost importance, underscoring the significance of sustainable energy, fuel, organic chemical, and polymer production from biomass within an integrated biorefinery framework (Maity, 2015). The concept of a "biorefinery" refers to a system designed for the commercial conversion of biomass into various products, including fuels, chemicals, polymers, materials, food, feed, and valuable ingredients (Koutinas et al., 2014).

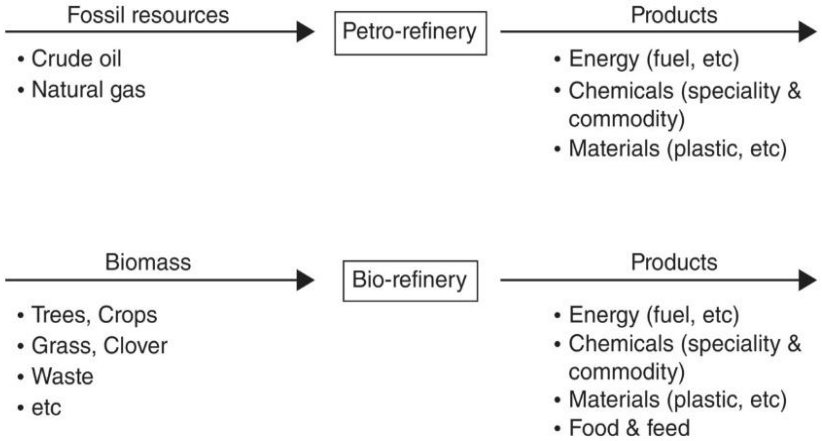

Figure 7. Comparison of biorefinery vs petrorefinery (Clark & Deswarte, 2015).

The fundamental idea behind a biorefinery is relatively straightforward: a biorefinery facility utilizes biomass as its feedstock, aiming to convert this biomass, ideally in its entirety,

into a diverse array of commercial products. This concept serves as an analogy to traditional petroleum-based refineries, where crude oil is processed to yield various valuable products (Wagemann & Tippkötter, 2019). **Fig. 7** demonstrates the comparison of biorefineries with petrorefineries (Clark & Deswarte, 2015).

Based on the conversion technologies for the production of various bioproducts, there are the three following types of biorefineries (Clark & Deswarte, 2015; Fernando et a1., 2006; Kamm & Kamm, 2004; Maity, 2015):

- Phase I biorefinery (single feedstock, single process and single primary product).
- Phase II biorefinery (single feedstock, multiple processes and multiple major products).
- Phase III biorefinery (multiple feedstocks, multiple processes and multiple major products).

Currently, biorefineries are facing several major challenges. These include their acceptance in the current market, which is mainly based on fossil fuels, the availability and composition of feedstocks, resource adequacy to fulfil market demands, efficient resource recovery, techno-economic viability and environmental sustainability (Katakojwala & Mohan, 2021). The fact that the availability and composition of feedstocks is a challenge for biorefineries makes the selection of the right feedstock a factor of utmost importance. The valorisation of wastes such as those from fruit and vegetable industries, breweries, wineries, sugar beet processing, crude glycerol from biodiesel production, waste from pulp and paper industries and the organic fraction of municipal waste seem to be ideal feedstocks for the production of products in biorefineries (Ioannidou et a1., 2020).

1.4. Sugar Beet Pulp

Sugar beet, scientifically designated as *Beta vulgaris*, falls under the botanical family *Amaranthaceae* and the order *Caryophyllales*. It holds significant economic value as a cash crop, with diverse applications in industries and commercial sectors. Within the *Beta* genus, there are four distinct sections: *Beta, Nanae, Procumbentes, and Corollinae*. Among these, the *Beta* section includes the cultivated varieties of beets, such as fodder beets, leaf beets, garden beets, and sugar beets, all of which belong to the sub-species *vulgaris* (Subrahmanyeswari & Gantait, 2022). *Beta vulgaris* subsp. *maritima* acts as the ancestor of the *Beta vulgaris* complex. It is distributed along the Mediterranean coast and extends along the Atlantic coast up to

Scandinavia. Additionally, this subspecies can be found in various regions, including the Middle East, India, Iran, and Azerbaijan (Goldman & Janick, 2021). Its wide geographical range indicates its significance in the evolutionary history of the cultivated beets within the *Beta vulgaris* complex. Sugar beets are composed of approximately 75% water, making up the majority of their content. They also contain around 18% sugar and 5% cell walls (Joanna et al., 2018).

Sugar beet holds a position of great importance among global crops (Alexandri et al., 2019) and in conjunction with sugarcane (*Saccharum officinarum*), sugar beet serves as one of the two primary worldwide sources of sucrose. (Adiletta et al., 2020). Approximately 270 million tonnes of sugar beet were cultivated worldwide in 2021. Europe accounts for almost 70% of the global sugar beet production. Germany stands as a prominent sugar beet producer in Europe, generating 28 million tonnes in 2021, accounting for nearly 12% of the global production (**Fig. 8**) (Our World in Data, 2023).

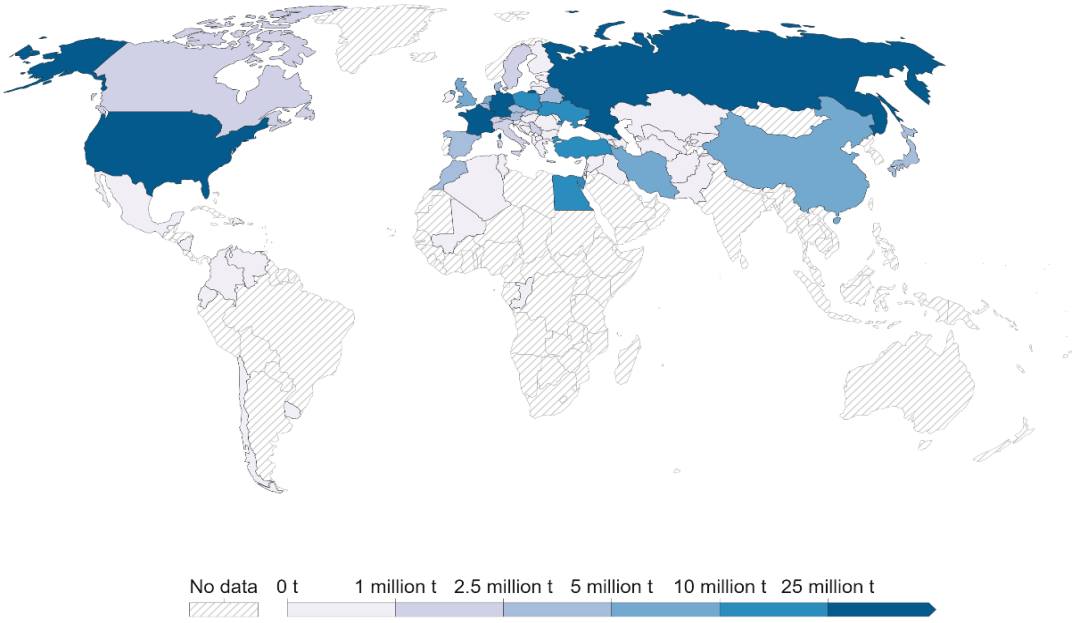

Figure 8. Sugar beet production measured in tonnes, 2021 (Our World in Data, 2023).

The sugar industry produces significant quantities of diverse waste materials, including sugar beet pulp, leaves, and molasses (**Fig. 9**). However, these co-products can be highly valuable substrates in the field of biotechnology (Joanna et al., 2018). Sugar beet pulp (SBP) is the primary solid co-product of the sugar industry, acquired following the extraction of sucrose from the sugar beet crop (Adiletta et a1., 2020; Alexandri et al., 2019).

SBP consists primarily of three structural carbohydrates: cellulose, hemicellulose and pectin. Additionally, it contains protein, lignin, ash, and fats in varying amounts. (Adiletta et a1., 2020; del Amo-Mateos et a1., 2022; Narisetty et a1., 2022; Usmani et a1., 2022). Despite being rich in valuable biomass and nutrients, sugar beet pulp (SBP) is primarily employed as animal feed in livestock farming or discarded in landfills without being utilized for animal feed (Adiletta et a1., 2020; Usmani et a1., 2022). SBP is abundant in nutrients and minerals, rendering it a viable raw feedstock for the industrial production of high-value products, such as bioethanol, biogas, biopolymers, and bioplastics (**Fig. 10**) (Usmani et al., 2022).

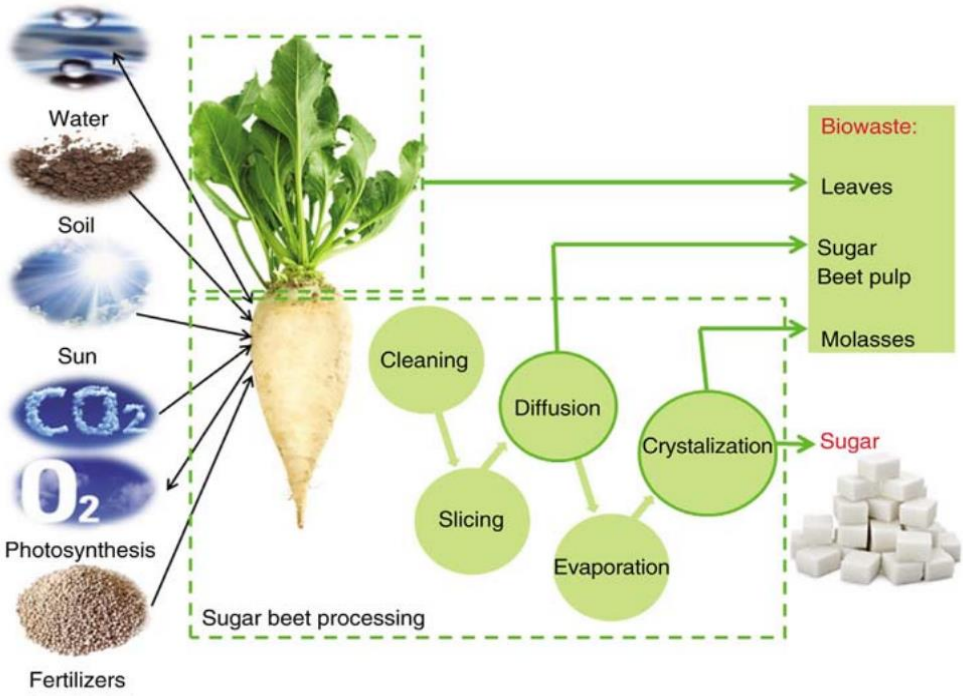

Figure 9. Sugar beet processing procedures (Joanna et a1., 2018).

Recently, a number of studies have concentrated on the development of innovative biorefineries using raw feedstocks, such as SBP. For instance, Adiletta et al. (2020) proposed a streamlined biorefinery approach aims to harness sugar beet pulp (SBP) for making commodity chemicals, specifically pectins, as well as solid fuels through the creation of torrefied pectin-free SBP (**Fig. 11**) and Alexandri et a1. (2019) researched the fractionation of SBP to produce pectins, phenolic components as well as a sugar-rich hydrolysate which is utilized for the making succinic acid.

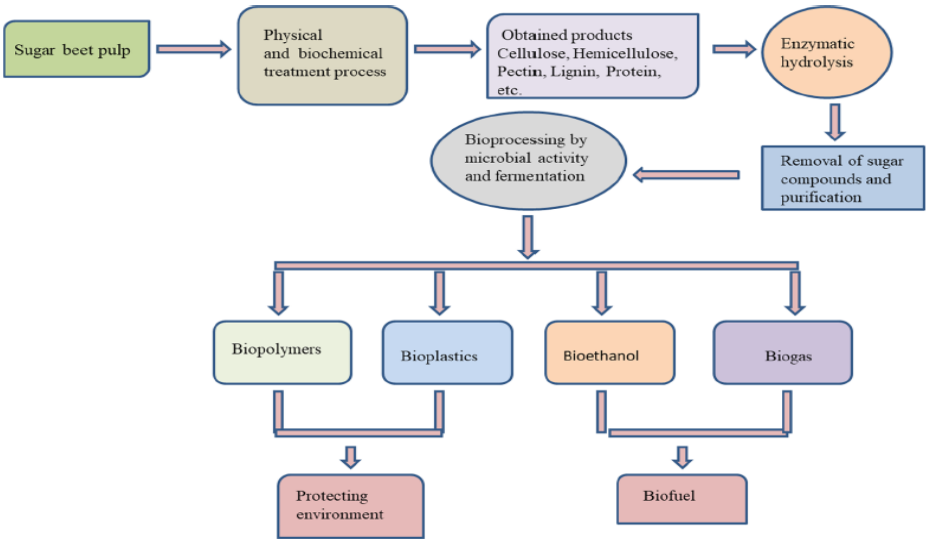

Figure 10. Transforming processed SBP into high-value products using secondary conversion (Usmani et al., 2022).

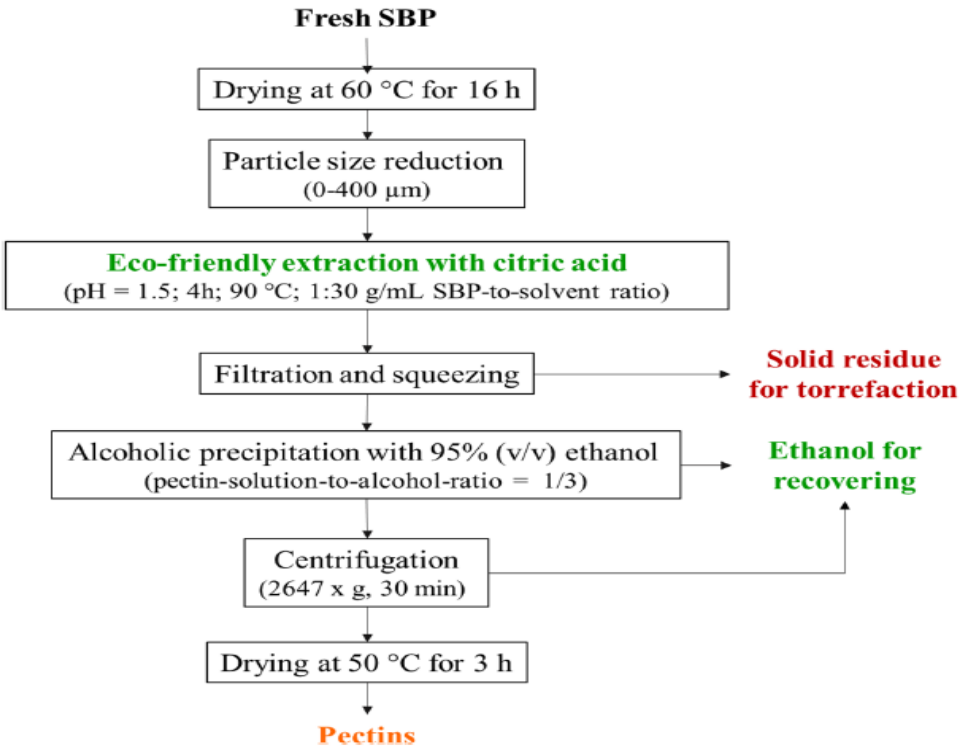

Figure 11. A simplified laboratory process flowchart outlining a streamlined biorefinery approach for converting SBP into valuable commodity chemicals and solid fuels (Adiletta et al., 2020).

1.5. Pectin

1.5.1. Structure and Classification of pectin

Pectin is a category of intricate polysaccharides primarily located in the cell walls of plant cells (Andersen et al., 2017). Pectin is present in nearly all plant varieties, but in commercial production, it is predominantly derived from citrus fruits. In recent times, there has been an exploration of novel pectin sources, with a notable interest in extracting pectin from waste co-products obtained from various industries. Although not widely used in commercial applications, some examples of these alternative sources include residues from sunflower heads, mangoes, amaranth, seed oils, and sugar beet pulp (Lara-Espinoza et al., 2018). Their presence in the cell is vital for a number of key functions: (a) adhesion between cells, (b) mechanical strength of the cell wall, (c) capacity to form stabilizing gels and (d) important role in plant cell growth (Valdes et al., 2015). Pectins are heterogeneous polysaccharides comprising three primary structural segments: homogalacturonan, which alternates with two types of highly branched rhamnogalacturonan domains, referred to as RG-I and RG-II. Additional structural categories of pectic polysaccharides are also encompassed, such as xylolacturonans, arabinogalactans and arabinans (**Fig. 12**) (Lara-Espinoza et al., 2018).

i. **Homogalacturonan (HG):** HG is a linear polymer composed of D-galacturonic acid and have the ability undergo acetylation or esterification with methyl groups. Depending on the extent of esterification, HG is categorized into: pectin, pectinic acid, and pectic acid or polygalacturonic acid. Pectin consists of a minimum of 75% methylated carboxylic groups, pectinic acid has less than 75% of methylated carboxylic groups, while pectic acid or polygalacturonic acid lacks methylated carboxylic groups. The term "pectin" is generally used to refer to substances with gelling properties (Kohli & Gupta, 2015). Pectins are categorized based on their degree of esterification, depending on the number of carboxyl groups that can be esterified with methyl groups. Pectins having more than 50% of esterified carboxyl groups are referred to as high methoxyl (HM), while those with less than 50% of esterified carboxyl groups are termed low methoxyl (LM). This characteristic is strongly associated with the gelatinization mechanism (Jong et al., 2023; Lara-Espinoza et a1., 2018; Lazaridou & Biliaderis, 2020). The key differentiation between HMP and LMP lies in their gel formation mechanisms, which are critical properties in various pectin applications. Typically, high-ester pectins create gels under acidic conditions (with a pH range of 2.0–3.5) when there are soluble solids like sugar at

concentrations exceeding 55% by weight. On the other hand, low-ester pectins can form gels within a wider pH variability (2.0 - 6.0) and in the presence of divalent ions, as calcium (Adiletta et al., 2020).

ii. **Rhamnogalacturonan I (RGI):** RG-I refers to a category of pectic polysaccharides composed of the recurring disaccharide unit rhamnose-galacturonic acid (Kohli & Gupta, 2015). More specifically, it contains a backbone composed of up to 100 repeating units [→4)-α-d-GalpA-(1→2)-α-l-Rhap-(1→] (Lara-Espinoza et a1., 2018). This backbone is partially branched at the O-4 (predominantly) and/or O-3 positions of the α-L-Rhap residues, with short side chains of various types and sizes, such as (1,5)-α-L-arabinane, (1,5)-β-D-galactan, galactoarabinan and arabinogalactans (Lazaridou & Biliaderis, 2020).

iii. **Rhamnogalacturonan II (RGII):** RGII includes a chain of homogalacturonans, with more intricate side chains attached to the galacturonan residues (Kohli & Gupta, 2015). In particular, RG-II has a highly conserved structure, with an oligogalacturonic acid backbone with no residues of rhamnose. This backbone is branching with four distinct side chains (A, B, C and D) containing six unusual sugars, notably apiose, 2-O-methyl-fucose, 2-O-methyl-xylose, aceric acid, 3-deoxy-manno-2-octulosonic acid (kdo) and 3-deoxy-D-lyxo-heptulosaric acid (dha) (Lazaridou & Biliaderis, 2020). The RGII structure is commonly preserved in numerous plants. Cross-linking between the RGII chains of two adjacent pectin molecules improves the integrity of the pectin network. Due to its configuration, RG-II can form borate ester dimers (Kohli & Gupta, 2015).

iv. **Xylogalacturonan (XGA):** XGA is a less commonly found type of pectin that is typically only expressed in the reproductive plant's tissues. It belongs to the substituted homogalacturonan structure, where the β-linked xylose at the O-3 position is additionally substituted with another β-linked xylose at O-4 (Roy et al., 2023).

v. **Apiogalacturonan (AP):** AP is one more infrequently occurring pectin type that results from d-Apiofuraose substitution at either the O-2 or O-3 positions. Which can be found in aquatic monocots (Roy et al., 2023).

