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DEVELOPMENT OF BIOPOLYMER FILMS FROM *OPUNTIA* SPP. MUCILAGE FOR FOOD PACKAGING APPLICATION

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Master in Food Technology and Safety

DOCTORATE IN FOOD SCIENCES

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To my family.

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ABSTRACT

This Ph.D thesis explores the valorization of *Opuntia ficus-indica* residues, cladodes and fruit peels, as sustainable sources of polysaccharides and natural pigments for the development of bio-based intelligent food packaging. The work combined extraction, material characterization, and functional testing to demonstrate the feasibility of transforming these agro-industrial by-products into value-added biopolymer systems.

Initially, polysaccharide-based edible coatings were evaluated on prickly pear fruits. Coatings formulated from pectin, alginate, chitosan, and starch effectively delayed weight loss, maintained firmness and color, and reduced microbial growth during storage, confirming the potential of natural polymers in food preservation.

Subsequently, pectin was extracted from *Opuntia* cladodes using optimized conditions established through response surface methodology. The extracted pectin exhibited a low degree of esterification and high galacturonic acid content. Betalain-rich extracts were obtained as natural colorants with high pH sensitivity.

Composite films were developed by blending pectin extracted from cladodes and commercial pectin (OFIPC) incorporating montmorillonite (MMT), betalain-rich extracts with or without calcium crosslinking. MMT addition improved mechanical resistance and barrier properties, crosslinking modulates the microstructure and permeability, while betalains conferred colorimetric sensitivity to pH changes. The films demonstrated distinct color transitions in response to ammonia released during salmon spoilage, confirming their functionality as freshness indicators. The films combined strong mechanical performance with low water vapor permeability and rapid chromatic response. exhibited balanced barrier properties, and a color transition from pink to yellow in response to volatile amines generated during fish spoilage.

Overall, this work represents a step toward waste valorization and the circular use of plant residues. The research demonstrated that *Opuntia ficus-indica* is a promising source of biopolymers and pigments for sustainable smart packaging. It highlights the potential of circular approaches that convert agricultural residues into intelligent materials, contributing to reduced plastic dependency and improved food quality monitoring.

Keywords: *Opuntia ficus-indica*; pectin; mucilage; betalains; biopolymer films; intelligent food packaging; circular economy; sustainable materials

RESUMO

Esta tese explora a valorização dos resíduos de *Opuntia ficus-indica*, nomeadamente cladódios e cascas dos frutos, como fontes sustentáveis de polissacáridos e pigmentos naturais para o desenvolvimento de embalagens alimentares inteligentes à base de biopolímeros. O trabalho combinou etapas de extração, caracterização de materiais e ensaios funcionais, demonstrando a viabilidade de transformar estes subprodutos agroindustriais em sistemas biopoliméricos de valor acrescentado.

Inicialmente, foram avaliados revestimentos edíveis à base de polissacáridos aplicados em figo-da-índia. As formulações de pectina, alginato, quitosano e amido mostraram-se eficazes na redução da perda de massa, na manutenção da firmeza e da cor, e na diminuição do crescimento microbiano durante o armazenamento, confirmando o potencial dos polímeros naturais na conservação de alimentos. Posteriormente, a pectina foi extraída dos cladódios sob condições otimizadas por metodologia de superfície de resposta. A pectina obtida apresentou baixo grau de esterificação e elevado teor de ácido galacturónico. Extratos ricos em betalaínas foram igualmente extraídos dos frutos demonstrando elevada sensibilidade ao pH.

Foram então desenvolvidos filmes combinando pectina extraída dos cladódios com pectina comercial (OFIPC), montmorilonite (MMT), extratos ricos em betalaínas e, com reticulação com cálcio. A adição de MMT melhorou a resistência mecânica e as propriedades de barreira, a reticulação modulou a microestrutura e a permeabilidade e as betalaínas conferiram sensibilidade colorimétrica a variações de pH. Os filmes apresentaram mudanças visíveis de cor de rosa para amarelo em resposta à amónia libertada durante a deterioração de salmão, comprovando a sua funcionalidade como indicadores de frescura.

De forma geral, este trabalho representa um avanço na valorização de resíduos vegetais e na aplicação dos princípios da economia circular. Demonstrou-se que *Opuntia ficus-indica* é uma fonte promissora de biopolímeros e pigmentos para o desenvolvimento de embalagens

sustentáveis e inteligentes, capazes de reduzir a dependência de plásticos e melhorar a monitorização da qualidade dos alimentos.

Palavras chave: *Opuntia ficus-indica*; pectina; mucilagem; betalaínas; biopolímeros; embalagem alimentar inteligente; economia circular; materiais sustentáveis

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ABBREVIATIONS

AP	Apiogalacturonan
BC	Betalain content
CAM	Crassulacean Acid Metabolism
CE	Catechin equivalent
CP	Commercial pectin
DE	Degree of esterification
DPPH	2,2-Diphenyl-1-picrylhydrazyl assay
EAB	Elongation at Break
EDTA	Ethylenediaminetetraacetic Acid
GRAS	Generally recognized as Safe
HG	Homogalacturonan
HM	High-methoxyl pectin
HPLC-DAD	High Performance Liquid Chromatography with Diode Array Detection
LC-MS	Liquid Chromatography – Mass Spectrometry
LM	Low-methoxyl pectin
MMT	Montmorillonite.
MMT	Montmorillonite

OFI	<i>Opuntia ficus-indica</i>
OFIPC	Film containing 3% (w/v) total polymer (1:1 blend of <i>Opuntia ficus-indica</i> cladode pectin and commercial pectin)
OFIPC_100EXT	OFIPC film incorporating 100% (w/w, relative to pectin) betalain-rich extract powder
OFIPC_100EXT_R	OFIPC_100EXT film crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_50EXT	3% <i>Opuntia ficus indica</i> cladode Pectin/Commercial Pectin, 50% betalain-rich extract powder
OFIPC_50EXT_R	3% <i>Opuntia ficus indica</i> cladode Pectin/Commercial Pectin, 50% betalain-rich extract powder with Reticulation
OFIPC_MMT	OFIPC film incorporating 2.5% (w/w, relative to pectin) montmorillonite (MMT)
OFIPC_MMT_100EXT	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 100% (w/w, relative to pectin) betalain-rich extract powder
OFIPC_MMT_100EXT_R	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 100% (w/w, relative to pectin) betalain-rich extract powder, crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_MMT_50EXT	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 50% (w/w, relative to pectin) betalain-rich extract powder
OFIPC_MMT_50EXT_R	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 50% (w/w, relative to pectin) betalain-rich extract powder, crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_MMT_R	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_R	OFIPC film crosslinked with 2% (w/v) CaCl ₂ solution
PC	Film containing 3% (w/v) commercial pectin

PC_R	PC film crosslinked with 2% (w/v) CaCl ₂ solution
RG I	Rhamnogalacturonan I
RG II	Rhamnogalacturonan II
RSM	Response Surface Methodology
SDGs	Sustainable Development Goals
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis
TPC	Total phenolic content
TS	Tensile Strength
TTA	Total titratable acidity
TVB-N	Total volatile basic nitrogen
WVP	Water Vapor Permeability
XG	Xylogalacturonan
YM	Young's Modulus

SYMBOLS

Ca	Calcium
CaCl₂	Calcium Chloride
CO₂	Carbon Dioxide
db	Dry basis
e	Film thickness
h°	Hue angle
HCl	Hydrochloric acid
K	Potassium
L/S	Liquid-to-solid ratio
Mg	Magnesium
Na	Sodium
NH₃	Ammonia
NH₄OH	Ammonium hydroxide
RH	Relative Humidity
T	Temperature
t	Time
Tg	Glass transition temperature
wb	Wet basis

ΔE	Total color difference
ΔP	Water vapor pressure difference
ε	Strain
λ	Wavelength
σ	Stress

INTRODUCTION

1.1 Background and motivation

The development of sustainable packaging solutions has become an urgent priority in response to the environmental impact of fossil-based plastics and the growing demand for eco-friendly alternatives. Biobased polymers, derived from renewable raw materials, have emerged as a promising alternative due to their biodegradability and compatibility with food systems. Polysaccharides, proteins, and lipids obtained from natural sources such as alginate, chitosan, agar, and gelatin have demonstrated potential for packaging applications for edible films and coatings due to their biodegradability and film-forming capacity (Razavi et al., 2015; V. G. L. Souza et al., 2017). These materials address consumer demand for safe and sustainable packaging (Hassan et al., 2018; V. G. L. Souza & Fernando, 2016a), but their scalability, mechanical resistance, and poor barrier properties remain ongoing challenges.

Opuntia spp. is a crop widely distributed in tropical/subtropical regions, particularly in arid climates such as those of the Mediterranean and Central America and, remains underexploited despite its high potential for sustainable valorization (Bayar et al., 2016; Ginestra et al., 2009). This plant is used to prevent soil erosion and degradation and to control desertification (Ben Salem-Fnayou et al., 2014), problematic issues in Mediterranean Region. Its fruits, commonly known as prickly pears, are consumed worldwide and are a rich source of betalain pigments with antioxidant and coloring properties (Aruwa et al., 2018; Guerrero-Beltrán & Ochoa-Velasco, 2018), while the cladodes are commonly used as animal feed and often discarded (Bacchetta et al., 2024; Barba et al., 2017; Bayar et al., 2016). Of particular interest is the cladode's mucilage (pectin) fraction, a carbohydrate-rich polymer capable of forming viscous colloids and acting as a water barrier (Allegra et al., 2017; Contreras-Padilla et al., 2016). These

functional properties make cactus mucilage attractive for industrial applications in cosmetics, pharmaceuticals, and food products (C. Rodrigues et al., 2023).