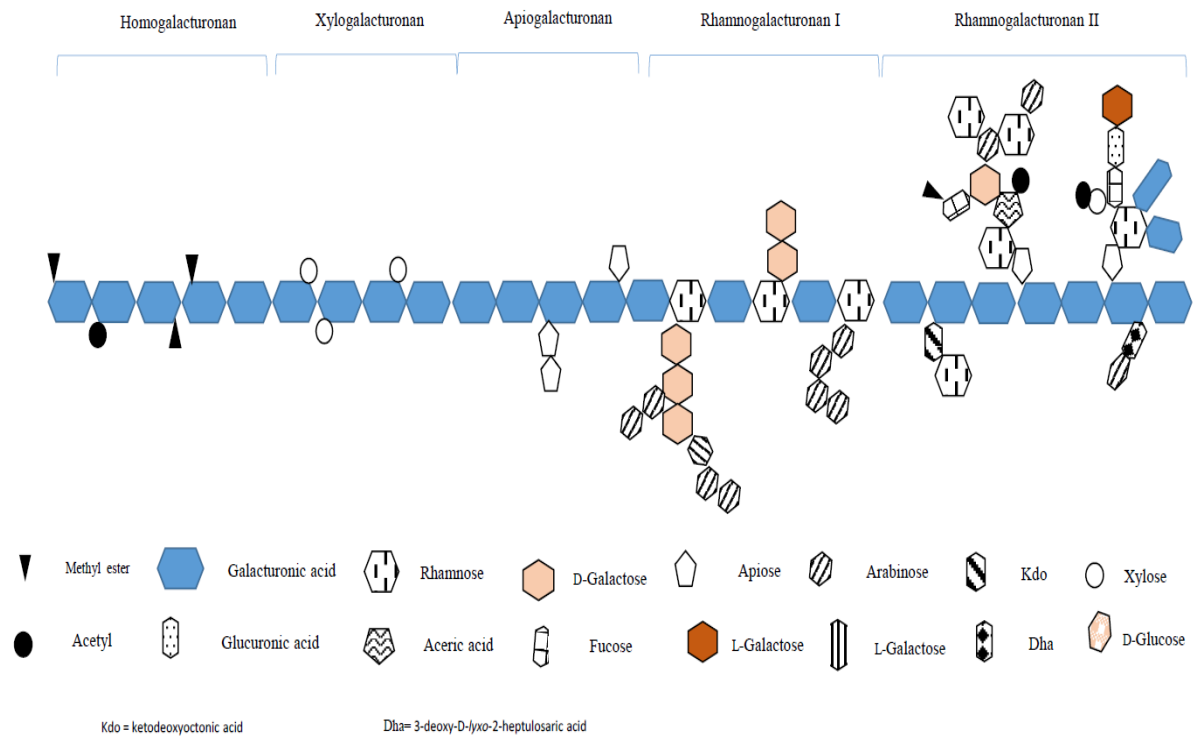

Figure 12. Pectin structure (Belkheiri et al., 2021).

1.5.2. Extraction of pectins

Several efficient methods for extracting pectin rely on mass transfer occurring within the solvents employed in the extraction process. The more substantial the yield achieved in terms of quality, the more advantageous the extraction method is considered. Following the principles of "green chemistry," various innovative techniques, including high-pressure processing, microwave, ultrasound, and enzyme-assisted extraction, are utilized for pectin extraction, alongside traditional methods (Singhal & Swami Hulle, 2022).

1.5.2.1. Conventional extraction

Traditionally, pectin is extracted in an acidic aqueous solution with a pH ranging from 1.5 to 3. This extraction process typically takes place at temperatures between 75 and 100°C, lasting for approximately 1 to 3 h. Several factors, including extraction temperature, the ratio of solids to liquid, pH, particle size, and duration of extraction, affect both the yield and quality of the extracted pectin. However, the use of mineral acids like sulfuric and hydrochloric acids for pectin extraction has raised concerns due to their environmental impact and increased costs. In response to the growing emphasis on "green chemistry" and "green technology," efforts are

being made to address these issues associated with mineral acids. Consequently, there is a shift towards using "food"-compatible acids, e.g. organic acids, for pectin extraction. Acetic and citric acids, in particular, have garnered significant attention as alternative extractants. While organic acids may have lower hydrolyzing capabilities compared to mineral acids, their adoption aligns with the development of "clean label" compounds, promoting environmentally friendly practices within the food industry. (Picot-Allain et al., 2022).

1.5.2.2. Microwave assisted extraction (MAE)

MAE is an expedited and eco-friendly technique that employs electromagnetic radiation in the microwave frequency range to generate thermal energy within a polar solvent. In this process, the solvent absorbs microwave energy, leading to the oscillation of electric and magnetic fields perpendicularly. These oscillations cause the electrophoretic transfer of ions and electrons, creating an electric field that facilitates particle movement. Additionally, polar molecules undergo rotation, contributing to heat generation. Through these mechanisms, MAE significantly improves extraction efficiency compared to traditional heating methods by enhancing diffusion rates. The extraction rate and quality are influenced by the dielectric properties of the sample and solvent, as well as the solubility of the target compounds. By applying the microwave field to a dielectric substance, MAE efficiently releases energy and generates heat, leading to higher mass transfer rates in the solvents used. Notably, MAE has demonstrated promising results in pectin extraction, resulting in reduced extraction time, solvent volume, and increased pectin yield. With its eco-friendly nature and improved efficiency, MAE holds immense potential for scientific and industrial applications, including the extraction of pectin and other bioactive compounds (Belkheiri et al., 2021; Singhal & Swami Hulle, 2022).

1.5.2.3. Ultrasonic-Assisted Extraction

Ultrasonic extraction has emerged as a versatile and valuable technique in the food industry with numerous advantages, finding applications in a number of processes (e.g. extraction, emulsification, filtration, etc.) due to its various chemical and physical effects. Operating within the frequency range from 20 to 100 kHz, ultrasonic waves play a central role in ultrasonic extraction. The passage of sound waves through a liquid medium leads to compression and expansion, resulting in cavitation - the creation, growth and collapse of

unstable microscopic bubbles in proximity to the surface of the target substance. This phenomenon creates high temperatures and pressures, forming microjets that disrupt cellular structures and improve solvent penetration, thus enhancing extraction efficiency. Consequently, ultrasonic extraction offers reduced extraction durations and increased yields in comparison with conventional methods. In addition, the method requires less equipment, consumes less energy and uses reduced amounts of solvents, making it more environmentally friendly than traditional extraction techniques. Nonetheless, it's important to acknowledge that polysaccharides obtained through ultrasonic extraction may demonstrate reduced viscosity, molecular mass, and DE (Belkheiri et a1., 2021; Picot-Allain et al., 2022).

1.5.2.4. Dielectric Barrier Discharge Plasma Extraction (DBD)

Plasma, often regarded as the fourth state of matter, comprises partially ionized gases containing various reactive components, including negative and positive ions, electrons, gas atoms, free radicals, and photons. Atmospheric cold plasma can be created by methods such as dielectric barrier discharge (DBD), radio frequency, slip arc discharge and corona discharge. DBD, in particular, has the ability to modify the side chains of biomacromolecules through the action of chemically active species present in the plasma. It can also cleave specific bonds, thereby disrupting the secondary structure of biomacromolecules. In addition, DBD is used to degrade biopolymers such as proteins and polysaccharides. During this process, high-energy electrons produce hydroxyl free radicals, which attack and break down pectin chains into smaller molecules. Although DBD has shown promising pectin degradation, its application in pectin extraction has not received extensive attention and there has been a scarcity of research conducted in this particular field. (Belkheiri et a1., 2021).

1.5.2.5. Enzymatic Extraction

EAE is a valuable method utilized to make better the extraction process by breaking down the plant cell wall matrix through the action of pectinases, primarily extracted from fungi. This enzymatic treatment increases cell wall permeability, allowing for improved extraction efficiency. Pectinases consist of various enzyme species, such as esterases, hydrolases, and lyases, which target the glycosidic bonds of pectin. By doing so, they reduce solution viscosity,

making filtration and centrifugation easier. Compared to conventional methods, EAE is considered less environmentally polluting and exhibits specific reactivity to pectin. However, the production of enzymes can be costly and challenging to control, potentially leading to pectin degradation and loss of desirable properties. Some commonly used enzymes in EAE include cellulases, hemicellulases, xylanases, proteases, and protopectinases. EAE's efficiency relies on the enzyme's potential to enable selective and specific reactions, resulting in reduced extraction time and enhanced pectin yield. Additionally, EAE offers advantages such as preventing equipment corrosion caused by strong acids, improving pectin quality, and requiring minimal energy input as a result of processing at reduced temperatures. However, the cost of enzymes remains a significant consideration. Buffers are employed as solvents in EAE to maintain the enzymes sensitivity to pH, ensuring their optimal activity throughout the extraction process (Belkheiri et al., 2021; Singhal & Swami Hulle, 2022).

1.5.3. Pectin in food packaging

Pectin is a adaptable substance with plent of uses in the industry of food, acting as a thickening and gelling agent, providing colloidal stabilization, contributing to texture modification, and functioning as an emulsifying agent. Beyond food processing, pectin also finds applications in packaging, coatings and as microencapsulation agents (Mellinas et a1., 2020). Pectin-derived materials in food packaging serve to prolong shelf life, safeguard food attributes, and satisfy contemporary market requirements. Pectin, being flexible and biodegradable, finds application in edible films and coatings, functioning as protective barriers against moisture, oil, as well as oxidation. Moreover, it effectively retains the nutritional aspects of the food and provides defense against potential quality deterioration(Vanitha & Khan, 2020). Pectin-based films act as excellent gas barriers but have limited water resistance. However, the addition of lipids in the pectin-matrix can enhance water barrier properties. Composite materials with other biopolymers mimic conventional films, while active ingredients like antimicrobials or antioxidants can be integrated for controlled release, improving preservation and the extenion of shelf life of food items (Lazaridou & Biliaderis, 2020). The fundamental stages in the production and utilization of pectin-based edible films and coatings include homogenization, de-gassing, casting, and subsequent use as either wrapping or coating materials (**Fig. 13**) (Nastasi et al., 2022).

The literature provides several examples of pectin-based films and coatings used in various food packaging applications. Chiarappa et al (2018) used an edible pectin film as a packaging to mathematically model the release of ascorbic acid (an antioxidant) from the film to a food simulation (hydrogel agar). Lei et al (2019) developed active food packaging by incorporating tea polyphenol into composite edible films composed of pectin and konjac glucomannan. In addition, Alvarez et al (2019) used the mixture of oregano and rosemary essential oils in fresh cut broccoli coated with pectin to examine their antimicrobial, antioxidant and sensorial impact. Ezati and Rhim (2020) had the primarly objective of their study was to prepare an active and smart packaging film based on pectin, curcumin and sulfur nanoparticles. The combination of these ingredients is expected to create a film that exhibits a color change in response to pH changes, indicating changes in food quality. In addition, the film is expected to have antimicrobial and antioxidant activity. The aforementioned examples are just a few instances of how pectin is applied in food packaging. There are many other uses of pectin in the food packaging, showcasing its versatility and importance in this field.

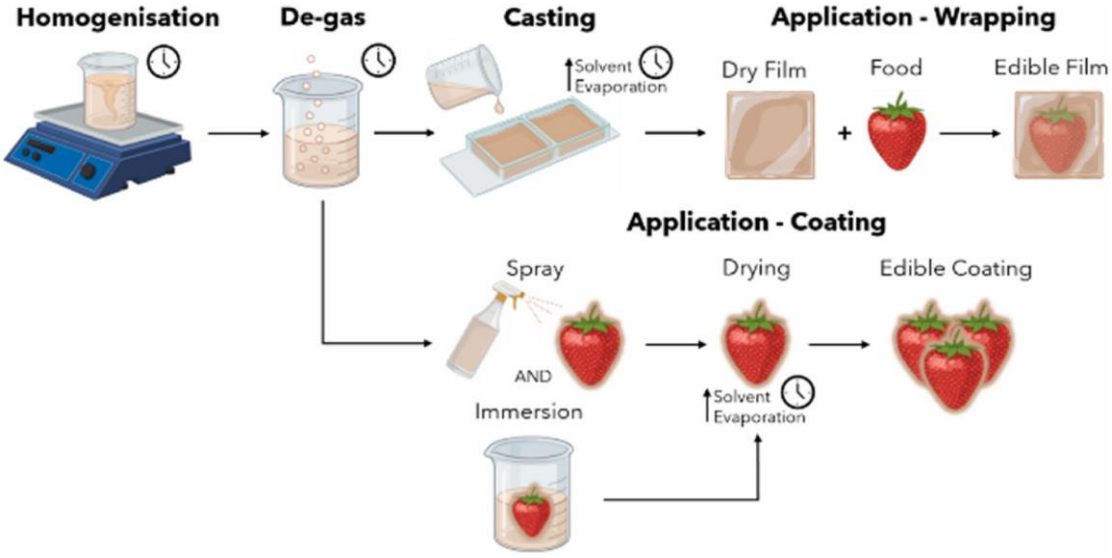

Figure 13. A basic sequential diagram illustrating the laboratory process of creating and applying pectin-based edible films and coatings infused with extracts derived from plant (Nastasi et al., 2022).

1.6. Bacterial cellulose

Cellulose ranks as one of the most prevalent biopolymers present on our planet, accounting for approximately 1.5 trillion tons of the yearly biomass production. It can be found in various sources such as cotton, wood and other plant-derived materials, and has a significant role as the primary reinforcing component in plant structures. In addition to plant sources, cellulose is also synthesized by certain bacteria, algae, and tunicates (Moniri et a1., 2017).

Bacterial cellulose (BC) is a structural carbohydrate and as its name suggests it is produced as an extracellular metabolic product by bacteria. Some commonly recognized bacterial strains that produce cellulose include *Komagataeibacter*, *Sarcina,* etc. Among these, *Gluconacetobacter xylinus* is known for its large-scale production of BC (Blanco Parte et al., 2020).

Plant cellulose and BC the same molecular formula $(C_6H_{10}O_5)_n$, however they are different in their physicochemical properties. BC has a higher purity, due to its lack of hemicellulose and lignin, which leads to superior water retention ability, hydrophilicity, degree of polymerisation (DP), mechanical strength, crystallinity and porosity in comparsion with plant cellulose (Tsouko et a1., 2015). Consequently, the process for the purification of BC is simpler, more cost-effective and eco-friendly in comparison to plant-origin cellulose. In addition, BC possesses nearly linear glucan chains, resulting in well-organized nanofibrils with nano-sized cross-sectional dimensions, owing to intra- and inter-molecular hydrogen bonds. These nanofibrils have the ability to aggregate into microfibrils with specific width and thickness, ultimately forming a 3D network structure. The DP of BC is reported to be in the range of 16,000 to 20,000, while plant-origin cellulose has an average DP varying from 13,000 to 14,000. BC exhibits superior mechanical properties compared to cellulose derived from plants, showcasing higher tensile strength (ranging from 200 to 300 MPa) and Young's modulus. Moreover, BC has become significant as a degradable substance, demonstrating no worth noting that BC also has advantages in its production compared to plant cellulose. In particular, its production is not limited by regional or climatic conditions, providing greater flexibility in its production. The growth rate of the micro-organisms can be regulated, allowing precise control of the quantity and timing of BC production. Furthermore, microorganisms can be genetically modified to produce BC with specific desirable properties, offering adaptive capabilities. While finally agro-industrial waste can serve as a growth medium for microbial cellulose, making the process economical and sustainable (Shi et a1., 2014).

Various techniques are employed for the preparation of BC, including static, agitated/shaking, and bioreactor cultures. The resultant macroscopic morphology, microstructure, and characteristics of BC exhibit significant differences. In the static culture method, a gelatinous cellulose membrane accumulates on the surface of the nutrient solution, while the agitated/shaking culture produces asterisk-like, sphere-like, pellet-like, or irregular masses. The choice of the method relies on the intended uses of BC and the particular attributes needed. In addition, the conditions of the environment of the culture, such as bacterial strain,

nutrition, pH and oxygen supply, play a vital role and influence the characteristics of BC (Wang et a1., 2019). The key determinant of the overall cost of BC production is associated with the culture media used. Therefore, a crusial facet of BC production is the identification of a cost-effective growth media capable of enhancing BC yield and offering an economically feasible option for diverse applications. Recent studies have predominantly consentrated on optimizing the use of industrial and agricultural residues as nutrient alternatives to do the synthesis process more environmentally sustainable and financially efficient (Moniri et a1., 2017).