The potential of *Opuntia* spp. has also been recognized within the ERANETMED-funded project *MediOpuntia*, which promoted cactus cultivation in marginal Mediterranean lands with minimal water requirements, and that was coordinated by Universidade NOVA de Lisboa. Within the framework of this project, several studies explored the opportunities associated with this crop, underscoring its relevance for sustainable agriculture and biomass valorization. Building on that experience, the present thesis investigates the development of *Opuntia*-based biopolymer films for food packaging applications.

In food packaging, *Opuntia* mucilage (pectin) has demonstrated potential as a coating and film-forming agent (Allegra et al., 2016, 2017; Del-Valle et al., 2005). However, current extraction protocols for pectin are limited and require optimization to improve yield and functionality (Rodríguez-González et al., 2014). Furthermore, films produced exclusively from mucilage often exhibit weak mechanical and barrier properties (Espino-Díaz et al., 2010; Gheribi & Khwaldia, 2019). Strategies to overcome these limitations, such as the addition of calcium, gelatin, beeswax, or plasticizers (e.g., glycerol, sorbitol, polyethylene glycols), have improved certain characteristics but limitations remain (Espino-Díaz et al., 2010; Gheribi et al., 2018; Lira-Vargas et al., 2014).

To address these challenges, this work explores the development of novel smart (active and intelligent) active and eco-friendly food packaging materials based on *Opuntia* spp. (*Opuntia ficus-indica*) mucilage/pectin and bioactive compounds. Therefore, the aim is the optimization of the extraction processes for mucilage from the cladodes, for the production of the films, and betalains from the fruits, to enhance the activity and sensory capacity of the films. Besides, different formulations need to be tested to achieve the best combination of polymers (single mucilage or blends), incorporating plasticizers, nanofillers, and bioactive compounds which need to be investigated to enhance the mechanical, physicochemical, and barrier properties of the films. Those biomaterials need also to be tested in food packaging applications, focusing on fresh fruits and fish. Additionally, the incorporation of betalains will be evaluated not only for bioactive properties but also for their potential use as natural spoilage indicators through pH-dependent color changes.

By advancing the valorization of *Opuntia* biomass, this work aims to contribute to crop sustainability, reduce the dependence on fossil-based plastics, and improve food security and safety.

Its outcomes are expected to generate fundamental knowledge on *Opuntia*-based biopolymers and support the transition of the food-packaging sector toward environmentally responsible materials.

1.2 Research objectives

The main goal of this project is to obtain an optimized formulation for producing films from *Opuntia* spp. mucilage/pectin and betalains, and to apply them as active and/or intelligent food packaging materials.

In doing so, the project aims to add value to cactus cladodes and fruit residues, while offering sustainable alternatives to fossil-based plastics.

To achieve the main goal, the following objectives have been defined:

1. **Development of efficient and sustainable extraction methods** for mucilage/pectin from *Opuntia* cladodes and betalains from fruit residues. Different solvents and process parameters will be tested, with optimization based on yield and the physicochemical characteristics required for biopolymer production.
2. **Production and characterization of biopolymer films** obtained from *Opuntia ficus-indica* mucilage/pectin.
3. **Enhancement of functional properties** of the films through incorporation of betalain rich-extract, to provide antioxidant, antimicrobial, and color-sensing activity.
4. **Application of the most promising films to a food matrix** (salmon) in order to evaluate the ability of the films to be used as sensor for monitoring food quality and safety.

Furthermore, this Ph.D. thesis is aligned with the 2030 Agenda for Sustainable Development of the United Nations fitting the Sustainable Development Goals (SDGs): 2 (End hunger, achieve food security and improved nutrition and promote sustainable agriculture), 12 (Ensure sustainable consumption and production patterns), and 15 (Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss). This project follows those SDGs by exploring a crop that is highly suitable to adverse environments, providing an alternative to fossil-based sources commonly used in food packaging and by helping the consumer to take safe choices from a food safety point of view.

1.3 Thesis outline

This Ph.D. thesis is organized as it follows:

1 – Introduction

Introduces the research outlining context, motivation, objectives and organization of the research.

2 – Literature review

Presents a comprehensive literature review on *Opuntia* spp. constituents, their compounds extraction methods and potential applications. Also, an extensive review was conducted on the current state of bio-based sensors for food packaging applications. This chapter is based on two review papers already published:

- i. C. Rodrigues, C.D. Paula, S. Lahbouki, A. Meddich, A. Outzourhit, M. Rashad, L. Pari, I. Coelho, A.L. Fernando, V.G.L. Souza. *Opuntia* spp.: An Overview of the Bioactive Profile and Food Applications of This Versatile Crop Adapted to Arid Lands. *Foods*. 2023; 12(7):1465. <https://doi.org/10.3390/foods12071465>
- ii. C. Rodrigues, V.G.L. Souza, I. Coelho, A.L. Fernando. Bio-Based Sensors for Smart Food Packaging—Current Applications and Future Trends. *Sensors*. 2021; 21(6):2148. <https://doi.org/10.3390/s21062148>

3 – Quality Preservation and Shelf-Life Extension of Prickly Pear (*Opuntia ficus-indica* L. Mill) Using Edible Coatings

Consists in a preliminary study of the application of polysaccharide-based coatings for prickly pear fruit preservation and shelf-life extension. The resulting outcomes of this work were published in an international peer reviewed scientific journal: C. Rodrigues, C. Polesca, I. Bicalho, V.G.L. Souza, I. Coelho, A.L. Fernando. Quality Preservation and Shelf-Life Extension of Prickly Pear (*Opuntia ficus-indica* L. Mill) Using Edible Coatings. *Foods*. 2025; 14(2):161. <https://doi.org/10.3390/foods14020161>

4 – Pectin extraction from *Opuntia* spp. Cladodes: Process Optimization and Characterization

This chapter describes the optimization of pectin extraction from *Opuntia ficus-indica* cladodes and the characterization of the resulting materials. This work is being prepared to be submitted to *Sustainable Food Technology*: C. Rodrigues, V.G.L. Souza, B. S. Börekçi, I. Coelho, A.L. Fernando. Pectin extraction from *Opuntia* spp. Cladodes: Process Optimization and Characterization.

5 – Development and Characterization of *Opuntia*-Based Films for Intelligent Food Packaging

This chapter focuses on the development and characterization of *Opuntia* pectin based films, with addition of betalain-rich extracts. The application of the films into a food matrix was also tested to evaluate their ability to work as a sensor. This work is being prepared to be submitted to *Food Packaging and Shelf-Life*: C. Rodrigues, V.G.L. Souza, I. Coelho, A.L. Fernando. Development and Characterization of *Opuntia*-Based Films for Intelligent Food Packaging.

6 – General Conclusions

Provides an overview of the Ph.D. thesis and summarizes the main findings.

7 –Future work

Proposes perspectives for future research.

LITERATURE REVIEW

This chapter provides a comprehensive review of the literature relevant to the development of this Ph.D. work. It integrates information from two previously published review articles, highlighting both the fundamental background and the most recent advances in the field. The first paper, *Opuntia spp.: An Overview of the Bioactive Profile and Food Applications of This Versatile Crop Adapted to Arid Lands* (C. Rodrigues et al., 2023) , addresses the nutritional, functional, and technological potential of *Opuntia* species, with emphasis on their suitability for food-related applications. The second paper, *Bio-Based Sensors for Smart Food Packaging—Current Applications and Future Trends* (C. Rodrigues et al., 2021), discusses the development and application of bio-based sensing systems in smart food packaging. Together, these papers synthesize current knowledge, identify research gaps, and provide the contextual foundation of this PhD project while establishing the framework for the experimental chapters that follow.

2.1 *Opuntia* spp.: An overview of the bioactive profile and food applications of this versatile crop adapted to arid lands

2.1.1 Introduction

Prickly pear is a xerophytic plant belonging to the *Cactaceae* family (Ahmed et al., 2021). This family comprises about 2000 species belonging to 130 genera (Abouseadaa et al., 2020).

Opuntia is amongst the most important and widely distributed genera of the family *Cactaceae* (Agüero et al., 2018). It is a native plant of arid and semi-arid lands owing to its particular adaptation mechanism to hostile climatic conditions (Edvan et al., 2020). It is originally from tropical and subtropical regions of America (Khodaeiaminjan et al., 2021). The species were recognized in Europe as early as the 15th century (Anderson, 2001; Arba et al., 2017). Afterwards, the cactus was introduced in North Africa, essentially in Morocco, Tunisia and Algeria, around the 16th century (Khatabi et al., 2016). It has been cultivated later in South Africa, Madagascar, India, Australia, Canada, Brazil and Argentina (Arba et al., 2017). Yet, the cultivation of the cactus it is not limited to those areas, being currently present in more than 30 countries due to its importance and multiple applications (Arba et al., 2017), which are reflected in its environmental, nutritional, and economic benefits (de Sá Souza et al., 2022; Dubeux et al., 2021; Stavi, 2022).

From an environmental point of view, cactus cladodes provide effective means to fight against desertification, erosion and soil-related problems (M. A. Iqbal et al., 2020; Rocha Filho et al., 2021). They have an exceptional capacity to adapt to drought, thanks to their ability to preserve water in the parenchyma through their specialized photosystem called Crassulacean Acid Metabolism (CAM) (Elbana et al., 2020; Khodaeiaminjan et al., 2021; Lahbouki et al., 2022; Scalisi et al., 2016).

In addition to their environmental importance, this valuable plant is also of great nutritional interest. The cactus fruit, is considered a nutritionally complete food, which can also be described as a nutraceutical food (Piga, 2004; Yahia & Mondragon-Jacobo, 2011). It's known for its very high nutritional value in vitamins, mainly ascorbic acid, and minerals, such as magnesium (Mg), calcium (Ca), and potassium (K), as well as being also rich in antioxidants like phenolic compounds and flavonoids (Aruwa et al., 2019; Bakar et al., 2020; Barba et al., 2020). Typically, the cactus fruit is consumed fresh, yet it can be variously formulated into jams, drinks, tea, tinctures and dietary supplements (C. A. Gouws et al., 2019, 2022; Lamia et al., 2018). The cladodes have multiple applications as food and feed, most commonly consumed as nutritious fresh vegetables, processed into juice or bread from their flour (Barba et al., 2020; Hernández-Becerra et al., 2022).

Several studies showed that the cactus could be used as a good source of bioactive compounds, due to their richness in secondary metabolites, especially phenolic compounds (Hernández-Becerra et al., 2022; Lahbouki et al., 2021). Among the reported compounds, eucomic acid, kaempferol 3-O-robinobioside-7-O-arabinofuranoside, isorhamnetin 3-O-

galactoside, and isorhamnetin 3-*O*-rhamnoside-7-*O*-(rhamnosyl-hexoside), are those with high antioxidant activities (Astello-García et al., 2015). In addition, different health benefits have been associated with cactus consumption and richness of bioactive compounds, e.g. diabetes prevention (C. A. Gouws et al., 2022; Pandit et al., 2020), hypercholesterolemia (Ennouri et al., 2006; C. Gouws et al., 2020), obesity (Sirotkin, 2022) and hypertension (Pandit et al., 2020).

Opuntia spp. plants can be used in full, as all vegetative parts are edible (cladodes, fruits, seeds, and flowers). Therefore, this paper represents a bibliographic review of the bioactive composition of prickly pear plants (cladodes, fruits/seeds, flowers, and roots) (Figure 2.1).

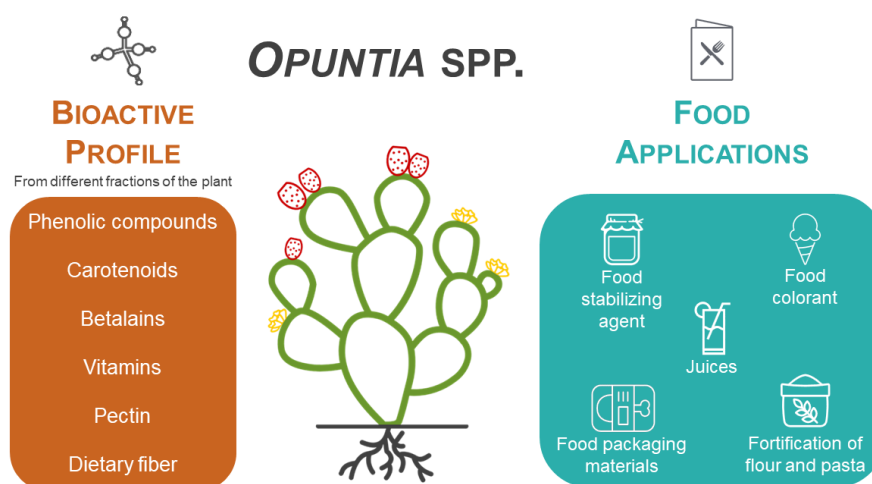


Figure 2.1 - *Opuntia* spp. bioactive profile and food applications.

As knowledge on the extraction procedures of the bioactive compounds of *Opuntia* plant and fractions is not commonly reviewed, highlights and main findings, from multiple studies, was also a goal of this work. As this plant is gaining more attention, due to its adaptability to marginal soils and arid conditions, which is increasing the area of cultivation, and the yearly production, postharvest treatments and different processing options are being also implemented to increase its shelf life and maintain its quality. Hence, recent and innovative food applications of this valuable crop are also addressed in this analysis, with a focus on the full use of this crop, that is, by including examples of application of by-products, following a circular bioeconomy approach.

2.1.2 Prickly pear plant as a source of bioactive macromolecules

2.1.2.1 Cladodes

Cladodes from *Opuntia* spp. are mainly used as food, i.e. in Mexico the younger cladodes, called nopalitos, are consumed as fresh vegetables in several dishes or transformed into several food products, forage and used as ornaments (Dubeux et al., 2021). Moreover, they find applications in building construction, cosmetics, medicine and wastewater treatment (Contreras-Padilla et al., 2016; Nharingo & Moyo, 2016).

Cladodes have a high nutritional value due to their minerals, dietary fiber and phytochemical content (del Socorro Santos-Díaz et al., 2017). The chemical composition of cladodes varies depending on the type of cultivar, species, maturity stage, environmental conditions, harvesting and post-harvesting conditions and treatments (Astello-García et al., 2015). Cladodes are mainly composed of water (80-95 %), carbohydrates (3-7 %) and, fiber (1-2 %), with low contents of protein and lipids (Astello-García et al., 2015; del Socorro Santos-Díaz et al., 2017; Ginestra et al., 2009; Stintzing & Carle, 2005). Also, the pads are a great source of minerals with a major presence of calcium and potassium and minor quantities of magnesium, manganese, iron, zinc and copper (Silva et al., 2021). The dietary fiber content in cladodes is composed of cellulose, hemicellulose, pectin, lignin and mucilage (Hernández-Urbiola et al., 2011). The ingestion of cladodes due to their dietary fiber content may help in reducing body weight, playing an important role in the excretion of lipids (Aragona et al., 2018; Uebelhack et al., 2014). Vitamins are also present in cladodes mainly in the form of ascorbic acid and carotenoids (Feugang et al., 2006; L. P. Méndez et al., 2015).

Cladodes are an important source of bioactive compounds such as phenolic compounds (phenolic acids and flavonoids) and carotenoids (Ciriminna et al., 2019; El-Mostafa et al., 2014). The most common phenolic compounds found are kaempferol, quercetin, isorhamnetin and isorhamnetin glucosides (del Socorro Santos-Díaz et al., 2017).

Several studies have been performed using different extraction procedures to assess the phytochemical profile of cladodes; the most recent research is summarized in Table 2.1.

Table 2.1 - Bioactive composition and extraction procedures of *Opuntia* spp. cladodes.

Species/Variety	Extraction Procedure	Main Compounds Found	Conclusions	Ref
<i>Opuntia ficus-indica</i> f. <i>inermis</i>	Cladodes were washed, cut, dried, ground and sieved. Maceration was performed with ethanol. The solvent was evaporated under vacuum. The extract was dissolved in methanol and properly kept in Liquid Cromatography-mass spectrometry (LC-MS) vials until further analysis.	Quercetin	The analysis of cladodes extracts allowed the identification of 13 bio-active compounds, several of them with anti-oxidant potential.	(Msaddak et al., 2017)
		Quercetin 3- <i>O</i> -glucoside		
		Kaempferol		
		Kaempferol 3- <i>O</i> -glucoside		
		Kaempferol 3- <i>O</i> -rutinoside		
		Isorhamnetin		
		Isorhamnetin 3- <i>O</i> -glucoside		
		Isorhamnetin 3- <i>O</i> -glucoside		
		Isorhamnetin 3- <i>O</i> -neohesperidoside		
		3,3',4',5,7-Pentahydroxy-flavanone		
<i>Opuntia dillenii</i>	Aqueous extract: Extraction with water (24h, room temperature). Macerates were filtered, lyophilized, and kept at -20°C.	p-Coumaric acid	Both aqueous and ethanolic cladode extracts presented a good	(Ben Lataief et al., 2020)
		Zataroside-A		
		Indicaxanthin		
		β-Sitosterol		
		Quinic acid		
		Protocatechuic acid		
		Caffeic acid		

Species/Variety	Extraction Procedure	Main Compounds Found	Conclusions	Ref
		Syringic acid P-coumaric acid Naringin Trans ferulic acid Cinnamic acid	polyphenol profile, with the ethanolic cladode extract presenting highest variability of these bioactive compounds.	
		Quinic acid Gallic acid Protocatechuic acid Rutin Hyperoside P-coumaric acid Naringin Quercetrin 1,3-di-O-caffeoylquinic acid Apeginin-7-O-glucoside Trans ferulic acid Salviolonic acid Quercetin Kampherol Naringenin Apeginin	The aqueous cladode extract was poor in flavonoid compounds.	
	Ethanolic extract: Extraction with ethanol (24h, room temperature). The macerates were filtered and evaporated in a vacuum rotary evaporator and kept under -20°C.			

Species/Variety	Extraction Procedure	Main Compounds Found	Conclusions	Ref
		Luteolin Cirsiliol Cirsilineol Acacetin		
<i>Opuntia ficus-indica</i> (L.) Mill	Cladode powder was extracted in an ultrasonic bath with a mixture of methanol/water 80:20 v/v. Then it was centrifuged, diluted and filtered.	Piscidic acid I Piscidic acid II 2 Ferulic acid derivative I Eucomic acid Ferulic acid derivative II Kaempferol derivative I Isorhamnetin derivative I Isorhamnetin derivative II Kaempferol derivative II Isorhamnetin derivative III Isorhamnetin 3-O rutinoside (a narcissin)	The analysis of cladode extracts through HPLC-DAD allowed the identification of 11 classes of bioactive compounds. The authors highlighted the presence of Piscidic acid (a polyphenol) and isorhamnetin (a flavonoid).	(Missaoui et al., 2020)
<i>Opuntia ficus-indica</i> (L.) Mill	Extraction was performed in an Ultra-turax with a mixture of methanol/water 80:20 v/v and 0.1% formic acid. The resulting extracts were centrifuged, filtered, and finally collected until analysis.	Cyanidin-Glu Pelargonidin-Glu Petunidin-Glu Delphinidin-Glu Malvidin-Glu	The analysis of cladode extracts through UHPLC-ESI-QTOF-MS allowed the identification of the principal	(Rocchetti et al., 2018)