Because of its exceptional characteristics, BC finds extensive application as a renewable natural polymer in various domains. It is utilized in food packaging, transparent coatings or films and others (**Fig. 14)** (Wang et a1., 2019).

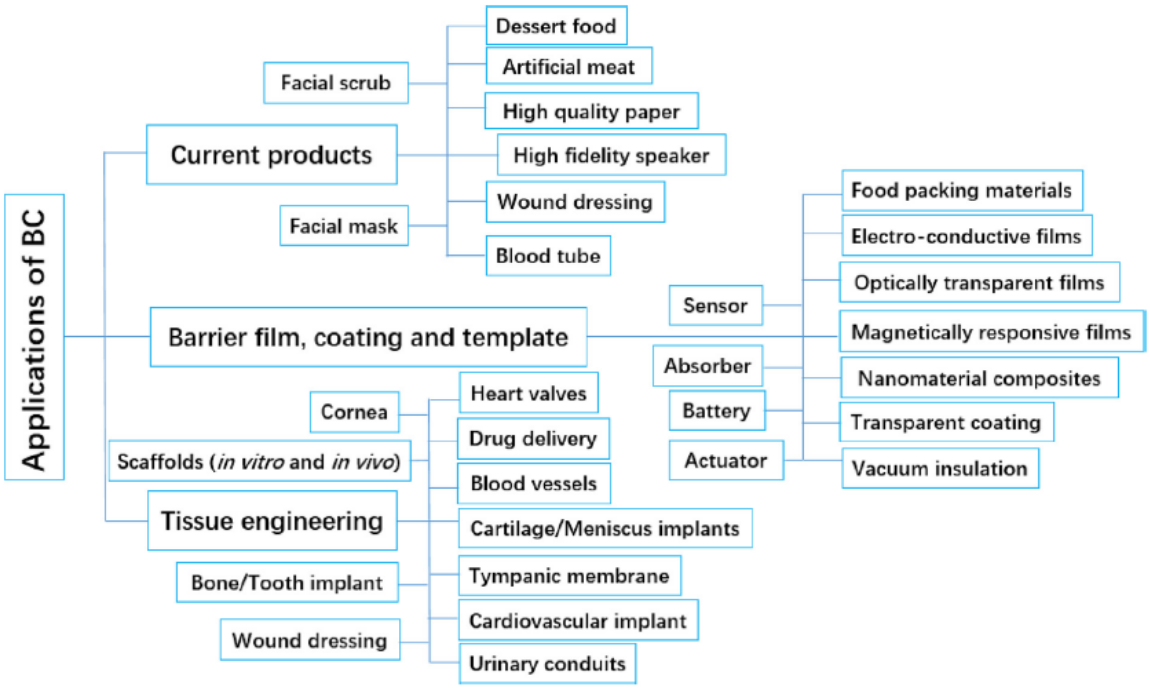

Figure 14. Utilization of Bacterial Cellulose in diverse sectors (Wang et al., 2019).

1.7. Pacific white shrimp

The combined output of fisheries and aquaculture hit a historic peak in 2020, reaching 214 million tonnes. Historically, shrimp and prawns are among the most extensively traded aquatic resources. Presently, these are primarily cultivated through intensive shrimp farming in regions like Latin America, East Asia, and Southeast Asia. Most of the produced stock is directed toward consumers in affluent markets of North America, Europe, and Japan. Pacific

white shrimp was among the leading species in 2020, with a production of 5.8 million tonnes (FAO, 2022).

The Pacific white shrimp (or Whiteleg or *Litopenaus vannamei* or *Penaeus vannamei*) **(Fig. 15)** holds significant prominence in global shrimp aquaculture and enjoys extensive trade, constituting over 90% of the total shrimp production. Its popularity has surged worldwide owing to its rich nutritional composition. The preference for this species over recent years can be attributed to its heightened productivity, relatively low susceptibility to diseases, increased yield during processing, sustained demand, and elevated market prices (Xavier & Khansaheb Balange, 2023).

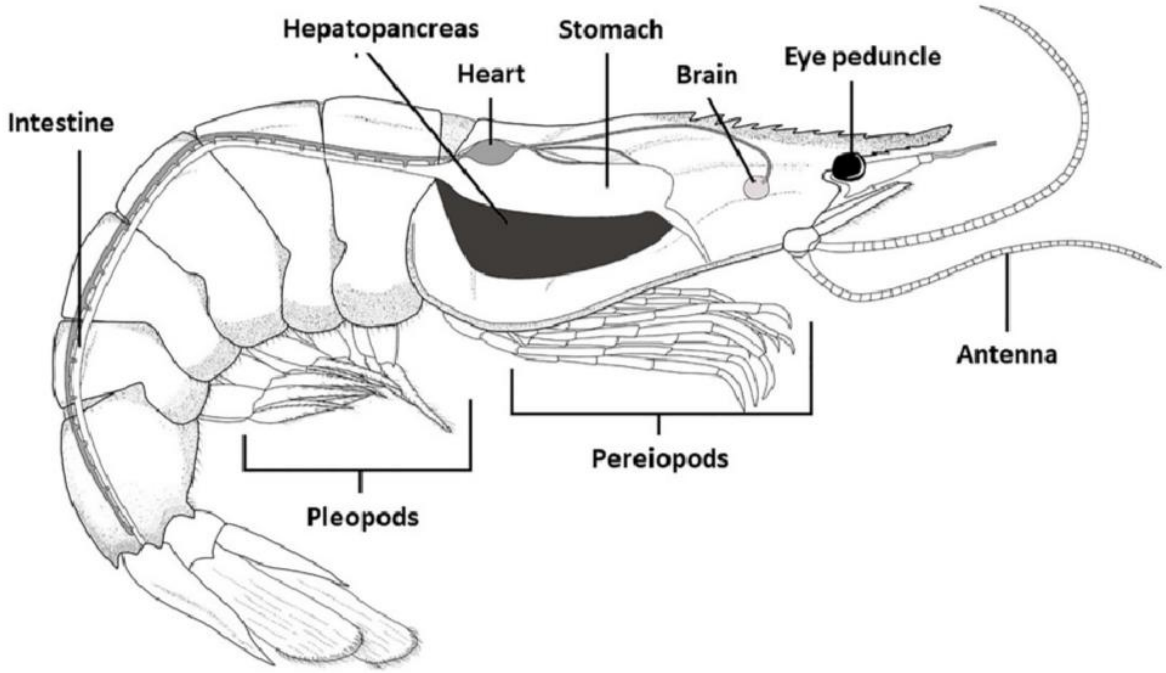

Figure 15. Anatomy of *Litopenaeus vannamei* (Duarte-Restrepo et al., 2020).

Black spot formation (melanosis) is a significant issue that arises in crustaceans during post-mortem handling and storage. This phenomenon considerably diminishes consumer acceptance and market value of shrimp products, resulting in substantial financial setbacks. Melanosis is triggered by a biochemical process in which polyphenoloxidase (PPO) enzymes catalyze the oxidation of phenols into quinones. Also referred to as phenoloxidase, tyrosinase, and catechol oxidase. PPO is an enzyme that contains copper and serves a bifunctional role, catalyzing two primary reactions in the presence of molecular oxygen. These reactions encompass the o-hydroxylation of monophenols, producing o-diphenols and the subsequent oxidation of o-diphenols into o-quinones (Nirmal & Benjakul, 2011). These o-quinones can

undergo spontaneous polymerization, forming compounds of high molecular weight or brown coloration known as melanin. Additionally, they have the capacity to interact with amino acids and proteins, which can further enhance the formation of brown pigmentation. Numerous strategies exist to counteract enzymatic browning. These approaches often involve eliminating essential substances from the reaction, such as oxygen, enzyme, copper, or substrate. Over the years, a number of techniques and mechanisms have been devised to prevent PPO-induced reactions in seafood (Gonçalves & de Oliveira, 2016). The use of reducing agents is mentioned as one of the ways to prevent melanosis. The role of reducing agents involves inducing chemical reduction of pigment precursors (such as ascorbic acid), acidulants (like citric acid) or chelating agents (e.g., ethylenediaminetetraacetic acid (EDTA)). This reduction process aims to lower the accessible copper levels, and common commercial antimelanotic products often include citric acid, ascorbic acid, and/or EDTA (Gómez-Guillén et al., 2005). Ascorbic acid functions to decrease the formation of quinones produced by polyphenol oxidase, thereby slowing down the browning process (Gonçalves & de Oliveira, 2016).

2. Purpose of the study

The purpose of the current study was the valorization of the co-products of the sugar industry (SBP) through the development of a biorefinery for pectin extraction and bacterial cellulose production, focusing on their applications in the food industry. Pectin coatings were manufactured and subsequently applied to frozen shrimp under freeze-thaw cycles, with ascorbic acid serving as a browning inhibitor.

Initially, free sugar extraction was performed on the initial solid. Subsequently, the effect of time and temperature on pectin isolation was studied in both the residual solid and the isolated pectin. The optimal process was selected on a large scale in order to produce a significant quantity of pectin. In the solid obtained post-pectin extraction, hydrolysis was conducted using commercial enzymes to generate a hydrolysate, which served as a carbon source for the production of bacterial cellulose utilizing the microorganism *Komagataeibacter sucrofermentans*.

Pectin coatings were subsequently prepared by adding various concentrations of ascorbic acid. These coatings were applied to frozen shrimps subjected to freeze-thaw cycles, and their effect on shelf life was assessed through a series of physicochemical analyses (color measurement, sensory analysis and polyphenoloxidase activity).

3. Materials and Methods

3.1. Raw material

In the current study, sugar industry wastes and Pacific white shrimps were used as raw materials.

3.1.1. Sugar Beet Pulp (SBP)

The pellets of SBP were generously supplied by Dimitriaki S.A. (Thessaloniki, Greece). SBP pellets were blended using a blender, homogenized prior to use, and remain at room temperature until utilization.

3.1.2. Shrimps

Whole frozen Pacific white shrimps (*Litopenaeus vannamei*) of varying sizes, ranging from 66 to 88 shrimps per kilogram, were obtained from a local market. These shrimps were cultivated through aquaculture practices and originated in Ecuador within FAO fishing zone 87. The shrimps were immediately stored at a temperature of -30°C in a freezer, ensuring preservation until their intended utilization.

3.2. Biorefinery development

Aqueous free sugar extraction was conducted using an initial solid (SBP) to liquid ratio of 1:20 (w/v) at 40°C for a duration of 2 hours on a hotplate with stirring at 180 rpm. This process was repeated three times for successive extractions. Following extraction, the mixture was filtered through Whatman filter paper N°1 to eliminate the solids. The identified and quantified free sugars were then analyzed in the collected supernatant. In the solid residue after removal of free sugars, a compositional analysis was performed. Furthermore, an acid hydrolysis procedure was conducted with the aim of extracting pectin from the solid matrix.

The study focused on the pectin-rich extract. In brief, the extraction of the pectin-rich content was took place using a solid to liquid ratio of 1:20 (w/v) at 60°C, 70°C and 80°C for extraction durations spanning 1 to 4 hours. In order to find the best conditions for pectin extraction, the pH of the suspension was modified to 1.5 using 2 M nitric acid. Once the acidified mixture reached room temperature, the supernatant was subjected to vacuum filtration. The pH of the liquid phase was subsequently raised to 3.5 using 5 M NaOH. The pectin portion

was then precipitated by adding twice the volume of absolute ethanol (97%, v/v). This precipitate was collected through filtration, washed with a solution of ethanol (97%, v/v), freeze-dried, and stored for further analysis. In order to identify the most effective procedure for subsequent developments, various cases were evaluated based on their physicochemical attributes. The pectin extraction procedure is summarized in **Fig. 16**. In the solid residue after the removal of free sugars and pectin, enzymatic hydrolysis was undertaken, and the resulting hydrolysis was used as carbon source in fermentation for the production of BC.

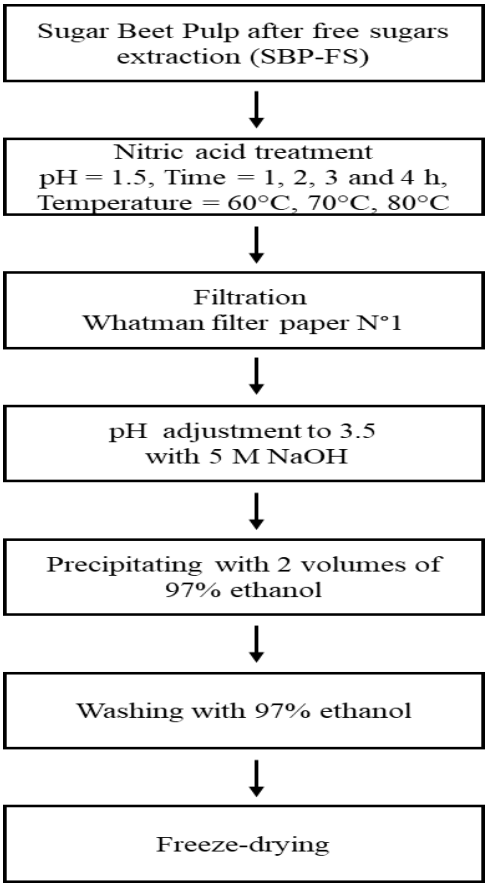

Figure 16. Illustration of the process of SBP pectin extraction.

3.3. Compositional analysis

The solid obtained after extraction of free sugars (SBP-FS) was subjected to compositional analysis.

3.3.1. Moisture content determination

For the calculation of the percentage of moisture content, a quantity of SBP-FS is put in a pre-weighed crucible, the total is weighed before it is put in the oven at 105ºC for 24 hours. The sample is subsequently moved to a desiccator until it reaches a constant weight, and its weight is recorded. The percentage of moisture content is calculated according to equation (1):

$$Moisture\ content\% = \frac{Initial\ weight - Dried\ weight}{Initial\ weight} * 100 \quad (1)$$

3.3.2. Ash determination

For ash determination, SBP-FS sample is transferred to pre-weighed porcelain crucibles and put in the oven at 80ºC until complete removal of moisture. After the sample has been weighed, the porcelain crucibles are placed in a muffle furnace at 575ºC for 6 hours. The porcelain capsules are then transferred to a desiccator and after returning to ambient temperature, they are weighed, and the ash is calculated as the mineral fraction remaining in the porcelain crucible. The percentage of ash is calculated as the weight of ash divided by the weight of the sample after removal of moisture at 80ºC, according to the following equation (2):

$$Ash\% = \left(\frac{(Weight\ of\ crucible + Ash)(g) - Weight\ of\ crucible\ (g)}{(Weight\ of\ crucible + Dry\ sample)(g) - Weight\ of\ crucible(g)}\right) \times 100 \quad (2)$$

3.3.3. Determination of extractable ingredients

The extraction of extractable components from SBP-FS was performed by Soxhlet extraction. In the implementation of the classical form of Soxhlet extraction, the sample is placed in a special porous pouch (thimble), which is transferred to a special compartment of the Soxhlet device, the extraction chamber. A spherical flask containing the solvent used by the extraction is placed at the bottom of the Soxhlet apparatus. The solvent is heated by a heating mantle and the vapours reach the extraction chamber through a side tube, where they condense and wet the sample. When the solvent reaches a certain height in the extraction chamber, siphoning is generated and the solvent, together with the extracted substance, is returned to the spherical flask. The whole process continues until all the extractable components are taken up by the solvent. Soxhlet extraction presents some important advantages. As the sample comes

into repeated contact with freshly unsaturated solvent, the mass transfer coefficient is constantly changing. At the same time, the sample does not come into direct contact with a heat source, which means that there is no risk of burning the sample (Luque de Castro & Priego-Capote, 2010).

The extraction procedure was carried out according to Sluiter et al. (2005). Firstly, the thimble is weighed, and the dry SBP-FS sample is placed inside it. The thimble is then plugged with cotton wool, folded in order to seal it and the whole is weighed. Depending on the component of the sample to be extracted, a suitable solvent is selected. Thus, for the extraction of fatty substances the solvent used is hexane, for the extraction of water-soluble components distilled water, and for the extraction of components soluble in ethanol, the latter is chosen. The solvent is placed in the spherical flask and transferred to the Soxhlet apparatus, where the extraction process starts. The heating mantle is set at a temperature such that 4-5 siphoning/h are achieved and the extraction is conducted for 24 hours. If the solvent calculated for the extraction is water, the extract is placed in a volumetric flask, made up to 200 mL with distilled water and analyzed by high-performance liquid chromatography (HPLC). If it is necessary to follow the extraction of the ethanol-soluble components, then the thimble with the SBP-FS sample remains in the Soxhlet holder and 70% (v/v) ethanol is added to the spherical flask and the same procedure as described above is followed. On completion of the extraction, the thimble is removed, lyophilisation is performed and then weighing is carried out. The extractable matter content of the sample is expressed as follows (3):

$$extractives\% = \frac{\text{dry sample before extraction (g)} - \text{dry sample after extraction (g)}}{\text{dry sample before extraction (g)}} * 100 \quad (3)$$

3.3.4. Determination of structural polysaccharides and lignin

The method according to Sluiter et al. (2008) provided by the National Renewable Energy Laboratory ("NREL"), was used to analyze the composition of structural carbohydrates and lignin in SBP. The SBP sample used for the method should be free of extractables, so the sample has been subjected to Soxhlet extraction.