Species/Variety	Extraction Procedure	Main Compounds Found	Conclusions	Ref
		Luteolin-Glu Apigenin-Glu Isoflavonoids Myricetin-Glu Quercetin-Glu Kaempferol-Glu Isorhamnetin-Glu Furofurans Dibenzylbutyrolactone Alkylphenols Hydroxybenzaldehydes Hydroxycoumarins Tyrosols Hydroxybenzoics Hydroxyphenylpropanoics Hydroxycinnamics	phenolic classes and subclasses. The authors highlighted that cladodes are a good source of anthocyanins and phenolic acids, thus a good source of antioxi- dant compounds.	
<i>Opuntia ficus-indica</i> (L.) Mill	Cladode powder samples were mixed with enzymes Rapidase or Viscozyme in a mixture of ethanol /water (90: 10). The vessel was subjected to pressure with CO ₂ and	3-O-methyl quercetin 3-O-methyl kaempferol Luteolin Isorhamnetin tri- and di- glycosides:	The use of enzymes to assist the extraction with supercritical fluids improved the extraction	(Antunes-Ricardo et al., 2020)

Species/Variety	Extraction Procedure	Main Compounds Found	Conclusions	Ref
	then a supercritical fluid extraction was performed.	Isorhamnetin-3- <i>O</i> -glucosyl-rhamnosyl-rhamnoside Isorhamnetin-3- <i>O</i> -glucosyl-rhamnosyl-pentoside Isorhamnetin-3- <i>O</i> -glucosyl-rhamnosyl-methylpentoside Isorhamnetin-3- <i>O</i> -glucosyl-pentoside Isorhamnetin-3- <i>O</i> -glucosyl-rhamnoside	of isorhamnetin conjugates.	
<i>Opuntia ficus-indica</i> (L.) Mill	Cladodes powder was subjected to sequential extractions with different solvents. The resulting supernatants were mixed, and acid hydrolysis was performed under reflux using different concentrations of HCl and time.	Isorhamnetin Kaempferol Quercetin Total flavonoids Ferulic acid 4-Hydroxybenzoic acid	Acid hydrolysis was demonstrated to be advantageous for identifying and quantifying (poly)phenolic compounds through High Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD).	(De Santiago et al., 2021)

Species/Variety	Extraction Procedure	Main Compounds Found	Conclusions	Ref
			The highest amount of total (poly)phenolic compounds was obtained from acid hydrolysis under the following conditions: 2 h, 90°C, 1.5 M HCl.	

In *Opuntia ficus-indica*, it was possible to identify several bioactive compounds such as kaempferol, quercetin, and isorhamnetin glucosides (Antunes-Ricardo et al., 2020; De Santiago et al., 2021; Missaoui et al., 2020; Rocchetti et al., 2018). Ben Lataief and collaborators performed both aqueous and ethanolic extractions on *Opuntia dillenii* cladodes to evaluate the differences in the composition of the compounds (Ben Lataief et al., 2020). Ethanolic extracts allowed the detection of more phenolic and volatile compounds than the aqueous extract. The solvent choice displays an influence on the extraction efficiency and has impact on the properties and composition of the resulting extracts (Ammar, Ennouri, & Attia, 2015). Another important factor for bioactive compound extraction from cladodes is the high content in dietary fiber, that can retain those compounds and is where most of them are linked, so an appropriated solvent choice and extraction procedure is recommended (Hernández-Urbiola et al., 2011).

Along with the solvent extraction process for bioactive compounds extraction, new techniques are emerging, as is the case of enzyme-assisted extraction combined with supercritical carbon dioxide extraction. The use of supercritical carbon dioxide and enzymatic hydrolysis proved effective in the isolation of isorhamnetin conjugates (Antunes-Ricardo et al., 2020). The use of enzymes allowed the accessibility to the phenolic compounds through the breakage of the dietary fiber compounds that are eliminated by the action of CO₂ and co-solvent during the extraction (Antunes-Ricardo et al., 2018).

The high content of phenols, flavonoids and also ascorbic acid in cladodes is highly related to several biological effects such as antioxidant, antibacterial, antifungal and cytotoxic activities (Aruwa et al., 2018; Ben Lataief et al., 2020).

As previously mentioned cladodes have in their composition a hydrocolloid substance constituted by a complex polysaccharide of high molecular weight, named mucilage and that is produced in specialized plant cells (Contreras-Padilla et al., 2016; Dick et al., 2019). Mucilage represents about 14% of the cladode dry weight and its physiological function is to regulate the cellular water content during prolonged drought and the calcium fluxes of the plant (Ginestra et al., 2009; Sáenz et al., 2004). It is reported that cladodes in an older maturity stage have a decrease in mucilage content because, as a part of the soluble fiber, its content decreases along with the cladode maturation (Contreras-Padilla et al., 2016). The mucilage is a complex polysaccharide resulting from the polymerization of monosaccharides like arabinose, galactose, rhamnose, xylose and uronic acids (e.g. – galacturonic acid) and is present in the internal layer of cladodes (Rodríguez-González et al., 2014). The polysaccharide allows the plant to retain a large amount of water and has several functional properties like gelling, thickening

and emulsifying (Bayar et al., 2016) . The mucilage structure is composed of two different water-soluble fractions: pectin with gelling properties with Ca^{2+} and mucilage without gelling properties (Sepúlveda et al., 2007).

Pectin is a complex polysaccharide found in the cell wall of a variety of fruits and plants. It is present in the primary cells of the plants acting as an adhesive between them, enhancing the mechanical strength of the cell wall and playing an important role in plant growth (Valdés et al., 2015).

In terms of structure, pectin is a complex heterogeneous polysaccharide constituted mainly by homogalacturonan (HG), rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG II), xylogalacturonan (XG) and apiogalacturonan (AP) domains and its ratio may vary (Figure 2.2) (Hassan et al., 2018; Mao et al., 2019; Picot-Allain et al., 2022). Homogalacturonan (HG) is the most abundant form present in pectin, representing about 65% and, is comprised of a linear chain of 1,4-linked α -D-galacturonic acid monomer that can be acetylated, or methyl esterified (Picot-Allain et al., 2022). The methyl-esterification occurs at the C-6 carboxyl and the acetylation can be either at O-2 or O-3 positions (Mohnen, 2008). Rhamnogalacturonan I (RG-I) is a heteropolysaccharide that represents about 20-35% of pectin constitution, and it is constituted of repeated disaccharides of galacturonic acid residues and rhamnose (Nastasi et al., 2022). This latter can be substituted by neutral sugar side chains such as galactose and xylose. Rhamnogalacturonan II (RG-II) that takes 10% part of the pectin content and, includes an HG backbone of 1,4-linked α -D-galacturonic acid residues that can be substituted by groups of different hetero-oligomeric side chains (Cui et al., 2021; Mellinas et al., 2020). Xylogalacturonan (XG) and Apiogalacturonan (AP) can be substitutes for the galacturonic units in some parts of the pectin chain (Mohnen, 2008).

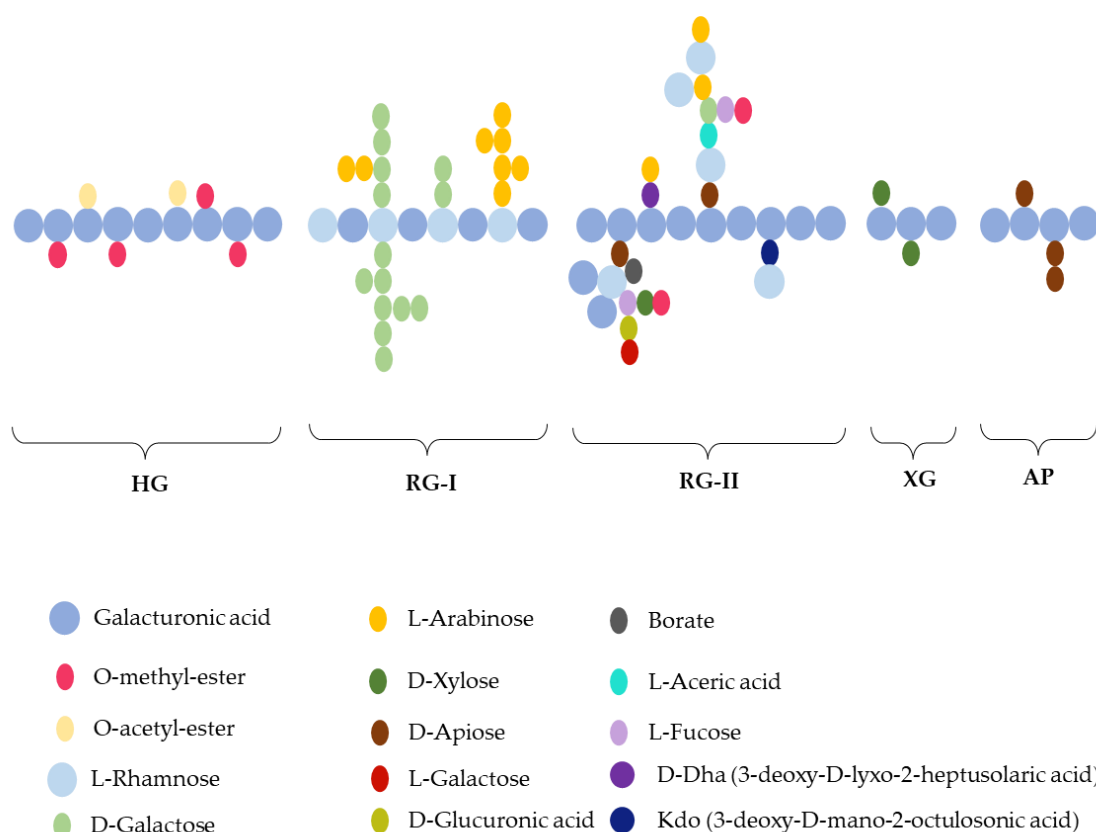


Figure 2.2 - Pectin structure, adapted from (Cui et al., 2021; Freitas et al., 2021; Picot-Allain et al., 2022) .