Initially, 300 mg of SBP-FS sample is weighed and underwent to a two-step acid hydrolysis. Specifically, the samples were put into tared pressure tube, to which 3 mL of 72% H_2S0_4 (v/v) was added and remained at 30ºC for 1 h under stirring in a magnetic stirrer. After the time elapsed, the H_2S0_4 was diluted to 4% v/v by incorporating 84 mL of distilled water to

each sample, bringing the total volume of each sample to 86.73 mL. The second stage of hydrolysis then follows, which involves placing the tared pressure tubes in the autoclave at a temperature of 121ºC for a duration of 60 min. In parallel, the whole procedure was also carried out for a sugar solution of known concentration (Calibration Verification Standard-CVS) to calculate the percentage of sugars removed during acid hydrolysis (sugar recovery standards-SRS). After 1 h, the samples were filtered under vacuum using special pre-weighed filters (Glass fibers). The filters together with the entire amount of solid samples were placed in pre-weighed porcelain crucibles, placed in the oven (80ºC, 24 h) for complete removal of moisture and then in the desiccator until they returned to room temperature at which time they were weighed. Finally, the samples were placed in a muffle furnace at a temperature of 575ºC for 6 h and the difference of the two weighing between 80ºC and 560ºC is used to calculate the amount of insoluble lignin (AIL, %).

The liquid fraction that was separated during vacuum filtration was volumetric and photometric determined by UV-Vis spectroscopy (Shimadzu UV – 1900i) at 280 nm after the absorbance maximum was photometrically checked. The calculation of the soluble lignin content (ASL, %) of the sample was carried out according to equation 4:

$$\mathrm{ASL}(\%) = \left(\alpha\mathrm{bs} * \mathrm{V} * \frac{\mathrm{Dil}}{\varepsilon} * \mathrm{M} * \mathrm{pathlength}\right) * \ 100 \quad (4)$$

Where,

abs: absorbance of the sample,

V: volume of the filtered sample,

Dil: Dilution = $\frac{Volume_{sample} + Volume_{diluting\ solvent}}{Volume_{sample}}$

ε: absorbance of the sample at the specified wavelength,

M: mass of the solid sample weighed at the initial stage and

Pathlength: thickness of the UV-V cell.

The total amount of lignin (extractant-free sample) is calculated as (5):

$$Lignin_{ext.free}\% = AIL\% + ASL\% \quad (5)$$

For the quantitative and qualitative determination of the structural carbohydrates of the sample, a HPLC equipped with a Shodex SP0810 column at 60ºC and a flow rate of 0.8 mL/min with distilled water as mobile phase was used. Analytically, 10 mL of filtrate from each sample

was isolated and neutralized with $CaCO_3$ until the pH reached the 5.0-5.5 limits to avoid precipitation of sugars at higher pH. The neutralized solution was centrifuged (10 min, 9000 rpm) and the resulting supernatant underwent filtration with a 0.02 μm filter, after which the sugar content was analyzed using HPLC.

To calculate sugars concentration, sugars recovery rate is first calculated by dividing the HPLC-identified concentration of the sugar standard solution before autoclave treatment by the observed concentration afterwards (sugar recovery standards - SRS) according to equation (6):

$$R_{\text{sugar}}\% = \frac{concentration\ detected\ by\ HPLC\ (\frac{mg}{L})}{known\ concentration\ of\ sugar\ before\ hydrolysis\ (\frac{mg}{L})} * 100 \quad (6)$$

The sugar recovery rate mentioned above is employed to adjust the measured concentration of each sugar in the analyzed sample, taking into account in the equation (7) the possible dilution of the sample:

$$sugar\ concentration_{hydrolysed\ sample} = \frac{sugar\ concentration_{HPLC} * dilution}{\frac{\%\ R_{\text{sugar}}}{100}} \quad (7)$$

Subsequently, for the calculation of the concentration of sugar polymers before hydrolysis, a correction factor is used to account for the removal of one water molecule during the synthesis of a glycosidic bond. This coefficient is equal to 0.88 (132/150) for sugars with 5 carbon atoms (xylose and arabinose) and 0.9 (162/180) for sugars with 6 carbon atoms (glucose, galactose and mannose). This gives the concentration of each polysaccharide in g/L, which is reduced to a percentage of the initial dry sample. The concentration of glucose, calculated as described above, is used to determine the concentration of cellulose, while the sum of the concentrations of the other polysaccharides constitutes the hemicellulose.

3.3.5. Determination of protein

The Kjeldahl method, which involves a three-step process consisting of digestion, distillation and titration, was used to estimate the total protein content of the sample. The organic material is digested by utilizing concentrated H_2SO_4, high temperature, Na_2SO_4, to increase the boiling point and a catalyst to accelerate the reaction. This procedure transforms all the N in the sample to $(NH_4)_2SO_4$. The residue is neutralized by introducing NaOH, which

transforms $(NH_4)_2SO_4$ into NH_3. This ammonia is then distilled and gathered in a flask that contains a surplus of H_3BO_3, resulting in the formation of ammonium borate. The boric acid anions produced are subsequently titrated with an appropriate endpoint indicator to assess the overall nitrogen content. Once the total nitrogen content is established, it's necessary to apply a specific conversion factor to translate the estimated nitrogen content into protein content. This is typically done using a conversion factor of 6.25, as most proteins contain approximately 16% nitrogen (Jiang et a1., 2014).

For protein quantification in the present study, a Kjeltek TM 8100 distillation unit (Foss, Denmark) was used. Dry sample was weighed to the accuracy of four decimal places on rice paper and positioned in a digestion tube. A 25 mL of H_2SO_4 was introduced with a bottle top dispenser and a Kjeldahl tablet containing Na_2SO_4 96.5%, $CuSO_4$ 1.5% and Se 2.0% was placed in. The same reagents were added for the blank sample. The digestion was carried out at 430ºC for 1h and once the tubes had returned to room temperature, the distillation process ensued. At this step, 30 mL H_2O and 100 mL NaOH (40%, w/v) are added automatically. The resulting liquid after distillation is collected in a conical flask to which 50mL of boric acid solution containing 40 g of boric acid, 7 mL of methyl red indicator (0.1%) and 10 mL of bromocresol green indicator (0.1%) have been previously added for one liter of solution. Finally, the borate anions were then titrated with a 0.1 N standard HCl solution. The volume of standard HCl solution consumed during titration is translated into total nitrogen according to equation 8:

$$Nitrogen\% = \frac{(mL\ sample - mL\ blank) * N * 1.4007}{mg\ dry\ sample} * 100 \quad (8)$$

Where,

N: normality of the HCl solution,
mL sample: mL of 0.1 N HCl used during the titration of the sample,
mL blank: mL of 0.1 N HCl used during the titration of the blank.

The percentage of protein contained in the sample is then calculated from equation 9:

$$Protein\% = Nitrogen\% * 6.25 \quad (9)$$

Where,

6.25: conversion factor of organic nitrogen to proteins as previously mentioned.

3.4. Pectin yield and characterization

The characterization of pectin-rich extracts in terms of equivalent weight (EW), methoxyl content (MeO), total anhydrouronic acid content (AUA) and Degree of esterification (DE) was conducted following the procedure detailed in the referenced method by Nguyen and Pirak (2019).

3.4.1. Yield

The pectin yield was ascertained through gravimetric assessment and presented as a percentage. This percentage represented the weight of the extracted dried pectins in relation to the total dry matter of the initial SBP utilized in the biorefinery assessment, calculated as follows (10):

$$Yield\% = \frac{mass\ of\ extracted\ pectin\ (g)}{mass\ of\ \ initial\ SBP\ sample\ (g)} * 100 \quad (10)$$

In addition, the pectin recovery yield expressed in galacturonic acid equivalents from the initial SBP solid (overall recovery yield) and from the SBP-FS solid (recovery yield of the process) was calculated according to the following equations (11) and (12):

$$Recovery\ Yield\% = \frac{mass\ of\ extracted\ pectin\ (g)^1}{mass\ of\ pectin\ in\ solid\ sample(g)^1} * 100 \quad (11)$$

$$Overall\ Recovery\ Yield\% = \frac{mass\ of\ extracted\ pectin\ (g)^1}{mass\ of\ pectin\ in\ initial\ SBP(g)^1} * 100 \quad (12)$$

[1]galacturonic acid equivalents

3.4.2. d-galacturonic acid content

The content of d-galacturonic acid of both the substrate and pectins were determined by the method of Melton & Smith (2001). Additionally, the purity of the extracted pectin was quantified as the proportion of d-galacturonic acid content. This method consists of two main parts: hydrolysis and colorimetric assay. In the hydrolysis stage, 5 mg of each sample is initially weighed (in duplicate) into borosilicate glass tubes. To establish a reagent control, a tube containing 1 mL of concentrated H_2S0_4 was also prepared alongside the samples. For all tubes, 1 mL of concentrated H_2S0_4 was placed in, and the tubes were securely capped. These tubes, along with the reagent control tube, were positioned in an ice bath. To facilitate the process,

small magnetic spin bars were introduced into each tube. The rack containing the tubes was placed within a vessel of ice slurry, positioned atop a magnetic stirrer. The contents were stirred for a duration of 5 minutes. An additional 1 mL of concentrated H_2S0_4 was introduced into all tubes, followed by another 5-minute stirring period on ice. Subsequently, 0.5 mL of water was incorporated into the tubes, and stirring was sustained for 5 minutes on ice. This step was repeated by introducing another 0.5 mL of water and stirring for an additional 5 minutes. To each tube, water was added to facilitate the dilution process, aiming for a final volume of 10 mL in each 10-mL volumetric flask. To perform the colorimetric assay, the following procedure was applied. For each hydrolysate, a setup was established comprising three borosilicate glass tubes. In addition, two tubes were set up for the reagent control. Aliquots of 400 µL were withdrawn from both the hydrolysate supernatant and the reagent control, with each aliquot being placed within the respective tubes. A volume of 40 µL of a 4 M sulfamic acid/potassium sulfamate solution at pH 1.6 was introduced into all the tubes. Subsequent to this addition, the contents of the tubes were subjected to vortexing. All tubes received the addition of 2.4 mL of a 75 mM sodium tetraborate solution in sulfuric acid. The contents were subjected to vigorous vortexing. Subsequently, the tubes were positioned within a water bath set to 100°C (boiling) for 20 min. Following this heat treatment, the tubes were immersed in an ice bath for a period of 10 minutes. Plastic tube stoppers were placed atop the tubes to forestall sample condensation. Within each sample, two of the tubes were supplemented with 80 µL of m-hydroxydiphenyl solution. In conjunction with this, the third tube of each sample received an addition of 80 µL of 0.5% NaOH (serving as the sample control). The contents of the tubes underwent three vortexing cycles to ensure thorough mixing. A pink coloration emerged within a span of 5 to 10 minutes, displaying stability for approximately 1 hour, after which it began to diminish. Between 10 minutes and 1 hour following complete mixing, absorbance readings at 525 nm were taken against the reagent control. The values corresponding to the sample controls were subtracted from their respective sample absorbances. Each batch of samples saw the preparation of a d-galacturonic acid standard curve. The samples' d-galacturonic acid concentration was assessed using the standard curve.

3.4.3. Equivalent weight of SBP pectin

The EW was determined as follows: Initially, 0.5 g of pectin was placed in a 250 mL conical flask and moistened with 5 mL of ethanol. To enhance the endpoint, 1 g of NaCl was placed into. Subsequently, 100 mL of distilled water and six drops of methyl red indicator were

introduced. Titration began gradually using 0.1 N NaOH up till the indicator turned pink (at pH 7.5), and it was allowed to stand for at minimum 30 seconds. The neutralized solution was then employed for MeO determination. The equation (13) was utilized to compute the EW:

$$Equivalent\ weight = \frac{weight\ of\ sample\ (g) * 1000}{mL\ of\ NaOH * Normality\ of\ NaOH} \quad (13)$$

3.4.4. Methoxyl content of SBP pectin

The MeO content of SBP pectin was calculated using the following method: Initially, 25 mL of 0.25 N NaOH was added to the titrated solution obtained from the EW experiment (2.4.2). This mixture was vigorously shaken and left to sit for 30 minutes at room temperature within a stoppered flask. Subsequently, 25 mL of 0.25 N HCl and titration was performed until the endpoint achieved the same pink coloration as previously. The subsequent equation (14) was employed for calculating the methoxyl content:

$$MeO\% = \frac{meq\ of\ sodium\ hydroxide * 31 * 100}{weight\ of\ sample\ (mg)} \quad (14)$$

Where,

31: methoxyl group molecular weight.

3.4.5. Total anhydrouronic acid content of SBP pectin

Calculating the AUA content was a critical step in assessing the purity and DE, utilizing EW and MeO. The total AUA of pectin was determined using the subsequent equation (15):

$$AUA\% = \frac{176 * 0.1z * 100}{w * 1000} + \frac{176 * 0.1y * 100}{w * 1000} \quad (15)$$

Where,

176: molecular unit of AUA (1 U) in g
z: mL of NaOH from equivalent weight determination,
y: mL of NaOH from methoxyl content determination,
w: weight of sample.

3.4.6. Degree of esterification of SBP pectin

To determine the degree of esterification of the pectin, a procedure was followed. Firstly, 50 mg of pectin powder was mixed with 65% isopropanol and dissolved in 10 mL of distilled water. The resulting pectin solution was then undergoing a titration using 0.1 N NaOH solution (a mL) until reaching pH 7.5. Afterward, the solution was treated with 30 mL of 0.1 N NaOH and left for 30 minutes and then 30 mL of 0.1 N HCl were added. Subsequently, the pectin solution underwent another titration with 0.1 N NaOH (b mL) to achieve pH 7.5. The DE for every pectin was then determined by the use of the following equation (16):

$$DE\% = \left(\frac{b}{a} + b\right) * 100 \quad (16)$$

3.5. Enzymatic hydrolysis of residual solids

The residual solids obtained after the completion of the biorefinery process underwent enzymatic hydrolysis. Initially, these solids were rinsed to neutralize and eliminate any excess acid. Subsequently, a starting concentration of 100 g/L underwent enzymatic hydrolysis within a 5-L bioreactor (Labfors 4, Infors HT) featuring a working volume of 4 L under conditions of (50°C, 48 h, pH 5.0). Specifically, the enzymatic hydrolysis of the pretreated SBP was performed using a commercial enzyme cocktail at a ratio of 0.05 mL per gram of SBP. This enzyme cocktail comprised several components, including endo-β-1,4-glucanase (CMCase) at a concentration of 3.5 U/g, exo-1,4-β-glucanase (Avicelase) at 3.9 U/g, β-glucosidase at 8.3 U/g, xylanase at 2.3 U/g, and total cellulase at 4.0 FPU/g. To provide some context, one unit (U) of CMCase, exo-1,4-β-glucanase (Avicelase), β-glucosidase, and filter paper unit (FPU) activities were referred as the amount of enzyme that could release 1 mg of glucose within 1 minute during the hydrolysis of 1% (w/v) carboxymethyl cellulose (CMC), 1% (w/v) microcrystalline cellulose, 0.5% (w/v) cellobiose, and 50 mg of Whatman No.1 filter paper strip (1.0 × 6.0 cm) in a solution of 0.1 M acetate buffer at pH 4.8 and 50°C, within time intervals of 30, 120, 30, and 60 minutes, respectively. Similarly, one unit (U) of xylanase activity was defined as the amount of enzyme capable of releasing 1 mg of xylose in 1 minute through the hydrolysis of 0.25% (w/v) xylan from birchwood, using a solution of 0.1 M acetate buffer at pH 5 and 50°C, within a 15-minute timeframe. After the designated hydrolysis period, the solid component was separated from the liquid to complete the enzymatic hydrolysis process.

3.6. Bacterial cellulose production

3.6.1. Bacterial strain

Komagataeibacter sucrofermentans DSM 15973 utilized for bacterial cellulose synthesis and was obtained from the Leibniz-DSMZ Institute in Germany. To initiate the culture, a preculture was established in shake flasks by introducing a single colony to a liquid medium in accordance with the Hestrin and Schramm (HS) media (Gromet-Elhanan & Hestrin, 1963). The composition of the medium was as follows: 20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L disodium phosphate (Na_2HPO_4), and 1.15 g/L citric acid. The pH was adjusted to 6 using either 5 M HCl or 5 M NaOH. The inoculum was then incubated in a rotary shaker incubator at 200 rpm with agitation at 30°C for a duration of 24 hours. For long-term storage of bacterial cells, cryovials containing a mixture of 50% (v/v) of grown cells in the pre-culture medium and 50% (v/v) pure glycerol were stored at -80°C.

3.6.1. Fermentation conditions

Batch fermentations were took place in 250-mL Erlenmeyer flasks with 50 mL working volume and incubated as the inoculum, followed by a 13-day period of static culture at 30°C. 10% of the inoculum was used and two different carbon sources were studied: commercial sugars simulate the sugars ratio of the SBP hydrolysate, as well as the produced SBP hydrolysate. The media contained the adjusted HS medium (20 g/L total sugars), whereas the pH during the fermentation was kept at 5.0, according to the results of Efthymiou et al. (2022).

3.7. Analytical methods

3.7.1. Determination of sugars and organic acids

Sugars as well as organic acids were quantified by the use of a Shimadzu HPLC system equipped with a Shimadzu RI detector and a Rezex ROA-Organic acid H+ column. The column was maintained at 65°C, and the mobile phase consisted of a 10 mM H_2SO_4 aqueous solution, flowing at a rate of 0.6 mL/min. Each sample was injected in a volume of 10 μL. Monosaccharides were also analyzed using a Shodex SP0810 (8.0 × 300 mm) column on a Shimadzu HPLC system with a Shimadzu RI detector. The column was set to a temperature of 65°C, and the mobile phase employed was HPLC grade water, flowing at a rate of 0.5 mL/min. Each sample was injected in a volume of 5 μL.

3.7.2. Free amino acid nitrogen determination (FAN)

FAN was assessed using the colorimetric ninhydrin method (Lie, 1973). According to this method, the diluted sample is heated with nynidine (2,2-Dihydroxyindane-1,3-dione) at pH 6-7 and the resulting color is measured at 570 nm. Ninhydrin is an oxidizing agent that causes oxidative decarboxylation of alpha-amino acids, in a reaction that produces ammonia (NH_3), carbon dioxide (CO_2), and an aldehyde with one less carbon atom than the original amino acid. The reduced ninhydrin then reacts with the unreduced form of ninhydrin and the ammonia released, forming a blue-colored complex with a maximum absorption at 570 nm. As ninhydrin is attached via its oxygen atom to the amino terminal of the amino acid, only ammonia and primary amines can react to produce the characteristic complex.