The degree of esterification (DE) refers to the percentage of carboxyl groups present in the main chain of galacturonic acid present in pectin esterified with methyl or acetyl groups and it is of extreme importance for its classification (Guandalini et al., 2019; Nastasi et al., 2022; Sevgi et al., 2022). The DE of the pectin is directly related to its source and has a fundamental influence on its future application (Sevgi et al., 2022). Pectin can be classified as a high-methoxy (HM) degree of esterification ($DE > 50\%$) or a low methoxy (LM) degree of esterification ($DE \leq 50\%$). The HM pectins have the ability to form gels in solutions with high sugar content (55-75%) and $pH < 3.5$ (Freitas et al., 2021; Gavahian et al., 2021). Under low pH, there is a reduction of the dissociation of carboxyl groups of galacturonic residues. The HM pectin is stabilized by intramolecular hydrogen bonds and hydrophobic bonds between methyl ester groups. LM pectin can form gels in pH ranging from 2-6 but need the presence of calcium to act as a crosslinking agent (Gawkowska et al., 2018). The concentration of calcium to be used depends on the carboxyl groups of the galacturonic acid residues and the pH of the medium (Freitas et al., 2021; Veisi et al., 2019). The binding between Ca^{2+} and non-esterified galacturonic acid units is the reaction that allows the gelation of LM pectins is explained by the "egg-box" and is represented in Figure 2.3 (Cardoso et al., 2003; Gawkowska et al., 2018). At a low pH, it

is not only the presence of calcium to bind pectin that influences the gelation process but also the hydrophobic interactions and the formation of hydrogen bonds (Cardoso et al., 2003). Also, at strong acidic solutions (pH=1) it is also stated that Ca^{2+} does not bound with pectin because of the presence of protonated carboxyl groups which prevents electrostatic interactions. It is also reported that a reduction in the DM leads to an increase in the gelation ability of LM pectins (Gawkowska et al., 2018).

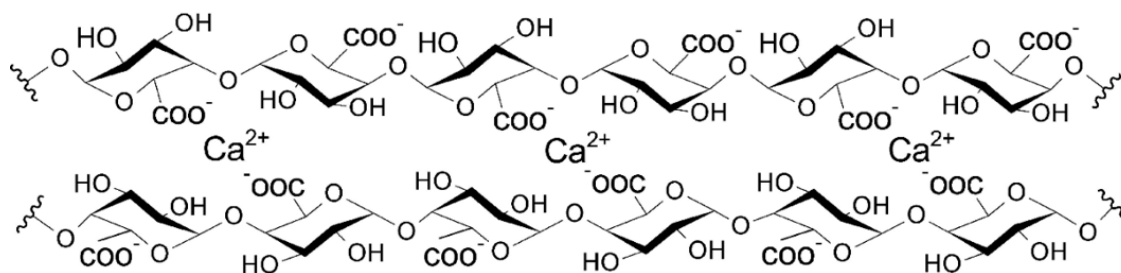


Figure 2.3 - Suggested binding mechanism between Ca^{2+} and non-esterified groups (Gawkowska et al., 2018).

In the food industry, HM pectin is usually used for the production of jam and jelly whereas LM pectin is used in the preparation of juice and dairy products, or products with low sugar content (dietary products) (Gavahian et al., 2021). HM pectin can be converted into LM pectin through the use of the enzyme pectin methylesterase or by chemical de-esterification by alkali treatment (E. H. Cho et al., 2019).

Pectin present in the cladodes is categorized as low-methoxy pectin and has a large application in the food industry due to its gelling and stabilizing properties (Aragona et al., 2018; del Socorro Santos-Díaz et al., 2017; Lefsih et al., 2016). It can be used in different areas due to its thickening, emulsifying and gelling properties and is considered generally recognized as safe (GRAS) (Espitia, Du, et al., 2013; Valdés et al., 2015).

The principal sources of commercial pectin are apple pomace, citrus peel and beetroot (Cazón et al., 2017a). The pectin extracted from these sources is generally derived from the by-products of the juice industries (Picot-Allain et al., 2022). Recently other sources such as tropical and sub-tropical fruits have been reported as good sources of pectin, such as mango peels (Rojas et al., 2020), jackfruit peels (Moorthy et al., 2017), passion fruit peels (C. F. De Oliveira et al., 2015), pomegranate peels (P. H. F. Pereira et al., 2016) and papaya peels (Koubala et al., 2014) to mention a few.

Despite the nutritional value of these bio-macromolecules, they are of good interest to the food industry due to their versatility, gelling and film-forming abilities. Thus, the extraction of mucilage and pectin is comprised of these general steps: removing outer layers of cladodes

to eliminate the spines and the peel; washing and cutting; mixing with a solvent; pressing/centrifugation; precipitation; and drying (C. Rodrigues et al., 2019). Table 2.2 summarizes the most recent research on the extraction of mucilage and pectin from cladodes.

Table 2.2 - Mucilage and pectin extraction procedures, yields and compositions from cladodes of *Opuntia* spp.

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
<i>Opuntia ficus-indica</i>	Cladode powder was subjected to a sequential extraction to obtain water-soluble pectins (WSP), chelating-soluble pectins (CSP) and acid-soluble pectins (ASP).	WSP: 5.75 % (w/w) of dry weight CSP: 0.21 % (w/w) of dry weight ASP: 0.11 % (w/w) of dry weight	Monosaccharide composition: Galactose Glucose Galacturonic acid Arabinose Xylose Rhamnose Mannose	All the pectin fractions obtained are mostly rich in galacturonic acid. Through the degree of methylation analysis, it was shown that all pectin fractions are low methylated (DM <50 %).	(Lefsihi et al., 2016)
<i>Opuntia ficus-indica</i>	Mucilage extraction: Cladodes were washed and cut, and the interior was removed and pressed. Mucilage was precipitated in ethanol in a ratio of 2:3 (extract: ethanol).	Not presented	Monosaccharide composition: Galactose Arabinose Xylose Rhamnose Glucose	The major sugars present in cactus mucilage are galactose and arabinose.	(Gheribi et al., 2018)

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
	The precipitate was collected, washed with ethanol, dried (50 °C, 24 h), and then pulverized.				
<i>Opuntia monacantha</i>	Mucilage extraction: Cladodes were washed, cut and crushed. The resulting pulp was mixed with water 1:2 w/v (pulp: water), stirred and heated (80 °C, 30 min). The mixture was filtered and centrifuged. The supernatant was precipitated in ethanol 95 % and kept overnight at 4 °C. The mucilage was filtered under vacuum, dried, and sieved.	12.0 % (w/w) of dry weight	Monosaccharide composition: Arabinose Galactose Glucose Rhamnose Xylose Galacturonic acid Glucuronic acid	The extraction method shows promising results to obtain mucilage powder that is mainly composed of carbohydrates.	(Dick et al., 2019)
<i>Opuntia ficus-indica</i>	Total pectic mucilage fraction extraction (TFC): Extraction was performed in a water bath using water as the solvent (adjusting the pH). The mixture was filtered and centrifuged, and the supernatant was concentrated. The solution	TFC: 13.1 % (w/w) of dry weight MC: 10.2 % (w/w) of dry weight PC: 6.13 % (w/w) of dry weight	High content in carbohydrates	Extraction conditions may affect the extraction yield. PC was richer in uronic acid content than the other two fractions. Mucilage	(Bayar et al., 2016)

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
	was precipitated in isopropanol (4 °C, overnight). The resulting precipitate was centrifuged, washed with ethanol, and dried. Mucilage fraction extraction (MC): The extraction procedure was performed under agitation with water as the solvent and centrifuged. The supernatant was precipitated with isopropanol (overnight, 4°C), then washed with absolute ethanol and dried. Pectin fraction extraction (PC): obtained by the same method used for total pectic mucilage fraction, using as starting material the residue resulting from mucilage extraction.			showed a lower content in uronic acid.	

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
<i>Opuntia ficus-indica</i>	Pectin extraction: extraction from cladode's powder was made with water, centrifuged and the resulting residues were dried. The residues were mixed with water, in an ultrasound water bath and centrifuged. The supernatant was precipitated in isopropanol and then dried.	18.6 % (w/w) of dry weight	High uronic acid content	The use of ultrasounds increased the pectin extraction and reduced the extraction time when compared with chemical extraction methods.	(Bayar et al., 2017)
<i>Opuntia ficus-indica</i>	Mucilage was extracted from cladodes by performing a microwave-assisted extraction (different powers were applied). Then the samples were filtered, centrifuged, and precipitated in ethanol. The resulting precipitate was washed with ethanol and lyophilized.	8.13 % (w/w) of dry weight with 500 W for 7 min	Monosaccharide composition: Galactose Arabinose Xylose Rhamnose Glucose	Microwave irradiation promotes the interaction between the solvent and the material enhancing the extraction.	(Felkai-Haddache et al., 2016)
<i>Opuntia dillenii</i> (Ker-Gawl) Haw	Mucilage extraction: fresh cladodes were extracted with water under stirring. The solution was filtered and concentrated. The concentrated	6.20 % (w/w) of dry weight	Monosaccharide composition: Arabinose Galactose	The major neutral sugars found were arabinose and galactose. The uronic acid	(Kalegowda et al., 2017)

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
	solution was precipitated with and dialyzed against water (24 h, membrane of 12-14 kDa) and lyophilized.		Rhamnose Xylose Glucose Uronic acid	was found in a lower percentage (2.5 %), demonstrating the neutrality of the mucilage	
<i>Opuntia ficus-indica</i>	Mucilage was previously extracted from cladodes and the resulting residue was used for pectin enzyme-assisted extraction. Xylanase and cellulase were added under optimal conditions. After enzyme inactivation, the mixture was centrifuged, and the supernatant was precipitated with isopropanol. The precipitate was centrifuged, dried at 50 °C, and reduced to powder.	16.7 % (w/w) of dry weight	Arabinose Xylose Fructose Glutamic acid Galacturonic acid Glucose Galactose Mannose Rhamnose	Enzyme-assisted extraction proved effective for enhancing pectin extraction without using acid treatments. The resulting pectin was low methylated and showed a galacturonic acid content of 65 %.	(Bayar et al., 2018)
<i>Opuntia ficus-indica</i>	Pectin extraction was performed under three different extraction	Acidic (pH = 2): 8.80 % (w/w) of dry weight	Neutral sugars Galacturonic acid	Neutral extraction obtained the highest total yield although	(Chaouch et al., 2016)

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
	conditions: acidic (pH = 2), neutral (pH = 6), and basic (pH = 10). The samples were submitted to ultrasound and then placed under mechanical stirring, filtered, and centrifuged. The residues were submitted to the same extraction, and the supernatant was mixed in the end. The resulting supernatants were precipitated in ethanol and filtered. The residues were dialyzed (30 kDa membrane) against water, concentrated and lyophilized.	Neutral (pH = 6): 24.5 % (w/w) of dry weight Basic (pH = 10): 4.60 % (w/w) of dry weight		acidic extraction presented a higher galacturonic acid content.	
<i>Opuntia ficus-indica</i>	Mucilage extraction was performed by extrusion. The solution was centrifuged, and the supernatant precipitated in ethanol and dried.	1.50 % (w/w) of dry weight	n/a	The yield of extraction was demonstrated to be dependent on mucilage synthesis which depends on edaphoclimatic conditions. (Quinzio et al., 2017)	

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
<i>Opuntia spinulifera</i> Salm-Dyck	Mucilage extraction was accomplished by mixing cladodes with water under stirring and filtered at the end (process repeated four times). The filtrate obtained was precipitated with ethanol, dried, and reduced to powder.	n/a	Arabinose Rhamnose Xylose Galactose Uronic acid	Cladodes from <i>Opuntia spinulifera</i> are rich in carbohydrates. Furthermore, the presence of pectins was found through Fourier-Transform Infrared Spectroscopy (FTIR) analysis. This could be explained by the ability of pectins to also precipitate in ethanol.	(Madera-Santana et al., 2018)
<i>Opuntia ficus-indica</i>	Residue from cladode's flour was obtained by depigmentation with ethanol and acetone, and centrifugation. The extraction was performed by mixing EDTA and water, adjusting the pH, and stirring. Then, a centrifugation was performed, and the	10.4 % (w/w) of dry weight under conditions of: pH = 11; 20 % EDTA; 80 °C	Galacturonic acid Arabinose Galactose Glucose Rhamnose	Pectin extractions under alkaline conditions demonstrated a higher yield of extraction than those performed under acidic conditions. The use of	(Pérez-Martínez et al., 2013)

Species/Variety	Extraction Procedure	Yield of extraction (%)	Main Compounds Found	Conclusions	Ref
	supernatant was precipitated. The precipitate was recovered through centrifugation and resuspended in water. The precipitated was filtered and the solution was mixed with ethanol (5 °C, overnight), filtered again, and then dried.			EDTA suggests that the chelation of calcium is an important factor for extraction.	

Conventional methods are used to extract mucilage and pectin from *Opuntia cladodes*, based on the extraction with solvents to isolate those compounds that are all mixed and compose dietary fiber (Dick et al., 2019; Gheribi et al., 2018; Lefsih et al., 2016). The solvents to be used in the extraction and precipitation can be ethanol, methanol, isopropyl alcohol, acetone or a combination of solvents and can influence the extraction yield so it is important to determine the best option (Inglese et al., 2017).

Other techniques employed to improve the extraction are the use of ultrasounds, microwave irradiation and enzyme-assisted extraction (Bayar et al., 2017, 2018; Felkai-Haddache et al., 2016). It is also reported by several authors that acidic, neutral or basic environments are possible to be used in the extraction (Chaouch et al., 2016).

The use of ultrasounds allows the reduction of the particle size by the enhancement of the surface area and the mass transfer (Chemat et al., 2017). In microwave-assisted extraction, the irradiation power helps with the diffusion of the solvent into the plant matrix by dissolving the compounds aimed to be extracted (Thirugnanasambandham et al., 2015). These techniques are helpful in the extraction process by enhancing the extraction efficiency and reducing the use of solvents and the time of extraction (Felkai-Haddache et al., 2016). The use of chelating agents such as EDTA that interact with Ca^{2+} also helps with the extraction, to improves the process (Pérez-Martínez et al., 2013). Since pectins present in cladodes are categorized as low methoxyl pectins, the use of chelating agents for calcium sequestration allows the decrease of the degree of methoxylation by improving the gelling capacity of this pectin (Gawkowska et al., 2018).

Low methoxyl pectins have a large application in the food industry due to their gelling and stabilizing properties (Aragona et al., 2018; del Socorro Santos-Díaz et al., 2017; Lefsih et al., 2016). Mucilage can be used as a functional additive and have application in different industries: as a water purifying agent, as an organic adhesive to lime in construction, as an inhibitor of the corrosion of aluminum and, in the food industry as an edible coating in fruits, while also performing as a stabilizer of emulsions, foams, and suspensions (Contreras-Padilla et al., 2016; Monrroy et al., 2017). One of the novel potential applications of mucilage is to be used as a material for alternative food packaging once it can replace fossil-based plastics or reinforce polymeric matrices (Gheribi & Khwaldia, 2019).

2.1.2.2 Fruits

Prickly pears are the succulent fruits from *Opuntia* spp., characterized by their high content in water (almost 92% wt), followed by carbohydrates (4-6 % wt), proteins (1-2% wt), and minerals (around 1 %wt), of which calcium, phosphorus and sodium are highlighted (Tegegne, 2001).

Opuntia spp. fruits are also a good source of bioactive compounds, especially phenolic compounds, and vitamins (A and C) (Table 2.3). Their composition depends on several factors, such as soil, place of planting, environmental conditions, age, and species (Monrroy et al., 2017), which explains the differences observed in the data found in the literature.

Table 2.3 - Bioactive composition and extraction procedures of prickly pears and seed oil.

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia ficus-indica</i> var. Gialla	The fruits were purchased in Sicily, Italy, and Portugal. They were separated according to color; i.e., orange-red (<i>Opuntia ficus-indica</i> var. Gialla) and red-violet (<i>Opuntia ficus-indica</i> var. Sanguigna)	Hydro-alcoholic extraction (ethanol:water) was performed under agitation and followed by filtration. The resulting extracts were lyophilized and their content in total phenolic compounds, antioxidants and betalain was evaluated by using High Performance Liquid Chromatography (HPLC).	Piscidic acid:1,061 mg/g fruit		
			Eucomic acid:1.40 mg/g fruit		
			Isorhamnetin- O - (di-deoxyhexosylhexoside):0.22 mg/g fruit		
			Isorhamnetin- O - (deoxyhexosyl-pentosylhexoside):0.77 mg/g fruit		
<i>Opuntia ficus-indica</i> var. Sanguigna			Isorhamnetin- O - (pentosylhexoside):0.10 mg/g fruit	<i>Opuntia ficus-indica</i> var. Sanguigna has more phenolic compounds and betacyanin compounds than	(Melgar et al., 2017)
			Total phenolic compounds:3.26 mg/g fruit		
			Betaxanthines: Indicaxanthin I isomer-25.3 mg/g fruit		
			Indicaxanthin II isomer-64.5 mg/g fruit		
<i>Opuntia ficus-indica</i> var. Sanguigna			Betacyanins: Betanidin-5-O- β - glucoside (betanine)-1.25 mg/g fruit	<i>Opuntia ficus-indica</i> var. Gialla	
			Total: 1.25 mg betacyanin compounds/g fruit		
			Eucomic acid: 2.2 mg/g fruit		
			Isorhamnetin- O - (di-deoxyhexosylhexoside): 0.56 mg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
			Isorhamnetin- O - (di-deoxyhexosylhexo- side): 0.21 mg/g fruit Isorhamnetin- O - (deoxyhexosyl-pen- tosylhexoside): 1,3 mg/g fruit Isorhamnetin- O - (pentosylhexoside): 0.67 mg/g fruit Isorhamnetin- O - (deoxyhexosylhexoside): 1,7 mg/g fruit Total phenolic compounds: 3.7 mg/g fruit Indicaxanthin I isomer: 56.0 mg/g fruit Indicaxanthin II isomer: 2.8 mg/g fruit Betanidin-5-O - β -glucoside (betanine): 3.44 mg/g fruit Isobetanine: 0.54 mg/g fruit Total beta-cyanine compounds: 3.97 mg/g fruit		
<i>Opuntia ficus-in- dica</i> (green peel)	Fruits were har- vested at Texas A&M University, Kingsville or- chard.	Flavonoid extraction: skin and pulp were ho- mogenized with 25 mL of methanol. Acid hydroly- sis was performed with	Flavonol content Kaempferol: 2.2 μ g/g fruit Quercetin: 43.2 μ g/g fruit Isorhamnetin: 24.1 μ g/g fruit	<i>Opuntia stricta</i> var. <i>stricta</i> has presented more Kaempferol; <i>Opuntia streptacantha</i> has more Quercetin;	(Kuti, 2004)