More specifically, for the implementation of the method, 1 mL of suitably diluted sample, 0.5 mL of Color Reagent (Color Reagent: 49.71 g $Na_2HPO_4 \cdot 2H_2O$, 5 g ninhydrin, 3 g fructose as reducing agent, 60 g KH_2PO_4 for pH adjustment and make up to 1 L with dH_2O) is added to a test tube. The mixture is stirred well in a vortex, the tubes are plugged and brought to the boil for 16 minutes. Cool in an ice bath for 20 minutes and add dilution reagent (dilution reagent-FAN Dilution: 2 g KIO_3 dissolved in 616 mL dH_2O and make up to 1 L with ethanol) The mixture is stirred in the Vortex for 20 seconds and then measured in a double beam UV-Vis spectrophotometer (Shimadzu UV – 1900i) at 570 nm. 1 mL of distilled water is used as a blank and the procedure is carried out twice for every sample. The nitrogen concentration of the sample was calculated from the standard curve using standard glycine solution. The nitrogen concentration of the samples was expressed in mg/L FAN based on the standard curve.

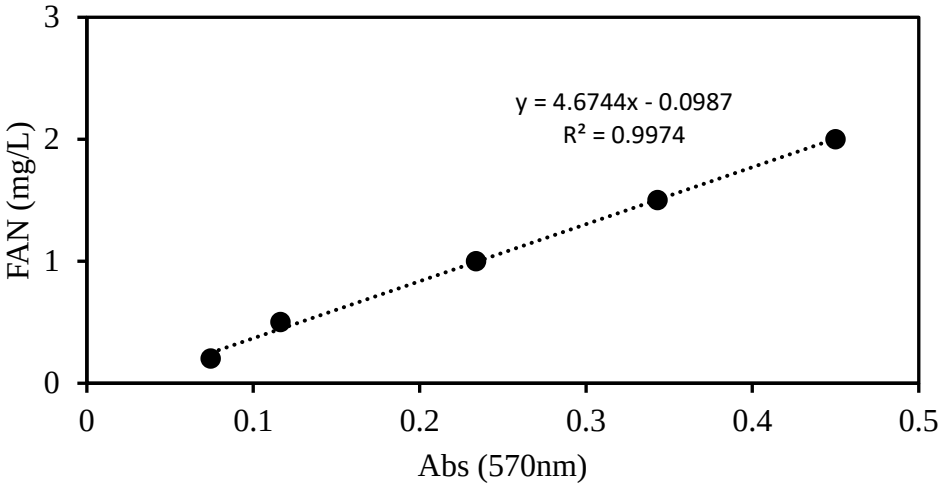

Figure 17. Glycine standard curve by the FAN method.

3.8. Preparation of pectin coating

The pectin coating was formulated by dissolving 3.5% (w/v) pectin and glycerol (25% w/w pectin) as a plasticizer in distilled water, with continuous stirring at 70°C until thorough homogenization was attained. Subsequently, ascorbic acid (AA) was incorporated into the solution to achieve final concentrations of 0.5%, 1%, and 2% (w/v) of AA in the coating solution.

3.9. Preparation and treatment of shrimp samples

Shrimp were divided into five groups which includes the control (uncoated) and the four extra groups were coated with either pure pectin forming solution or pectin forming solution enhanced with different concentrations of ascorbic acid (AA) (0.5%, 1% and 2%).

The five categories of samples named as follows:

- **C**: Control, no pectin coating was added to this category.
- **P**: 3.5% w/v pectin and 0.25 g glycerol/ g pectin.
- **P + 0.5% AA**: 3.5% w/v pectin, 0.25 g glycerol/ g pectin and 0.5% w/v AA.
- **P + 1% AA**: 3.5% w/v pectin, 0.25 g glycerol/ g pectin and 1% w/v AA.
- **P + 2% AA**: 3.5% w/v pectin, 0.25 g glycerol/ g pectin and 2% w/v AA.

Shrimp were coated by dipping in coating for 15 min and then the samples were permitted to drain for 3 h at 2°C. Subsequently, samples were packed in airtight bags and stored under cold chain simulated conditions for 28 days. Time-temperature data was meticulously recorded using a precise data recorder (Elitech RC-5 Temperature Data Logger). Chemical and sensory analyses were conducted at intervals of 28 days.

3.10. Color measurement

During the preservation of the shrimp samples, the color parameters (L*, a*, b*) were evaluated using the CIELab system and measured with a colorimeter (Eye-one Pro, X-Rite, Michigan, USA). Calibration of the instrument was performed using the white standard to ensure accuracy. An average of 12 measurements was taken for each group (three samples to each group). In particular, the parameters a*, b*, L* of each sample were determined. The L* value characterizes the brightness, purity of the color on a scale of 0-100, where 100 corresponds to the maximum brightness. The value a* characterizes the gradation of the color from green (-a*) to red (+a*) and the value b* characterizes the gradation from yellow (-b*) to

blue (+b*). ΔE is the color differences between the control samples and treated samples during the storage. ΔE is calculated according to the equation (17):

$$\Delta E = \sqrt{(L - L_o)^2 + (a - a_o)^2 + (b - b_o)^2} \quad (17)$$

Where L, a, b are the values for treated samples and L_0, a_0, b_0 are the values for control samples.

In addition, the color alteration of the shrimp was also measured using the ImageJ application (https://imagej.nih.gov/ij/download.html) utilized to analyze the image of the samples. In the ImageJ application, one shrimp from each group was analyzed. The color measurement took place for 28 days and samples were analyzed at days 0, 3, 7, 10, 13, 16, 21, 24 and 28.

3.11. Sensory analysis

The organoleptic characteristics of the shrimps were conducted by eight trained participants. A five-point scale was utilized to rate the samples for discoloration, brightness, and overall appearance. Three representative samples from each treatment were presented to each participant. Participants were unaware of the experimental approach and the samples were blind. The sensory analysis was conducted for 28 days and samples were analyzed at days 0, 3, 7, 10, 13, 16, 21, 24 and 28.

3.12. Measurement of PPO activity

The cephalothoraxes of the shrimps were isolated, combined, and turned into powder by grinding with liquid nitrogen. This resulting powder was employed for the analysis. The isolation of PPO was conducted in accordance to the method of Nirmal & Benjakul (2009) with a slight modification. A portion of the powder was mixed with three times the quantity of extracting buffer (0.05 M sodium phosphate buffer, pH 7.2, containing 1.0 M NaCl and 0.2% Brij 35). The mixture was stirred continuously at 4°C for 30 min and then underwent centrifugation at 8000g at 4°C for 30 minutes using a refrigerated centrifuge. Solid ammonium sulfate was subsequently added to the supernatant to achieve a 40% saturation level, and it was allowed to stand at 4°C for 30 min. The resulting precipitate was collected by centrifugation at 9000g at 4°C for 30 minutes using a refrigerated centrifuge. The pellet obtained was then

dissolved in a small quantity of 0.05 M sodium phosphate buffer at pH 7.2. Subsequently, it was dialyzed against 15 volumes of the same buffer at 4°C, with three changes of the buffer.

Insoluble materials were eliminated by centrifugation at 3000g at 4°C for 30 minutes. The resulting supernatant was identified as the 'crude PPO extract.' PPO activity was analyzed with L-DOPA as a substrate in accordance with the method of Nirmal & Benjakul (2009). The assay system composed of 100 µL of crude PPO extract, 600 µL of 15 mM L-DOPA in deionized water, 400 µL of 0.05 M phosphate buffer, pH 6.0, and 100 µL of deionized water. PPO activity was assessed over a 3-minute duration at 45°C by tracking the generation of dopachrome at 475 nm using a UV-Vis spectroscopy (Shimadzu UV – 1900i). One unit of PPO activity was established as a rise in absorbance of 0.001 at 475 nm/min/mL. Enzyme and substrate blanks were created by excluding the substrate and enzyme, respectively, from the reaction mixture, with deionized water replacing them.

3.13. Statistical analysis

The statistical analysis of the shelf-life test results and the mechanical properties was conducted using STATGRAPHICS Centurion XVII, specifically Version 17.2.00. This software platform was utilized to handle and interpret the data, facilitating the extraction of valuable insights and conclusions from the experimental results.

4. Results and discussion

4.1. Biorefinery development

4.1.1. Composition of Sugar Beet Pulp (SBP)

Chemical analysis of initial SBP is presented on **Table 1** in dry weight basis and compares it with existing literature. The analysis included the quantification of structural polysaccharides, pectin, lignin, protein, and ash content. Specifically, the structural polysaccharides were assessed, revealing a hemicellulose content of 25.0% w/w, comprised of 3.1% w/w xylan, 5.5% w/w galactan, 12.0% w/w arabinan, and 4.3% w/w mannan. Cellulose and lignin were found to constitute 27.9% w/w and 2.3% w/w, respectively. Additionally, pectin content was determined to be 19.1%, protein content at 9.1%, and ash content at 3.8%. Lastly, the determination of free sugars in SBP revealed their presence at 11%, consisting of 8.9% sucrose, 1.4% glucose, and 0.6% fructose.

Table 1. Composition of the SBP in this study and its comparison literature.

Composition (%, dry basis)	This study	Literature	Reference
Pectin (GalAE[1])	19.1 ± 3.0	15.0 – 25.0	Amo-Mateos et a1., 2022; Narisetty et a1., 2022
Ash	3.8 ± 0.3	3.7 - 4.24	Adiletta et al., 2020; Narisetty et a1., 2022
Protein	9.2 ± 0.1	7.15 – 15.0	Adiletta et a1., 2020; Amo-Mateos et al., 2022
Lignin	2.4 ± 0.3	2.6 - 5.9	Alexandri et a1., 2019; Narisetty et a1., 2022
Cellulose	27.7 ± 5.0	19.0 – 25.0	Amo-Mateos et al., 2022; Usmani et al., 2022
Hemicellulose	25.2 ± 3.1	19.5 – 30.0	Alexandri et a1., 2019; Narisetty et a1., 2022
Free sugars	10.9 ± 1.6		
Sucrose	8.9 ± 0.6		
Glucose	1.4 ± 0.7		
Fructose	0.6 ± 0.4		

[1]galacturonic acid equivalents

4.1.2. Free sugars extraction

Aqueous extraction of the free sugars was carried out at the initial solids of SBP. The free sugars identified in the collected supernatant comprised sucrose, glucose, and fructose at concentrations of 82.6%, 13.7%, and 3.7%, respectively in dry basis. The recovery yield pertaining to the recovery of free sugars from the initial solid was determined to be 89%. The composition on a dry basis of the solid obtained after extraction of the free sugars is shown in **Table 2**.

Table 2. Chemical analysis of the SBP after free sugars extraction.

Composition (%, dry basis)	
Pectin (GalAE[1])	21.2 ± 1.2
Ash	4.1 ± 0.1
Protein	10.1 ± 0.1
Lignin	2.5 ± 0.3
Cellulose	29.6 ± 5.0
Hemicellulose	26.9 ± 3.3
Xylan	3.2 ± 0.2
Galactan	6.1 ± 0.2
Arabinan	13.2 ± 2.1
Mannan	4.4 ± 0.9

4.1.3. Pectin extraction

The conventional method for extracting pectins from agro-industrial co-products involves the use of hot aqueous solutions of mineral acids, such as HCl. This procedure is typically conducted at temperatures ranging from 50 to 90°C and at a low pH level between 1 to 3, with an extraction duration lasting from 3 to 12 hours (Adiletta et a1., 2020). In this study, extraction of pectins from the solid obtained after extraction of free sugars (SBP - FS) was carried out with hot distilled water acidified using nitric acid, using a solid to liquid ratio of 1:20 (w/v) at three diverse temperatures (60°C, 70°C, and 80°C) for 1, 2, 3 and 4 h of extraction time. The pH of the suspension was brought to 1.5 using 0.03g nitric acid per g SBP.

Figure 18. Extraction of SBP pectins under different conditions.

4.1.3.1. Pectin Yield and Characterization

The pectin yield, which varied from 4.6% to 11.7% (w/w), was influenced by both temperature and extraction time (**Table 3**). Higher quantities of pectin were obtained with increased temperature and extraction duration. Consequently, the most significant pectin yield was noted at 80°C for 4 hours, while the lowest yield was observed at 60°C for 1 hour. This result is consistent with findings by Lv et a1. (2013) who carried out optimization of the

production yield of pectin extracted from SBP and similarly reported that the pectin yield, with a constant extraction pH of 1.5, rises with higher temperatures and longer extraction durations. Similarly, the overall recovery yield and the recovery yield of the process increases with higher temperature and longer extraction time (**Fig. 19).**

Table 3. Impact of extraction temperature and time on the yield and on the chemical synthesis of SBP pectin.

Extraction Temperature	Extraction Time	Yield (%)	DE (%)	MeO (%)	AUA (%)
60°C	1 h	4.6	96.6 ± 3.5	15.7 ± 0.4	95.1 ± 3.1
	2 h	5.3	97.0 ± 3.7	16.1 ± 0.6	96.4 ± 4.3
	3 h	5.3	98.0 ± 4.2	16.2 ± 0.5	94.6 ± 2.3
	4 h	6.6	97.1 ± 4.8	16.1 ± 0.3	94.8 ± 4.9
70°C	1 h	7.7	98.0 ± 4.9	16.3 ± 0.8	95.0 ± 5.1
	2 h	8.1	98.0 ± 4.5	16.3 ± 0.8	97.6 ± 3.5
	3 h	8.0	97.1 ± 2.3	16.2 ± 0.7	96.8 ± 3.9
	4 h	8.9	98.0 ± 4.7	16.1 ± 0.6	96.6 ± 4.0
80°C	1 h	8.7	94.1 ± 3.2	15.5 ± 0.6	92.2 ± 4.1
	2 h	10.4	97.6 ± 3.4	16.0 ± 0.7	94.9 ± 5.2
	3 h	10.7	97.1 ± 3.4	14.3 ± 0.8	89.3 ± 3.3
	4 h	11.7	98.0 ± 4.2	15.7 ± 0.5	97.6 ± 4.2

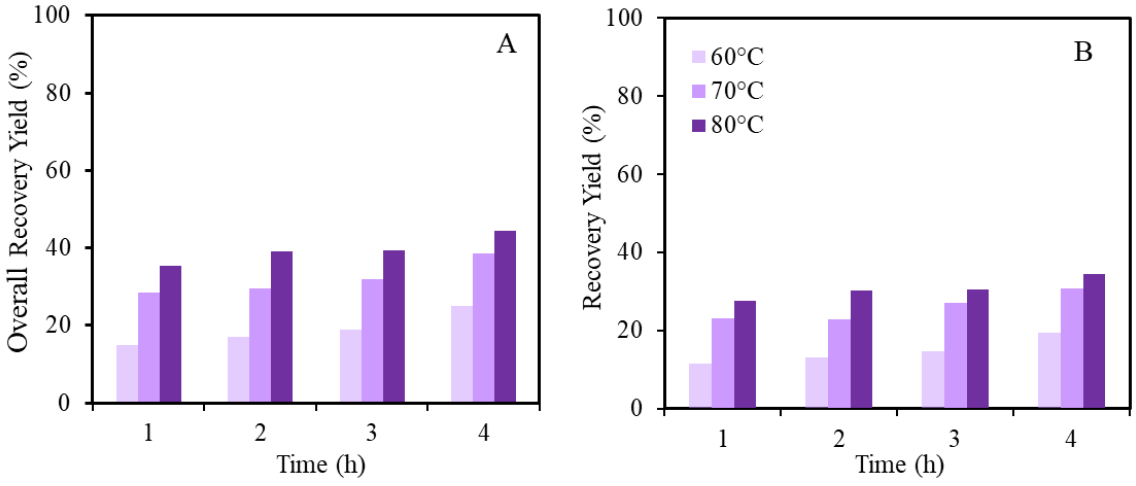

Figure 19. Impact of extraction temperature and time on the overall recovery pectin yield (A) and the recovery yield of the process (B) in galacturonic acid equivalents at pH 1.5.

To characterize the SBP pectins, several parameters were determined, including galacturonic acid content (GalA), MeO, AUA and DE (**Table 3**).

The galacturonic acid content of the pectins is illustrated in **Fig 20**. Their content of galacturonic acid ranged from 60.7% to 83.1% (on a dry-weight basis). These levels of galacturonic acid content align closely with data previously reported under comparable conditions, as documented in studies by Ma et a1. (2013) (ranging from 60.2% to 77.8%), and Huang et al. (2017) (between 35.2% and 76.3%). In order to comply with the requirements established in accordance with EU Regulation No. 231/2012 for food-grade pectin, it is imperative that the galacturonic acid content attains a minimum threshold of 65% (Frosi et al., 2023). According to the data presented in the figure below, it is evident that the galacturonic acid content is higher in SBP pectins extracted at temperatures of 70°C and 80°C compared to those extracted at 60°C. Additionally, for pectins extracted at 60°C and 70°C, a growth in extraction time corresponds to an increase in the percentage content of galacturonic acid. However, interestingly, for pectins extracted at 80°C, an extended extraction duration results in a reduction in the percentage content of galacturonic acid. Notably, pectin extracted at 80°C for just one hour exhibits a particularly high galacturonic acid content, reaching 78.3%.