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia streptacantha</i> (red peel)	Following that, the separation of the peels and pulp, the material was weighed and stored at -80 °C.	the flavonoids aglycones. Quantification was performed by using HPLC.	Ascorbic acid: 458 µg/g fruit Total carotenoids: 2.9 µg/g fruit Total flavonoids: 69.5 µg/g fruit	and the <i>Opuntia ficus-indica</i> has more Iso-rhamnetin. In addition to that, <i>Opuntia stricta</i> var. <i>stricta</i> has more ascorbic acid; <i>Opuntia lindheimeri</i> has more carotenoids; and <i>Opuntia streptacantha</i> has more flavonoids.	
		Ascorbic acid extraction: frozen tissues were sprayed with a dismembrator, centrifuged and the supernatant was filtered. Quantification was performed by using HPLC.	Flavonol content Kaempferol: 1.1 µg/g fruit Quercetin: 90.5 µg/g fruit Isorhamnetin: 1.9 µg/g fruit Ascorbic acid: 121 µg/g fruit Total carotenoids: 6.7 µg/g fruit Total flavonoids: 93.5 µg/g fruit		
<i>Opuntia stricta</i> var. <i>stricta</i> (yellow peel)		Carotenoids extraction: skin and pulp was mixed with a mixture of hexane/acetone/methanol, followed by centrifugation, and filtration. Total carotenoids determination was performed by using a UV-Vis spectrophotometer.	Flavonol content Kaempferol: 3.8 µg/g fruit Quercetin: 51.0 µg/g fruit Isorhamnetin: nd Ascorbic acid: 815 µg/g fruit Total carotenoids: 14.6 µg/g fruit Total flavonoids: 54.8 µg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia lindheimeri</i> (purple peel)			Flavonol content		
			Kaempferol: nd		
			Quercetin: 9.8 µg/g fruit		
			Isorhamnetin: nd		
			Ascorbic acid: 437 µg/g fruit		
			Total carotenoids: 23.7 µg/g fruit		
			Total flavonoids: 9.8 µg/g fruit		
<i>Opuntia ficus-indica</i> (Mexico, purple peel)	The fruits used in this study were obtained in Mexico and Spain. They were chosen based on their similar characteristics in terms of ripeness, uniformity, and quality of the material.	Extraction was performed in freeze-dried fruits using methanol and water. The mixture passed through an ultrasonic bath and was centrifuged. After removing the supernatant, the process was repeated twice. The supernatant was analyzed by HPLC.	Whole fruit	The whole fruits of the purple-colored species from Mexico and Spain were the richest in betacyanin and those of red coloration from Spain and yellow from Mexico have a higher content of betaxanthin. Regarding the peel, a large amount of	(García-Cayuela et al., 2019)
			Betaxanthins: 246.2 µg/g fruit		
			Betacyanins: 2176 µg/g fruit		
			Betalains: 2423 µg/g fruit		
			Phenolic acids: 27556 µg/g fruit		
			Flavonoids: 643.4 µg/g fruit		
			Phenolics: 28199 µg/g fruit		
			Pulp		
			Betaxanthins: 206.8 µg/g fruit		
			Betacyanins: 2070 µg/g fruit		
			Betalains: 2274 µg/g fruit		
			Phenolic acids: 18498 µg/g fruit		
			Flavonoids: 319.6 µg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia ficus-indica</i> (Mexico, red peel)			Phenolics: 18818 µg/g fruit	phenolic compounds were observed, mainly in the purple variety from Spain.	
			Peel		
			Betaxanthins: 69.1 µg/g fruit		
			Betacyanins: 630 µg/g fruit		
			Betalains: 699 µg/g fruit		
			Phenolic acids: 45587 µg/g fruit		
			Flavonoids: 3426 µg/g fruit		
			Phenolics: 49012 µg/g fruit		
			Whole fruit		
			Betaxanthins: 177.9 µg/g fruit		
			Betacyanins: 404.7 µg/g fruit		
			Betalains: 582.6 µg/g fruit		
			Phenolic acids: 23169 µg/g fruit		
			Flavonoids: 798 µg/g fruit		
			Phenolics: 23967 µg/g fruit		
			Pulp		
			Betaxanthins: 246.8 µg/g fruit		
			Betacyanins: 525 µg/g fruit		
			Betalains: 771.8 µg/g fruit		
			Phenolic acids: 13030 µg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia ficus-indica</i> (Mexico, yellow peel)			Flavonoids: 171.7 µg/g fruit		
			Phenolics: 13202 µg/g fruit		
			Peel		
			Betaxanthins: 118.3 µg/g fruit		
			Betacyanins: 389.4 µg/g fruit		
			Betalains: 507.6 µg/g fruit		
			Phenolic acids: 42106 µg/g fruit		
			Flavonoids: 2564 µg/g fruit		
			Phenolics: 44669 µg/g fruit		
			Whole fruit		
			Betaxanthins: 487.9 µg/g fruit		
			Betacyanins: 100.1 µg/g fruit		
			Betalains: 587.9 µg/g fruit		
			Phenolic acids: 19990 µg/g fruit		
			Flavonoids: 1073 µg/g fruit		
			Phenolics: 21063 µg/g fruit		
			Pulp		
			Betaxanthins: 390.8 µg/g fruit		
			Betacyanins: 89.2 µg/g fruit		
			Betalains: 479.9 µg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia ficus-indica</i> (Spain, purple peel)			Phenolic acids: 8902 µg/g fruit		
			Flavonoids: 139.5 µg/g fruit		
			Phenolics: 9042 µg/g fruit		
			Peel		
			Betaxanthins: 252.3 µg/g fruit		
			Betacyanins: 96.1 µg/g fruit		
			Betalains: 384.4 µg/g fruit		
			Phenolic acids: 28074 µg/g fruit		
			Flavonoids: 3218 µg/g fruit		
			Phenolics: 31292 µg/g fruit		
			Whole fruit		
			Betaxanthins: 278.7 µg/g fruit		
			Betacyanins: 1372 µg/g fruit		
			Betalains: 1651 µg/g fruit		
			Phenolic acids: 27252 µg/g fruit		
			Flavonoids: 615.3 µg/g fruit		
			Phenolics: 27867 µg/g fruit		
			Pulp		
			Betaxanthins: 209.8 µg/g fruit		
			Betacyanins: 1084 µg/g fruit		
			Betalains: 1293 µg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia ficus-indica</i> (Spain, red peel)			Phenolic acids: 23131 µg/g fruit		
			Flavonoids: 204.0 µg/g fruit		
			Phenolics: 23335 µg/g fruit		
			Peel		
			Betaxanthins: 175.7 µg/g fruit		
			Betacyanins: 1022 µg/g fruit		
			Betalains: 1197 µg/g fruit		
			Phenolic acids: 35163 µg/g fruit		
			Flavonoids: 3268 µg/g fruit		
			Phenolics: 38430 µg/g fruit		
			Whole fruit		
			Betaxanthins: 435.4 µg/g fruit		
			Betacyanins: 384.4 µg/g fruit		
			Betalains: 819.8 µg/g fruit		
			Phenolic acids: 17022 µg/g fruit		
			Flavonoids: 550.9 µg/g fruit		
			Phenolics: 17573 µg/g fruit		
			Pulp		
			Betaxanthins: 405.1 µg/g fruit		
			Betacyanins: 343.0 µg/g fruit		
			Betalains: 1293 µg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia ficus-indica</i> (Spain, yellow peel)			Phenolic acids: 14340 µg/g fruit		
			Flavonoids: 161.5 µg/g fruit		
			Phenolics: 14502 µg/g fruit		
			Peel		
			Betaxanthins: 210.3 µg/g fruit		
			Betacyanins: 379.3 µg/g fruit		
			Betalains: 589.6 µg/g fruit		
			Phenolic acids: 36183 µg/g fruit		
			Flavonoids: 3571 µg/g fruit		
			Phenolics: 39754 µg/g fruit		
			Whole fruit		
			Betaxanthins: 134.8 µg/g fruit		
			Betacyanins: 21.4 µg/g fruit		
			Betalains: 156.2 µg/g fruit		
			Phenolic acids: 18165 µg/g fruit		
			Flavonoids: 554.9 µg/g fruit		
			Phenolics: 18720 µg/g fruit		
			Pulp		
			Betaxanthins: 214.2 µg/g fruit		
			Betacyanins: 26.2 µg/g fruit		
			Betalains: 240.5 µg/g fruit		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
			Phenolic acids: 10437 µg/g fruit Flavonoids: 117.2 µg/g fruit Phenolics: 10554 µg/g fruit Peel Betaxanthins: 98.0 µg/g fruit Betacyanins: 27.8 µg/g fruit Betalains: 125.7 µg/g fruit Phenolic acids: 38820 µg/g fruit Flavonoids: 2860 µg/g fruit Phenolics: 41680 µg/g fruit		
<i>Opuntia ficus-indica</i> (green peel)	Fruits were harvested in Bejaia, Argelia, in August, 2008. They were selected based on spines, colors and shapes. This study compared the seed oil composition extracted from	Seed oil was obtained by Soxhlet extraction, using hexane. For the extraction of phenolic compounds from the oil, a solid-liquid extraction method was applied using ethanol. The content in phenolic compounds was assessed by the Velioglu method and	Total phenolic compounds: 61 mg GAE*/100 g seed Flavonoids: 1.5 mg/100 g seed Tannins: 4.5 mg/100 g seed	The orange variety has more phenolic compounds. The study concludes that the seeds from <i>Opuntia ficus-indica</i> have a good number of phenolic compounds that can be used by the industry.	(Chougui et al., 2013)
<i>Opuntia ficus-indica</i> (yellow peel)			Total phenolic compounds: 74 mg GAE*/100 g seed Flavonoids: 1.9 mg/100 g seed Tannins: 4.8 mg/100 g seed		
<i>Opuntia ficus-indica</i> orange peel)			Total phenolic compounds: 89 mg GAE*/100 g seed Flavonoids: 2.6 mg/100 g seed		

Species/ Variety	Provenance	Extraction Procedure	Main Bioactive Compounds	Main Conclusions	Ref
<i>Opuntia ficus-in-dica</i> (red peel)	<i>Opuntia ficus-in-dica</i> with different peel color (green, yellow, orange, and red).	also analyzed by High Performance Liquid Chromatography -Mass Spectrometry (HPLC-MS).	Tannins: 6.6 mg/100 g seed		
			Total phenolic compounds: 48 mg GAE*/100 g seed		
<i>Opuntia ficus-in-dica</i> (red peel)			Flavonoids: 1.5 mg/100 g seed		
			Tannins: 4.1 mg/100 g seed		

*GAE (Gallic Acid Equivalent).