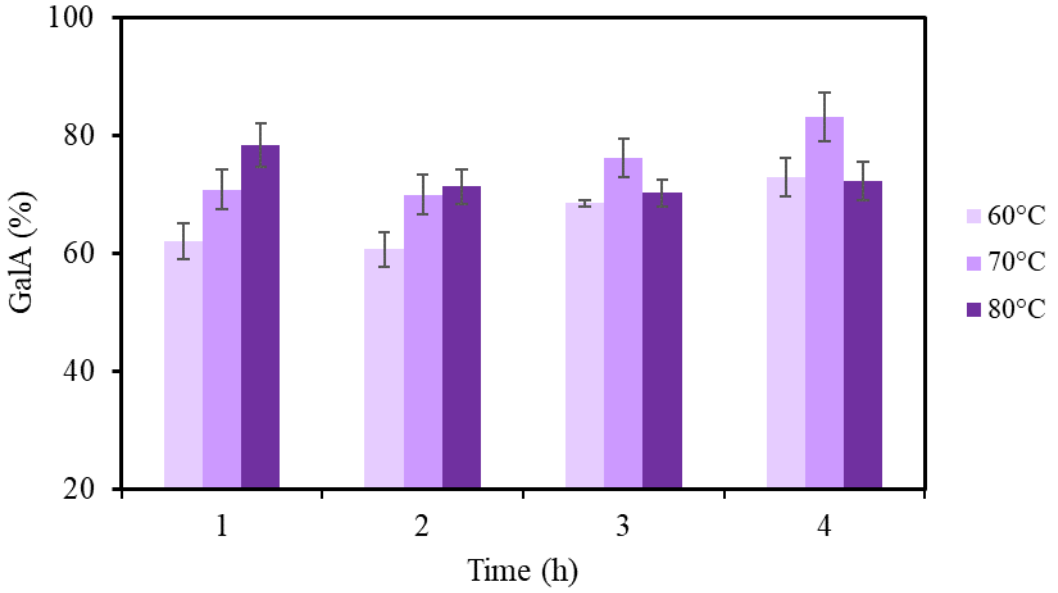

Figure 20. Impact of extraction temperature and time on the galacturonic acid content (%) of pectins from sugar beet pulp.

Table 3 indicates that regardless of temperature and extraction duration, the pectins recovered from SBP had a DE greater than 94%, classifying them as high methoxyl (HM) pectins. In a comparable study, Adiletta et a1. (2020) estimated the DE of SBP pectin extracted under similar conditions (pH = 1.5, 90°C, 4 h) to be around 59 ± 2.1% using a titrimetric method

and 69% using an FTIR method. Therefore, both this study and the one conducted by Adiletta et al. (2020) confirm that SBP pectins are indeed high methoxyl pectins. However, it is important to note that the DE percentage in the current study is notably higher.

The methoxyl content of pectin is a significant factor, representing the number of moles of methyl alcohol in 100 moles of galacturonic acid. Pectins are categorized into two groups based on MeO: High Methyl Pectins (HMP) with MeO > 7.12% and Low Methoxyl Pectins (LMP) with MeO 2.5-7.12% (Mamiru & Gonfa, 2023). This value has a notable role in regulating various characteristics of pectin, including gel strength, setting time, and the ability to form gels. The variability in methoxyl content depends on the source of the pectin and the extraction method employed (Nguyen & Pirak, 2019b). In the present study methoxyl content of SBP pectins varied from 14.3% to 16.3%, classifying them as high methoxyl pectins (**Table 3**). In the research of Yapo et a1. (2007) the MeO of the SBP pectins ranged from 2.0% to 4.2% a range of values corresponding to LMP. Whereas in the study of Sunt & Hughes (1998), the MeO of the SBP pectins ranged from 10.0% to 13.1% a range of values corresponding to HMP. The MeO determined in the current study is greater than in the literature. The range of values of MeO% in this study is confirmed since it is in the range of values obtained from the following equation, which relates DE to MeO: $MeO\% = \frac{16.32}{100} * DE$ (Polanco-Lugo et a1., 2019).

The AUA represents the purity of the pectin, and its value has to be not below < 65%. Having a low AUA value implies that the extracted pectin might have high content of protein, starch and sugars in the precipitated pectins (Wahengbam et a1., 2014). According to the (Jong et al., 2023), pectin of greater purity (AUA >65%) is efficiently extracted using mineral acids. As shown in **Table 3**, in the present study the AUA% values ranged from 89.3% to 98.2% which means that regardless of the extraction conditions of the pectins, the derived pectins were of high purity. Sunt & Hughes (1998) in their study extracted SBP pectins with HCl, at pH 1.5, temperature 85 for 1h and 4h which obtained AUA of 83.5% and 84.6%, respectively.

4.1.3.2. Residual solids

The residual solids obtained after the extraction of pectins were analyzed for cellulose and hemicellulose content. The mass balances of the residual solids are shown in **Fig. 21**. As anticipated, the mass of the solid residue obtained from the extraction conditions (temperature and time) that yielded greater amounts of precipitated pectins is comparatively lower. It should also be noted that with increasing temperature and extraction time, greater losses in the mass of

the residual solid are observed. This is possibly due to the fact that at stronger extraction conditions a certain amount of hemicellulose that is closely bound to the pectin is depredated.

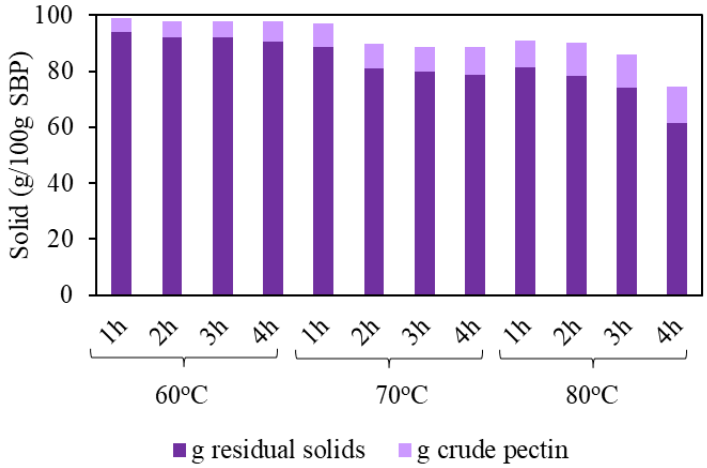

Figure 21. Mass balances of the residual solids after pectin extraction.

Regarding the impact of temperature and pectin extraction time on cellulose and hemicellulose, it's noticeable that the amount of cellulose remains unchanged under different extraction conditions. In contrast, hemicellulose tends to undergo hydrolysis, even under milder extraction conditions. Mass losses increases at both 70°C and 80°C, regardless of the extraction duration (1, 2, 3, or 4 h). At 60°C, mass decrease is somewhat less pronounced when the extraction lasts for 1 h. However, for extraction periods of 2, 3, or 4 h, the reduction in mass is consistent (**Fig 22).** This result is consistent with findings by Heux et a1., (1999) who carried out acid treatment (1 M HCl, 1.5 h and 80°C) in SBP microfibrils and similarly reported that the treatment had no effect in the quantity of the cellulose, but the overall amount of neutral sugars other than glucose decreased from 26% to 9% after treatment, indicating a substantial reduction in hemicellulose. The polysaccharide most affected by the extraction of the pectins was arabinan followed by galactan, which is confirmed by its reduced concentration in the solids obtained after extraction of the pectin. This is potentially due to the fact that in "hairy areas" of the pectins are located side chains containing large amounts of arabinan and arabinogalactan, which are extracted with the pectins (Ma et al., 2013).

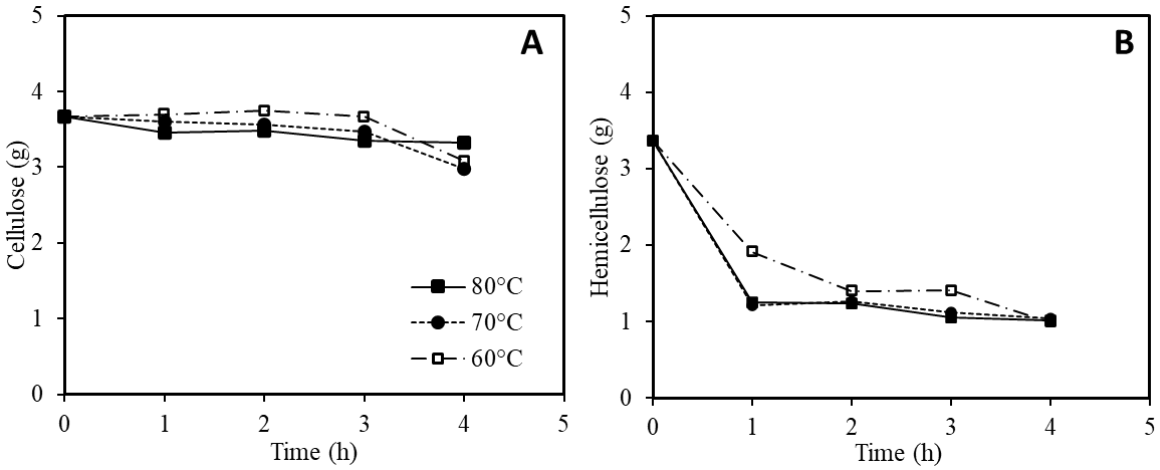

Figure 22. Impact of temperature and extraction time of pectin on the amount of cellulose **A.** and hemicellulose **B.** in (g) in the residual solids.

4.1.4. Selection of Pectin Extraction Conditions for the Biorefinery Development

The selected extraction conditions for the biorefinery involved using an extraction temperature of 80°C and an extraction time of 1 h. Specifically, the selection of extraction conditions was based on considerations of pectin yield and its galacturonic acid content. In relation to pectin yield the highest yields were consistently observed at 80°C for all tested extraction durations (ranging from 8.7% to 11.7%). Additionally, at 70°C with an extraction time of 4 h, a yield of 8.9% was obtained. In regard to galacturonic acid content, the most significant concentrations were identified at 80°C for 1 h, registering at 78.3%, and at 70°C for 4 h, reaching 81.3%. In conclusion, the extraction conducted at 80°C for a duration of just 1 hour yielded both a high pectin yield and a substantial galacturonic acid content. Opting to extract pectin at 80°C for 1 h, as opposed to 70 °C for 4 h, is not only more time-efficient but also more energy-efficient. The elevated temperature significantly reduces the processing duration, leading to energy savings by minimizing the need for prolonged heating. The shorter extraction time further contributes to reduced energy consumption, enhancing the sustainability and cost-effectiveness of the 80°C method. Importantly, this approach maintains pectin quality without any compromises. Consequently, these conditions were selected for the implementation of the biorefinery.

4.1.5. Scale-up of pectin extraction

Scale-up of the process was conducted in the selected extraction conditions from 13.7 g to 1000 g initial SBP solid. From 1000 g of the initial solid, 87.4 g of pectins were recovered.

Table 4 illustrates the characterization of the pectins obtained from the 13.7 g of initial solid compared to those of the pectins obtained from 1000 g initial solid. It is therefore noticeable that the properties of the pectins are not altered by the scale up of the process.

Table 4. Characterization of pectins derived from different quantities of initial SBP solid under the same extraction conditions.

Pectins derived from:	GalA (%)	DE (%)	MeO (%)	AUA (%)
13.7 g initial solid	78.3 ± 3.7	94.1 ± 3.2	15.5 ± 0.6	92.2 ± 4.1
1000 g initial solid	78.1 ± 2.5	95.1 ± 4.1	16.1 ± 0.8	95.7 ± 3.9

4.1.6. Enzymatic hydrolysis of residual solids

Residual solids of SBP after free sugars and pectin extraction were used for enzymatic hydrolysis. The resulting hydrolysate had a final sugar concentration of 27.3 g/L, primarily consisting of glucose (84.9%), with xylose (3.4%), arabinose (4.9%), galactose (4.7%), and mannose (2.2%) also present. Subsequently, this hydrolysate was employed as a carbon source in bacterial fermentation for the production of BC.

4.1.7. Bacterial cellulose Fermentation

BC fermentation was carried out by utilizing commercial sugars as the carbon source, simulating the sugar ratio found in the hydrolysate. Specifically, a 20 g/L carbon source was employed, following the Hestrin and Schramm fermentation media guidelines. The bacterial strain employed for the production of bacterial cellulose was *Komagataeibacter sucrofermentans* DSM 15973, and the fermentation process lasted for a duration of 17 days.

Fig 23A illustrates the sugar consumption and bacterial cellulose production over the entire 17-day fermentation period using the microorganism *K. sucrofermentans*. The highest concentration of BC was achieved on day 10, reaching 1.0 g/L. The peak productivity of BC occurred on day 2 at 0.11 g_{BC}/L/day, and by the end of the fermentation, it was at 0.05 g_{BC}/L/day. The yield of the fermentation on the last day was 0.06 $g_{BC}/g_{consumed\ sugars}$. Additionally, 51.6% of the FAN was consumed by the microorganism, resulting in a final FAN concentration of 105.5 mg/L. It's worth noting that a study by (Tsouko et al., 2023) utilized commercial sugars with the *Komagataeibacter rhaeticus* UNIWA AAK2 strain. When glucose was used at a concentration of 20 g/L for fermentation, they observed a BC concentration of 0.88 g/L, a productivity of 0.088 g/L/day, and FAN consumption of 39.7%.

The hydrolysate obtained after enzymatic hydrolysis was also used as carbon source. The starting FAN concentration was 273.1 mg/L, resulting in a C/FAN ratio of 32.7. The bacterial strain employed for the production of bacterial cellulose was *Komagataeibacter sucrofermentans* DSM 15973, and the fermentation process lasted for a duration of 13 days.

The concentration of BC peaked on day 13 of fermentation, reaching 2.1 g/L. The highest productivity of BC was observed on day 2 at 0.33 g_{BC}/L/day, and by the end of fermentation was 0.16 g_{BC}/L/day. The yield of the fermentation on the final day was 0.06 $g_{BC}/g_{consumed\ sugars}$. Additionally, the microorganism consumed 63.6% of the FAN, resulting in a final FAN concentration of 99.5 mg/L. When utilizing the SBP hydrolysate, carbon source was consumed on the third day of fermentation. This batch fermentation approach was subsequently transformed into a fed-batch process, involving two instances of feeding, as illustrated in **Fig 23**. The initial feeding occurred on day 3, introducing 10 g/L of total sugars. The second feeding took place on day 9, adding 8 g/L of total sugars to the fermentation.

The fermentation productivity achieved using the hydrolysate (**Fig 23B**) was notably higher than that observed when using commercial sugars. This difference in productivity might be attributed to the presence of essential vitamins, minerals, and proteins within the hydrolysate, which seem to yield superior results compared to fermentations involving commercial sugars (Puligundla & Mok, 2021).

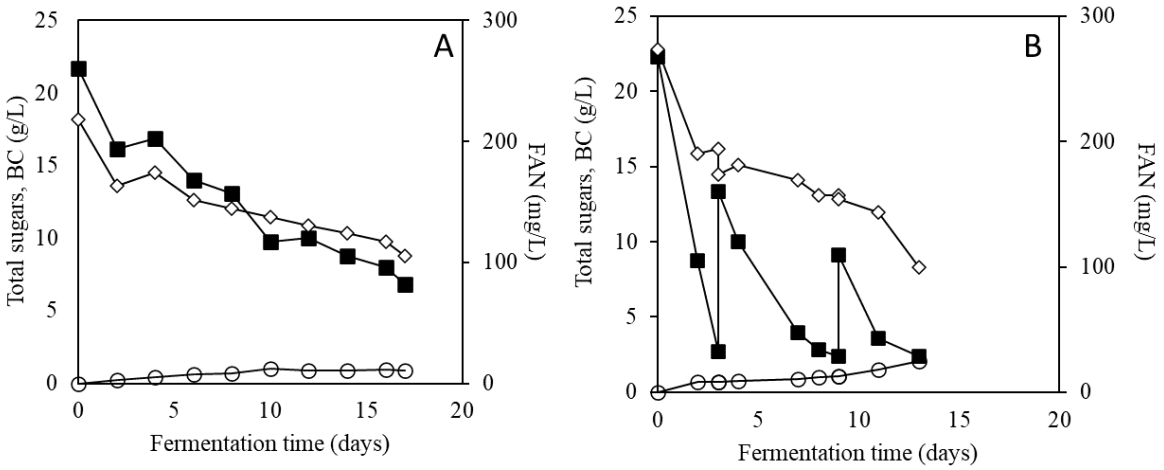

Figure 23. The kinetics of sugars consumption (■), bacterial cellulose production (○) and FAN (◊) during static fermentation **A.** with commercial sugars and **B.** with SBP hydrolysate.

Table 5. Production, yield and productivity of bacterial cellulose, under fermentation of commercial sugars and SBP hydrolysate.

	Simulation	SBP hydrolysate
BC (g/L)	1.0	2.1
Yield ($g_{BC}/g_{consumed\ sugars}$)	0.06	0.06
Productivity (g/L/day)	0.11	0.33

4.1.8. Proposed Biorefinery

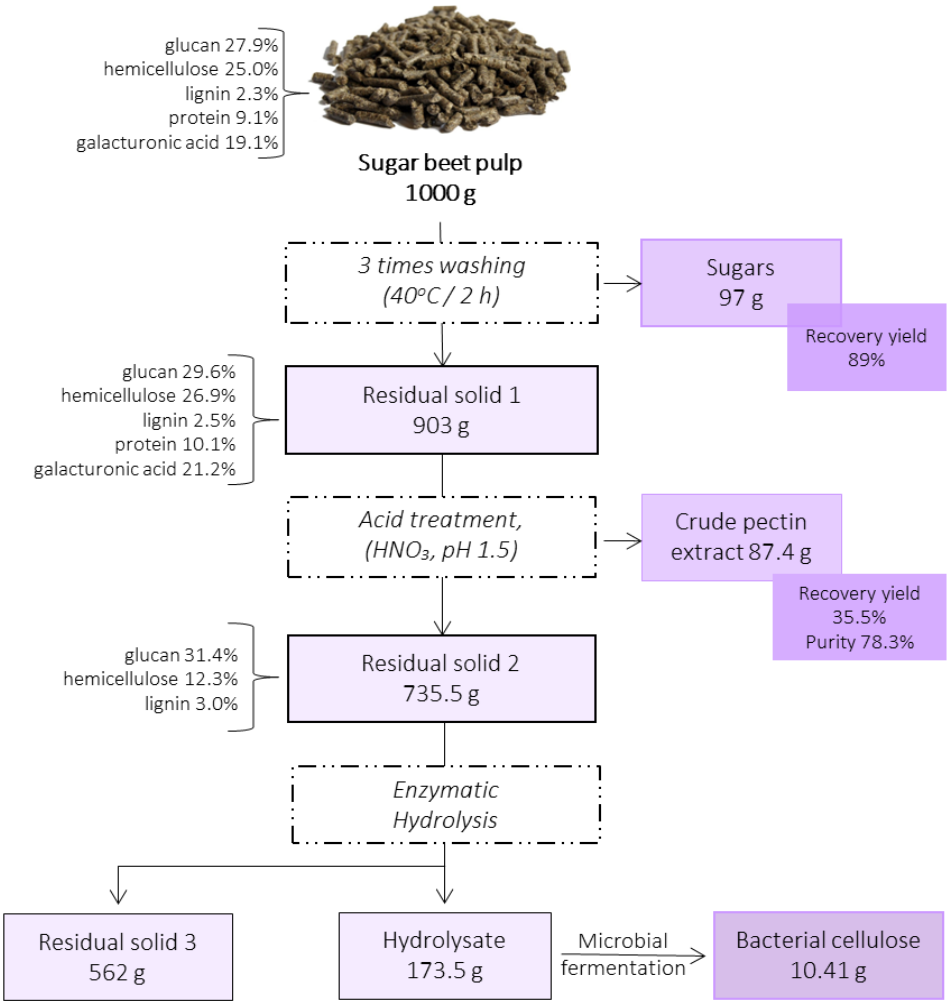

Figure 24. Mass balances and proposed process for the SBP biorefinery.

The calculation of the mass balances of the process proposed for the extraction of pectin and the production of bacterial cellulose from SBP, the whole process was carried out with 1000 g of dry initial SBP sample. Initially, the extraction of free sugars of SBP was carried out,

resulting in the recovery of 97 g of sugars, the solid fraction obtained from the extraction had a weight of 903 g. Subsequently, the extraction of the pectins was carried out under the conditions selected after optimization of the process. From the extraction 87.4 g of pectins were obtained, with a remaining solid of 735.5 g by using 31.21g nitric acid per 1000g initial SBP. A mass loss of 80.1 g was observed during this process. Subsequently, the SBP residue without free sugars and pectins was subjected to enzymatic hydrolysis to produce a hydrolysate. Utilization of the SBP hydrolysate for the production of bacterial cellulose from *Komagataeibacter sucrofermentans* strain DSM 15973 results in a yield equal to 10.41 g. The proposed process as well as the resulting mass balances are presented in **Fig. 24**.

4.2. Shrimp Packaging

4.2.1. Shrimp Maintenance Conditions

Once the shrimp were categorized into five groups, they underwent the coating and were subsequently sealed in airtight bags as outlined in section 3.9. They were thereafter stored under cold chain simulation conditions for 28 days. More specifically, they were subjected to freeze-thaw cycles throughout the study. Time-temperature data were recorded using an accurate data logger and are represented in **Fig. 25**.

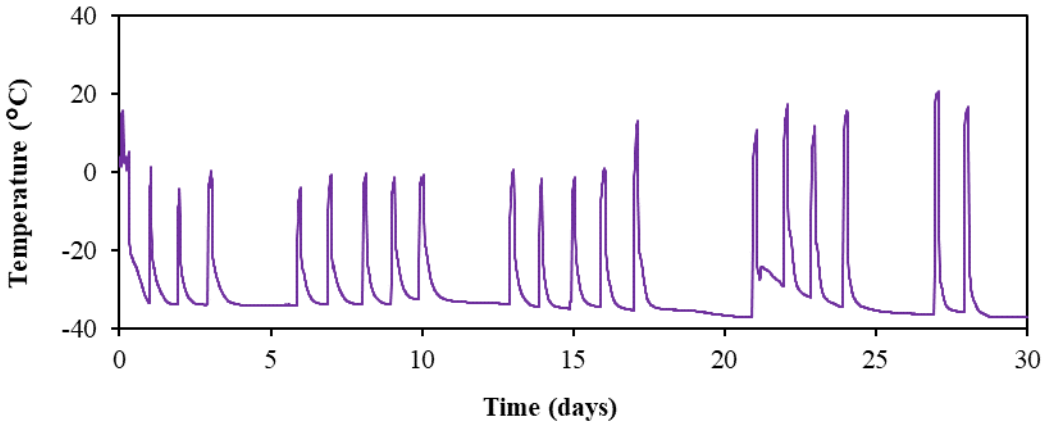

Figure 25. Time-temperature data of shrimp maintenance.

4.2.2. Color measurement

Color analysis of the samples was carried out on specific days over a 28-day period. The color parameters (L*, a*, b*) were evaluated using the CIELab system and measured with a colorimeter. Three samples were assigned to each category, and measurements were taken from each. This approach was adopted due to the inherent heterogeneity among shrimps, even

when subjected to similar treatments, owing to their complex nature as food systems. **Fig. 26** shows the evaluation of the parameter L* over time.

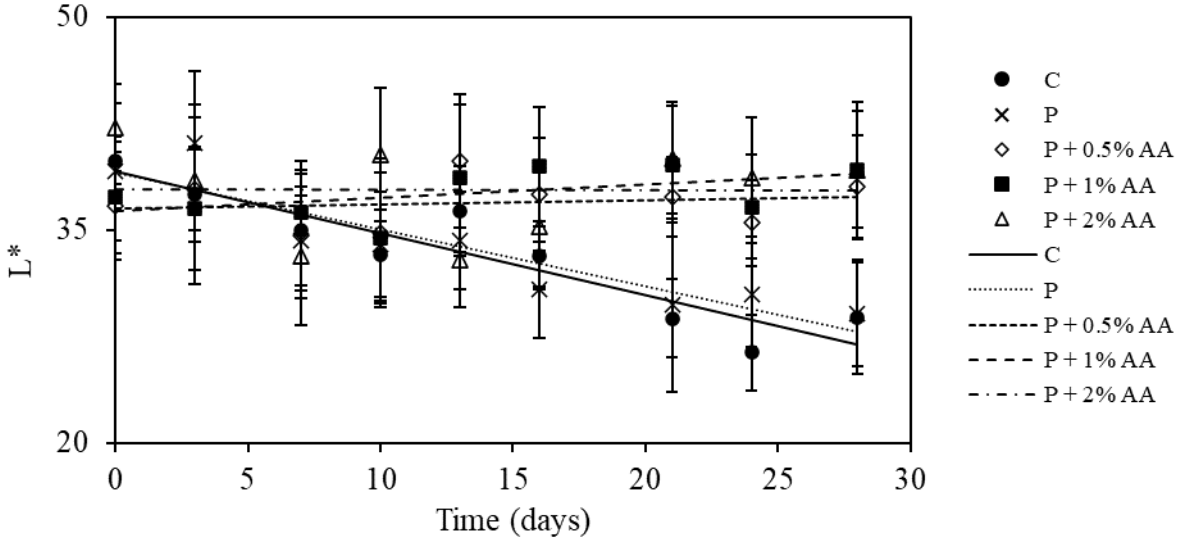

Figure 26. L* parameter evaluation over time for the different sample categories.

Table 6. L* parameter evaluation over time for the different sample categories.

Days	C	P	P + 0.5% AA	P + 1% AA	P + 2% AA
0	$39.9^{a,b,A}$	$39.1^{b,c,A}$	$36.7^{c,B,C}$	$37.3^{b,c,A,B}$	$42.2^{a,A}$
3	$37.5^{a,b,A,B}$	$41.1^{b,A}$	$37.8^{a,b,A,B}$	$36.5^{a,A,B}$	$38.5^{a,b,B,C}$
7	$35.0^{a,B,C}$	$34.3^{a,B}$	$34.6^{a,C}$	$36.3^{a,A,B}$	$33.2^{a,D}$
10	$33.2^{b,C}$	$34.0^{b,B,C}$	$34.8^{b,B,C}$	$34.4^{b,B}$	$40.3^{a,A,B}$
13	$36.3^{b,c,B}$	$34.3^{c,d,B}$	$39.8^{a,A}$	$38.6^{a,b,A}$	$32.9^{d,D}$
16	$33.2^{c,d,C}$	$30.8^{d,C,D}$	$37.5^{a,b,A,B,C}$	$39.4^{a,A}$	$35.3^{b,c,C,D}$
21	$28.8^{b,D}$	$29.7^{b,D}$	$37.3^{a,A,B,C}$	$39.6^{a,A}$	$40.1^{a,A,B}$
24	$26.3^{d,D}$	$30.4^{c,D}$	$35.5^{b,B,C}$	$36.6^{a,b,A,B}$	$38.7^{a,B}$
28	$28.8^{b,D}$	$29.1^{b,D}$	$38.0^{a,A,B}$	$39.2^{a,A}$	$39.3^{a,A,B}$

Two -Way ANOVA. Statistically significant differences are denoted by different letters within the same day (a–d, $p < 0.05$). Additionally, differences within the same sample are indicated with different capital letters (A-D, $p < 0.05$), as determined using the 95% LSD applied as a post-hoc comparison test.

In the CIELab color system, L* represents the lightness of a color, indicating how bright or dark a color appears. The L value ranges from 0 (representing black or no light) to 100 (representing white or the maximum amount of light). A value of 50 represents a mid-gray color, neither very dark nor very light. **Table 6** displays the average L* value for each sample during the measurement days. A statistical analysis was performed to evaluate significant

differences, both between different treatments and between different days for each treatment. From the first day it is observed that there are statistically significant differences between the samples. This is due to the high heterogeneity between the samples. It is important to note that, from day 16 onwards, the samples treated with pectin and ascorbic acid did not show statistically significant differences between them, but statistically significant differences are observed in comparison with the control and the samples treated with pectin alone. In addition, about the control and the samples treated with pectin alone, the L* values show a statistically significant decrease after day 16, descending below 30, which is not noticed in the samples coated with pectin combined with ascorbic acid. This trend is depicted in **Fig. 26**, where it can be observed that starting from day 16, the control and pectin-coated samples exhibit a decline, in contrast to the other samples.

ΔE represents the color differences between control and treated samples during storage and for the determination of ΔE the parameters L*, a* and b* were measured. Higher ΔE values represent wider variations among the samples and the control. The trend observed in the case of L* also appears to be applicable to ΔE, as shown in **Fig. 27**. More specifically, after day 16 the difference in color between control samples and samples coated with a combination of pectin and ascorbic acid has an increasing trend. In contrast, the difference in color between control samples and samples coated with pectin alone appears to be relatively stable.

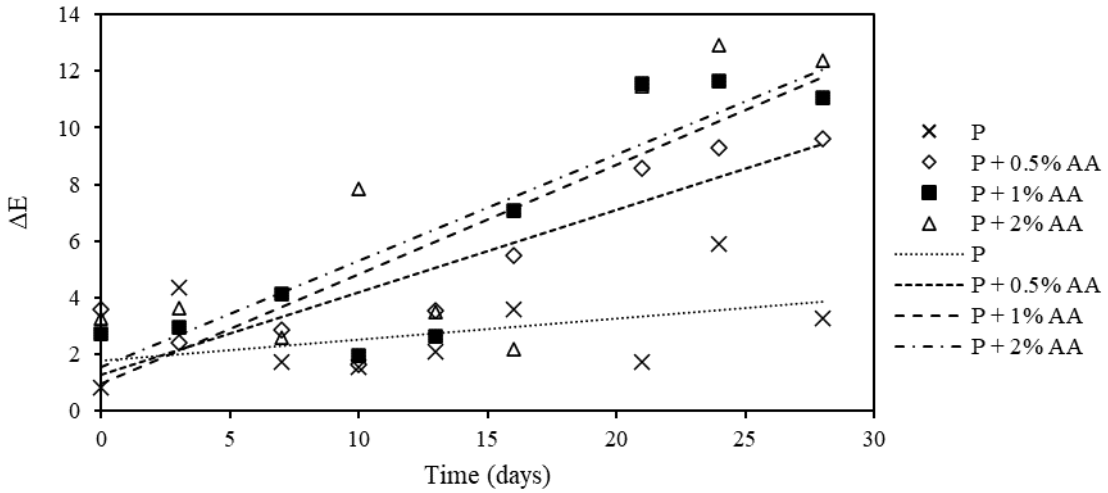

Figure 27. ΔE over time for the different sample categories.

In addition, the color change of the shrimp was also measured using the ImageJ application. For this analysis only one shrimp sample from each category was utilized. **Fig. 28** compares these samples on the first and last day of the analyses. As the figure implies, the samples exhibited high heterogeneity since day 0. As **Fig. 28** implies, the samples exhibited high heterogeneity since day one. The control and the sample with pectin and 1% AA had a

brighter and greyer color than the other three, while the sample with pectin and 2% AA appeared the darkest. In comparison to day 28, it's evident that the color of all samples becomes darker, and the cephalothorax turns redder. Nevertheless, it's important to highlight that even though the control initially looked better, its visual quality had noticeably declined by day 28, making it indistinguishable from the other samples. Conversely, the sample with pectin and 1% AA, which also exhibited superior visual appeal on the first day, continued to maintain a more favorable appearance on the final day due to experiencing less browning.

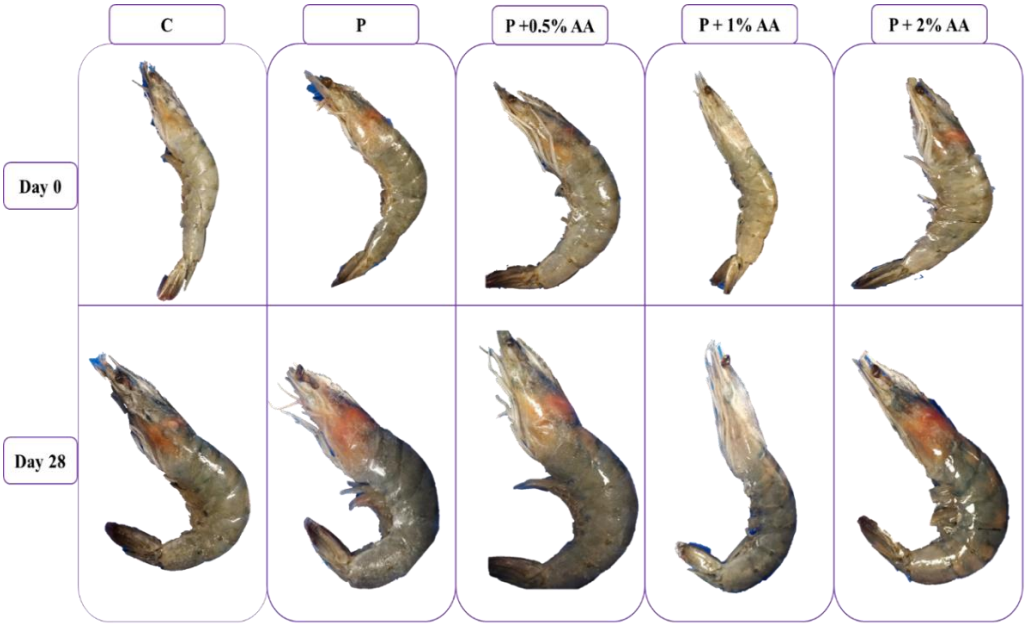

Figure 28. Evolution in the appearance of shrimps *Penaeus vannamei* over time.

The ImageJ software analyses did not reveal significant differences in either Intensity (weighted) or the sum of R+B+G among the samples or across different days (**Fig. 29 & Fig.30**).

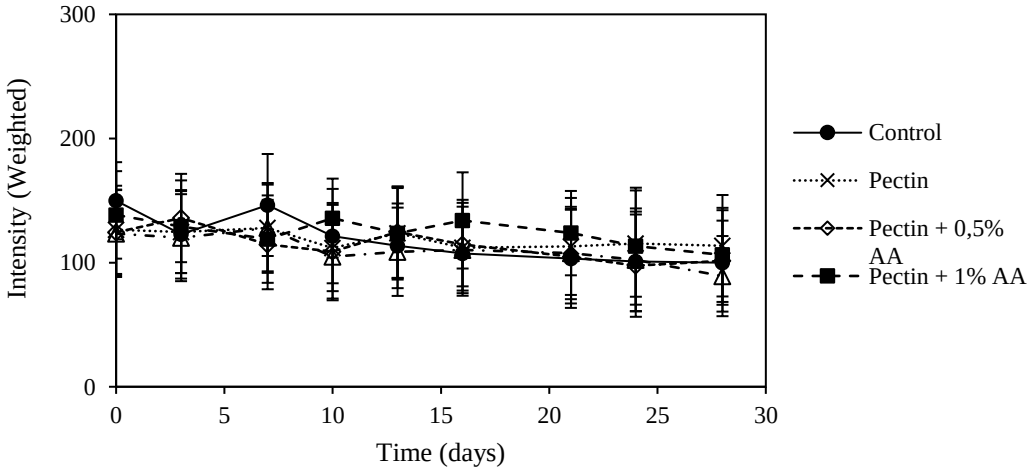

Figure 29. Intensity (Weighted) evaluation over time for the different sample categories.

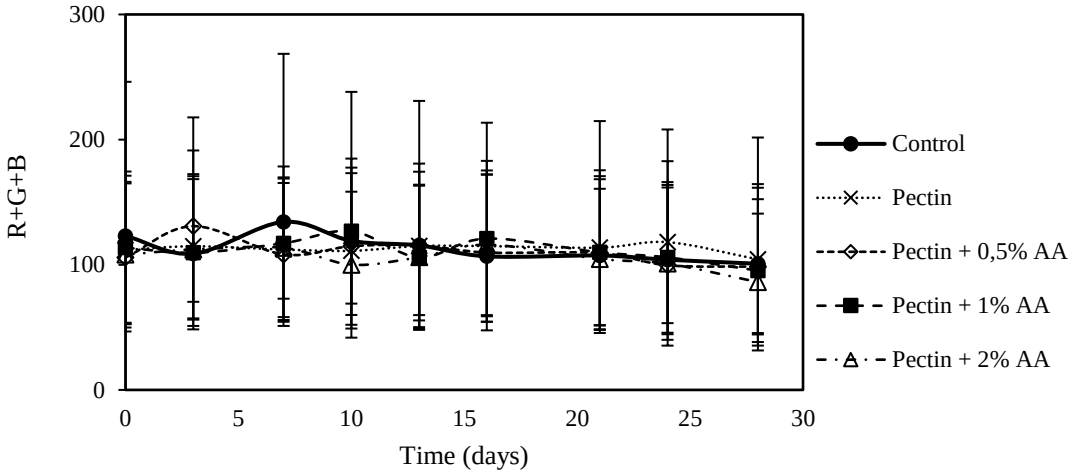

Figure 30. R+G+B evaluation over time for the different sample categories.

4.2.3. Sensory analysis

The sensory analysis conducted by eight trained participants. A five-point scale was used to evaluate the samples for discoloration, brightness and overall appearance at days 0, 3, 7, 10, 13, 16, 21, 24 and 28. Regarding discoloration, the sensory analysis scale used assigned a rating from 1 to indicate uniform color with no discolorations to a rating of 5 to signify complete discoloration. A 1 on the brightness scale corresponded to low brightness and a 5 to high brightness. Overall appearance evaluated form the panel in a scale 1-5 (worst - best).

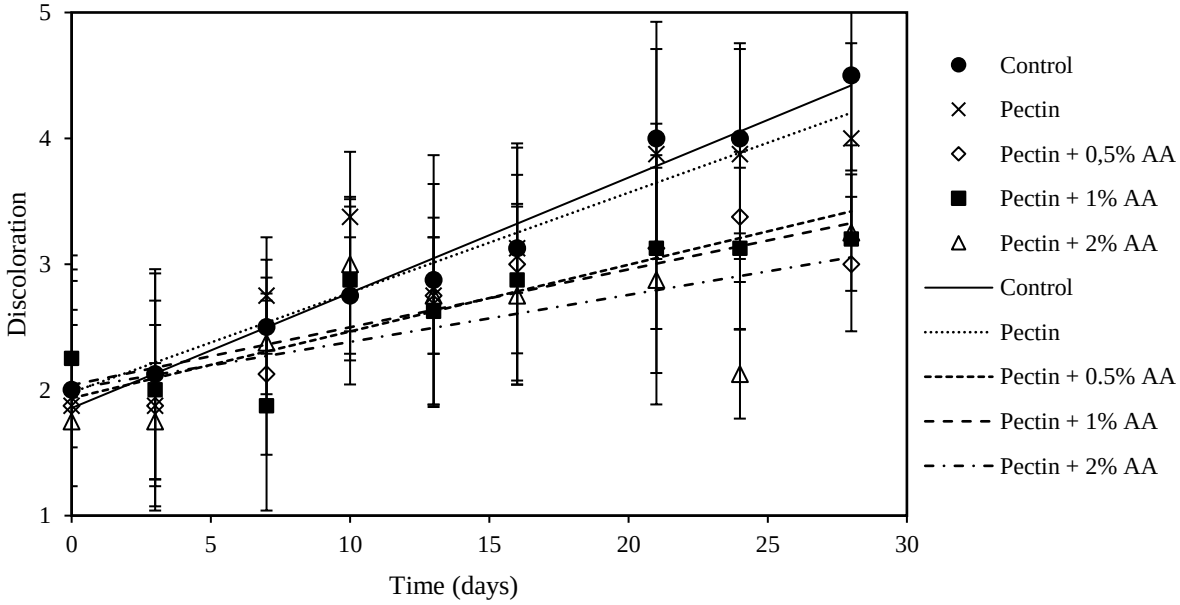

Figure 31. Sensory analysis scores of the discoloration in shrimps packaged with no coating (control) and with different coatings.

Table 7. Sensory analysis scores of the discoloration in shrimps packaged with no coating (control) and with different coatings.

Days	C	P	P + 0.5% AA	P + 1% AA	P + 2% AA
0	2.0^{a}	1.9^{a}	1.9^{a}	2.3^{a}	1.8^{a}
3	2.1^{a}	1.9^{a}	1.9^{a}	2.0^{a}	1.8^{a}
7	$2.5^{a,b}$	2.75^{a}	$2.1^{b,c}$	1.9^{c}	$2.4^{a,b,c}$
10	2.6^{b}	3.6^{a}	2.9^{b}	2.9^{b}	$3.1^{a,b}$
13	2.9^{a}	2.8^{a}	2.8^{a}	2.6^{a}	2.8^{a}
16	3.1^{a}	3.1^{a}	3.0^{a}	2.9^{a}	2.8^{a}
21	4.0^{a}	3.9^{a}	$3.1^{a,b}$	$3.1^{a,b}$	2.9^{b}
24	4.0^{a}	3.9^{a}	$3.4^{a,b}$	3.1^{b}	2.1^{c}
28	4.5^{a}	4.0^{a}	3.0^{b}	3.3^{b}	3.3^{b}

One-Way ANOVA. Significant statistical differences are represented by different letters (a–c, $p < 0.05$), as determined using the 95% LSD with post-hoc comparison.

Table 7 indicates how different treatments influence the discoloration sensory score over a 28-day period in shrimp samples, with statistical significance indicated by the superscript letters. It is worth noting that there were no statistically significant differences observed in the shrimp samples on days 0 and 3. However, on day 7, both the control sample and the sample coated with pectin alone exhibited a higher discoloration score compared to the samples coated with pectin combined with ascorbic acid. Among these, the samples with the addition of 1% ascorbic acid displayed a statistically significant difference, in comparison to both the control samples and the samples coated solely with pectin. Finally, on the last day of evaluation, all samples that had been coated with both pectin and ascorbic acid demonstrated lower discoloration scores. These scores showed a statistically significant difference when compared to both the control samples and the samples coated with pectin alone.

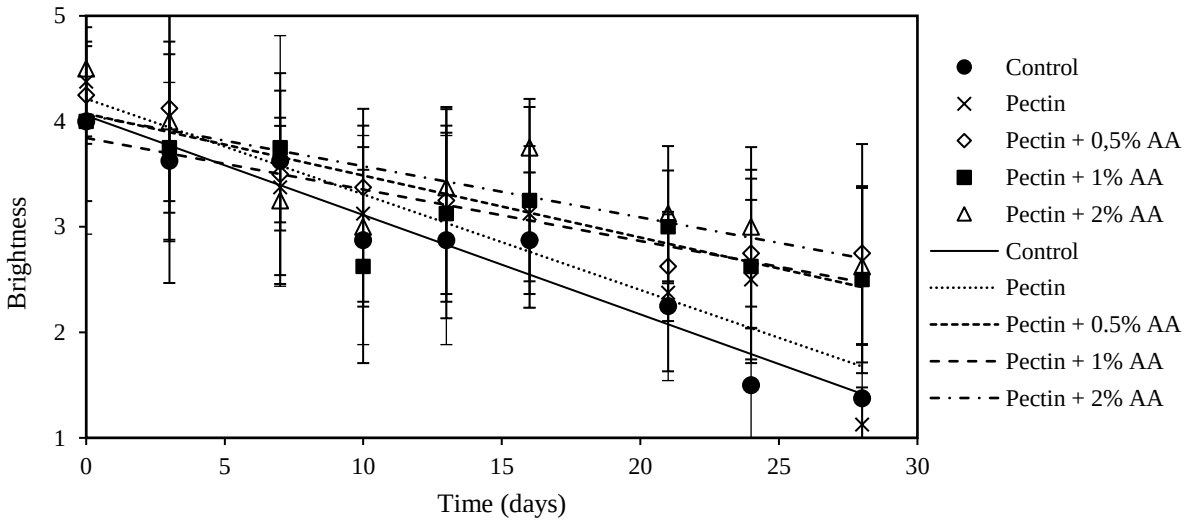

Figure 32. Sensory analysis scores of the brightness in shrimps packaged with no coating (control) and with different coatings.

Table 8. Sensory analysis scores of the brightness in shrimps packaged with no coating (control) and with different coatings.

Days	C	P	P + 0.5% AA	P + 1% AA	P + 2% AA
0	4.0^{a}	4.4^{a}	4.3^{a}	4.0^{a}	4.5^{a}
3	3.6^{a}	3.8^{a}	4.1^{a}	3.8^{a}	4.0^{a}
7	3.5^{a}	3.2^{a}	3.3^{a}	3.6^{a}	3.0^{a}
10	3.0^{a}	3.4^{a}	3.4^{a}	3.0^{a}	2.8^{a}
13	2.9^{a}	3.1^{a}	3.3^{a}	3.1^{a}	3.4^{a}
16	2.9^{b}	3.1^{b}	$2.9^{a,b}$	$3.3^{a,b}$	3.8^{a}
21	2.2^{c}	$2.4^{b,c}$	$2.7^{a,b,c}$	$3.0^{a,b}$	3.1^{a}
24	1.5^{b}	2.5^{a}	2.7^{a}	2.8^{a}	3.0^{a}
28	1.4^{b}	1.1^{b}	2.8^{a}	2.5^{a}	2.7^{a}

One-Way ANOVA. Significant statistical differences are represented by different letters (a–c, $p < 0.05$), as determined using the 95% LSD with post-hoc comparison.

Table 8 shows how different treatments influence the brightness sensory score over a 28-day period in shrimp samples, with statistical significance indicated by the superscript letters. It is therefore observed that until day 13 the shrimp samples do not exhibit statistically significant differences. On day 16, the samples with the addition of 2% ascorbic acid displayed a statistically significant difference, in comparison to both the control samples and the samples coated solely with pectin. On day 28, the samples that had been coated with pectin combined with ascorbic acid, exhibited a higher brightness score with statistically significant differences compared to the samples coated with pectin combined with ascorbic acid.

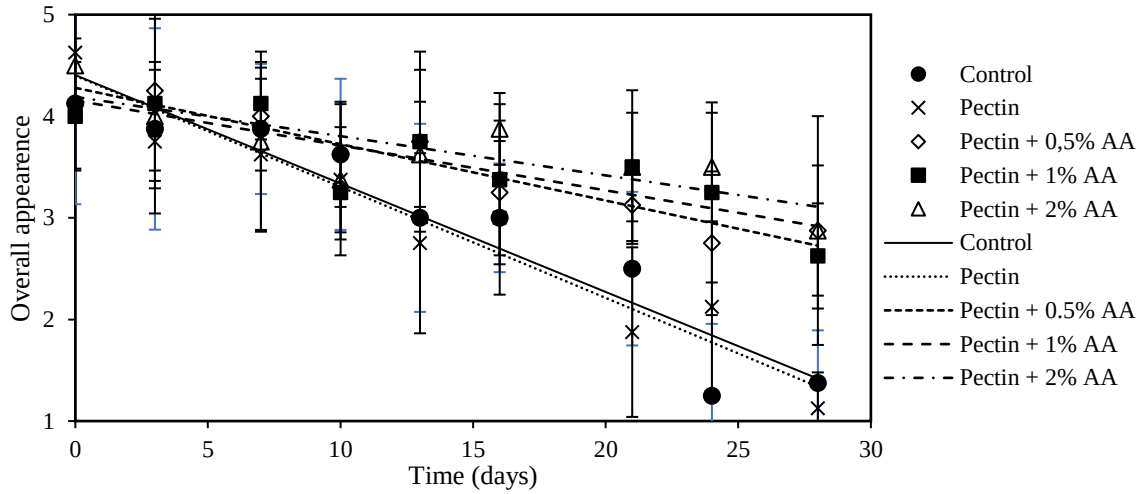

Figure 33. Sensory analysis scores of the overall appearance in shrimps packaged with no coating (control) and with different coatings.

Table 9. Sensory analysis scores of the overall appearance in shrimps packaged with no coating (control) and with different coatings.

Days	C	P	P + 0.5% AA	P + 1% AA	P + 2% AA
0	4.1^{a}	4.7^{a}	4.1^{a}	4.0^{a}	4.5^{a}
3	3.9^{a}	3.8^{a}	4.3^{a}	4.1^{a}	4.0^{a}
7	3.7^{a}	3.4^{a}	3.9^{a}	3.9^{a}	3.4^{a}
10	3.8^{a}	3.1^{a}	3.8^{a}	3.3^{a}	3.0^{a}
13	$3.0^{a,b}$	2.8^{b}	3.8^{a}	3.8^{a}	3.6^{a}
16	3.0^{b}	3.0^{b}	$3.3^{a,b}$	$3.4^{a,b}$	3.9^{a}
21	$2.5^{b,c}$	1.9^{c}	$3.1^{a,b}$	3.5^{a}	3.5^{a}
24	1.3^{c}	2.1^{b}	$2.8^{a,b}$	3.3^{a}	3.5^{a}
28	1.4^{b}	1.1^{b}	2.9^{a}	2.6^{a}	2.9^{a}

One-Way ANOVA. Significant statistical differences are represented by different letters (a–c, $p < 0.05$), as determined using the 95% LSD with post-hoc comparison.

Table 9 shows how different treatments influence the overall appearance sensory score over a 28-day period in shrimp samples, with statistical significance indicated by the superscript letters. It is therefore observed that until day 10 the shrimp samples do not reveal any statistically significant differences. On day 13, the samples with the addition of ascorbic acid displayed a statistically significant difference, in comparison to the samples coated solely with pectin. On day 28, the samples that had been coated with pectin combined with ascorbic acid, presented a greater overall appearance score with statistically significant differences compared to the samples coated with pectin combined with ascorbic acid.

In conclusion, as regards the sensory analysis, it is noted that approximately up to the midpoint of the sensory analysis duration there are no statistically significant differences between the samples. Moreover, it is worth noting that in all three parameters examined (discoloration, brightness, overall appearance) on the last day of the sensory analysis, the control and samples coated with pectin alone had statistically significantly worse scores than those coated with a combination of pectin and ascorbic acid.

4.2.4. Measurement of PPO activity

The impact of AA at varying concentrations on PPO from the cephalothorax of the shrimps is depicted in **Fig. 34**. Dopachrome formation showed a continuous increase in both the control and the samples coated with pectin alone as the reaction time was extended. Dopachrome formation was retarded in the presence of AA at all concentrations at which it was added. Hence, it seems that even at the lowest concentration added, AA inhibits the enzyme's activity. The results are supported by Nirmal & Benjakul (2012) who observed the inactivation of PPO from the cephalothorax of Pacific white shrimp by AA even in significantly lower concentrations.

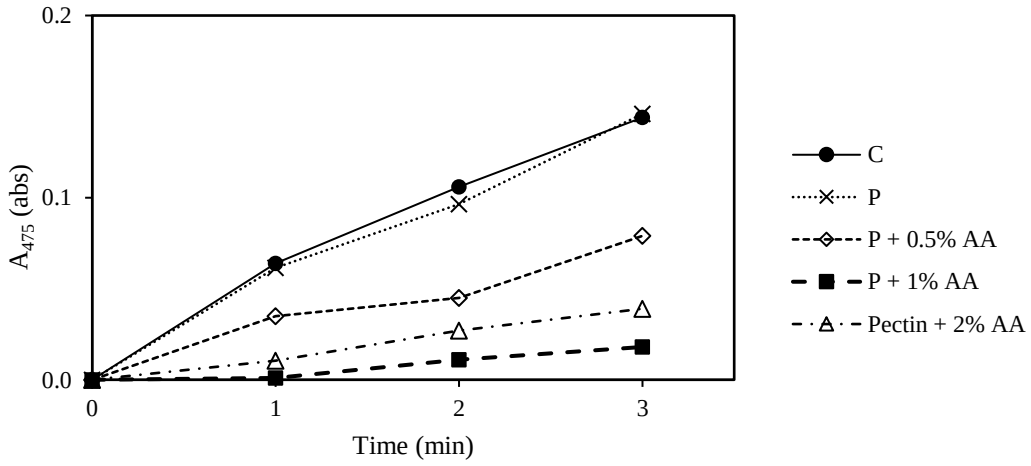

Figure 34. The impact of AA on the activity of PPO extracted from the shrimps' cephalothorax. The decline in A_{475} signifies the inhibition of DOPA-chrome formation by PPO.

5. Conclusions

In this study was carried out the valorization of sugar beet pulp through the development of a biorefinery for the production of biodegradable and bio-based coatings based on pectin, as well as bacterial cellulose. Pectin coatings were manufactured and subsequently applied to frozen shrimp under freeze-thaw cycles, with ascorbic acid serving as a browning inhibitor.

The most significant conclusions that were derived from this study were the following:

- The yield of pectins ranged from 4.6% to 11.7% (w/w) depending on the temperature and the extraction time and the greater pectin yield was noted at 80°C for 4 h.
- Their content of galacturonic acid ranged from 60.7% to 83.1% (on a dry-weight basis) depending on the temperature and the extraction time.
- The temperature and extraction time obtained by nitric acid do not affect the characterization of the recovered pectins. More specifically, all the pectins were HM with DE >94% and MeO >14.3% and AUA% values ranged from 89.3% to 98.2%.
- The extraction conditions chosen for the scale-up were 80°C for 1 h since they provided high pectin yield and significant galacturonic acid content.
- The enzymatic hydrolysis resulted in a hydrolysate with a sugar concentration of 27.3 g/L, with glucose being the predominant sugar (84.9%).
- The produced SBP hydrolysate was utilized as a carbon source for the production of BC using *Komagataeibacter sucrofermentans* DSM 15973, achieving a production of 2.1 g/L BC, with a yield 0.06 $g_{BC}/g_{consumed\ sugars}$ and productivity of 0.33 g/L/day.
- After day 16 a noticeable trend emerges: The average L* value did not show statistically significant differences among the shrimps coated with a combination of pectin and AA, but it differed significantly from both the control group and the shrimps coated with pectin alone. In addition, the color difference between control and pectin alone coated samples remains stable, while the color difference between control and pectin with AA coated samples steadily increases.
- The ImageJ software analyses did not reveal significant differences in either intensity (weighted) or the sum of R+B+G among the samples or across different days.

- Shrimps coated with a combination of pectin and ascorbic acid received higher scores in the sensory analysis after the midpoint of the sensory analysis duration.
- The presence of AA in all concentrations (0.5%, 1% & 2%) resulted in a reduction in PPO activity.
- The coatings produced from pectin derived from SBP with combination of AA exhibited promising characteristics for the extension of the shelf life of shrimps.

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