Regarding ascorbic acid (vitamin C), *Opuntia* spp. is rich in this compound due to the light intensity of the planting site. It may also be related to less irrigation and lower temperatures (Chiteva & Wairagu, 2013). Regarding the mineral content of *Opuntia ficus-indica*, the values obtained were, considering mg/ 100g, 63.4 Mg; 18.7 Na; 108.8 K; 316.5 Ca; 37.8 Mn; 25.9 Fe; 12.6 Zn; 0.01 Cu; and 0.05 P (Chiteva & Wairagu, 2013). Thus, it is noticeable that fruits have a significant amount of minerals, in addition to vitamin C, making them advantageous for use as a food supplement (Chiteva & Wairagu, 2013). Considering the pulp of the prickly pears, they are rich in biologically active compounds, such as vitamins, polyphenols, carotenoids, and betalains, among others, that can be extracted and used by the pharmaceutical and food industries (Kuti, 2004). It was found that the red-skinned fruit had a total content of phenolic compounds between 164.6 and 218.8 mg per 100 g. There is a large amount of quercetin, isorhamnetin, luteolin, and kaempferol, and it has a relevant content of flavonoids with higher concentrations than edible parts of papaya, banana, and watermelon, for example. Numerous polyphenolic acids such as ferulic acid, p-coumaric acid, 4-hydroxybenzoic acid, caffeic acid, salicylic acid, and gallic acid have also been identified (Barba et al., 2017). Peels are also rich in phytochemicals and have a high potential to serve as functional compounds, e.g., in active food packaging films, as it was observed with cranberry extracts (Severo et al., 2021), seaweed extracts (Andrade et al., 2021), different essential oils (V. G. L. Souza, Rodrigues, Ferreira, Pires, et al., 2019), and plant extracts (V. G. L. Souza, Rodrigues, et al., 2018a). The main compounds in the peels are cellulose, hemicellulose, pectin, proteins, antioxidants, flavonoids, minerals and other polysaccharides (Barba et al., 2017).

Moreover, their pH, taste, and color are other interesting characteristics that arouse interest for this fruit to be used as food, in addition to the absence of lead and cadmium, which brings greater safety for their consumption (Chiteva & Wairagu, 2013).

Yet, the amount of by-products reaches around 30% of the total weight after processing the fruits, making it feasible to look for ways to use these by-products in a circular bio-economy approach (Melgar et al., 2017).

According to the study by Elsy De Santiago et al. (2018) (De Santiago et al., 2018), cacti have a significant amount of fiber, such as pectin, lignin, mucilage, cellulose, and hemicellulose, which help in the metabolism of glucose and lipids (De Santiago et al., 2018).

In addition to the antioxidant properties, other actions are also attributed to the phenolic compounds, namely, anti-inflammatory, anti-diabetes, and anti-cancer (Melgar et al., 2017). In the composition of these fruits, betalain pigments are also present, which present a

red-violet (betacyanins) and yellow-orange (betaxanthins) color. This pigmentation is stable at pH between 3 and 7, making it possible to use it as a natural color in food and as nutraceuticals, for example (Melgar et al., 2017). In the study by Tomás García-Cayuela et al. (García-Cayuela et al., 2019), the composition of betalains and phenolic compounds of the peels, pulps and whole fruit was analyzed and quantified through the evaluation of three varieties from Spain and three from Mexico. The study made a complete comparison of the amounts of these compounds in *Opuntia ficus-indica*. In addition, betalains are natural pigments, with active properties (antioxidant, antimicrobial), sensitivity to pH, and other interesting features that could be applied as a bio-based sensor for smart packaging systems. As these compounds are more pH-stable than anthocyanins, their use in smart packaging constitutes a promising alternative (C. Rodrigues et al., 2021b). Tests made with the incorporation of amaranth leaf extracts, rich in betalains, in bio-based polymers, support this application. In the mentioned study, following the degradation of poultry meat and fish, total volatile basic nitrogen content increased, and pH was altered, modifying betalain chemical structure, which changed the films color, from red to yellow (Kanatt, 2020).

The seeds have a high number of compounds beneficial to health, such as unsaturated fatty acids, phytosterols, fat-soluble vitamins (vitamin E), and β -carotene, among others with antioxidant values. They can be used in the food and cosmetics industry, and also for the prevention of chronic diseases (Barba et al., 2017).

The extraction of oil from seeds is traditionally done using the solid-liquid extraction method with organic solvents such as hexane, chloroform, and petroleum ethers. With this procedure, about 13% of the seed oil can be extracted, depending on the species and the extraction conditions used. This extracted oil is rich in unsaturated acids such as oleic, vaccenic and linolenic acids. They also have a significant number of tocopherols and phenolic compounds that have antioxidant activity. The total phenolic acid contents in the seed ethanol extract range from 48 to 89 mg in 100 g; and the contents of total flavonoids vary from 1.55 to 2.64 mg in 100g; the total tannin contents vary between 4.1 and 6.6 mg in 100 g (Barba et al., 2017).

2.1.2.3 *Opuntia* flowers and roots

The flowers of *Opuntia* spp. are considered a vegetable, and the fruits and the young cladodes (or nopalitos) can be eaten as such (Sáenz et al., 2013). Moreover, flowers from different plants are known to have wide medicinal properties, being recognized for their

antioxidant properties; however, few data are reported in the literature regarding the phytochemicals and antioxidant properties of *Opuntia* spp. flowers (Ammar, Ennouri, & Attia, 2015). Traditionally, the flower from these *Cactaceae* are used for medical purposes; in Tunisia markets, dried flowers of prickly pear are sold and used as an infusion to treat kidney stones (Ammar et al., 2014). The data found in recent literature on the phytochemical composition and the extraction procedures from *Opuntia* spp. flowers and roots are summarized in Table 2.4.

Table 2.4 - Bioactive composition and extraction procedures of *Opuntia* spp. flowers and roots.

Provenance	Extraction Procedure	Main Bioactive Com- pounds and Levels Found	Main Conclusion	Ref
<i>Opuntia ficus-indica</i>	Flowers were collected during the post-flowering stage in June 2013, from wild populations located in Tunisia (latitude 34°46'29''N, longitude 10°39'73''E; elevation: 41 m), where the climate is semi-arid, characterized by a mean rainfall of 200 mm/year.	Total phenolic content (mg GAE*/g extract)	In terms of extract yield, maceration is more efficient than Soxhlet,	(Ammar, Ennouri, & Attia, 2015)
		Soxhlet extraction	and the two best solvents are water and methanol.	
		Water: 58.7	Regarding the bioactive com-	
		Methanol: 270.9	pounds and antioxidant activity,	
		Acetonitrile: 132.4	the solvent's polarity had a signifi-	
		Acetone: 67.5	cant effect on antioxidant activity.	
		Ethyl acetate: 122.3	Aqueous and methanolic extracts	
		Dichloromethane: 10.1	have proved to be best in terms of	
		Hexane: 7.5	high extraction of phenolic com-	
		Maceration extraction	pounds and antioxidant activity.	
		Water: 44.2	It was proven that the polyphenols	
		Methanol: 227.8	are thermostable, and once they are	
		Acetonitrile: 17.6	responsible for the antioxidant ac-	
		Acetone: 70	tivity of the flower extracts, the	
		Ethyl acetate: 70.8	Soxhlet method was the most	

Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
		Dichloromethane: 14.7 Hexane: 14.8	interesting to preserve the antioxidant activity.	
		Total flavonoid content (mg RE*/g extract) Soxhlet extraction Water: 9.7 Methanol: 60.81 Acetonitrile: 19.4 Acetone: 15.29 Ethyl acetate: 17.65 Dichloromethane: 1.73 Hexane: Not detected		
		Maceration extraction Water: 22.47 Methanol: 27.47 Acetonitrile: 3.25 Acetone: 4.6		

		Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
		Ethyl acetate: 15.75 Dichloromethane: 8.11 Hexane: Not detected		
<i>Opuntia ficus-indica</i> and <i>Opuntia stricta</i>	From May to June 2009, the flowers were collected in 4 different flowering stages (vegetative, initial flowering, full flowering, and post-flowering) from wild populations located in Tunisia (latitude 34°46'29''N, longitude 10°39'73''E; elevation: 41 m), where the climate is semi-arid and is	Maceration was used for extraction using hexane. After 7 days, extracts were filtered and concentrated and finally characterized by Gas Chromatography with flame-ionization detection (GC FID).	Fifty different compounds were detected, representing around 85–99.7% of the total integrated peak area. No remarkable differences were observed between the flower compositions. Phytochemical compounds present in the <i>Opuntia</i> flower extract essentially belong to carboxylic acids (28–97%), terpenes (0.2–57%), esters (0.2–27%), The extraction yield increased with the evolution of the flowering stage, with the highest yield found at the post-flowering stage (<i>O. ficus-indica</i>), and for <i>O. stricta</i> at the full flowering. Overall <i>O. stricta</i> presented more phenolic compounds than <i>O. ficus-indica</i> , which corroborates the superior antioxidant activity of its extract. However, the antioxidant power was not related to the number of phenolic compounds; once at the post-flowering stages, it presented the highest content but the lowest activity. This property was	(Ammar et al., 2012)

Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
characterized by a mean rainfall of 200 mm/year.		and alcohol classes (<1.8%). Aromatic compounds, mostly phenolic compounds (2-methoxy-4-vinylphenol and phenol) represented only 9.3% of the identified molecules. Monoterpenes d1-limonene and linalool were found in small quantities in the early stages of <i>O. ficus-indica</i> flowering, and only at post-flowering for <i>O. stricta</i> . Camphor represented 32.4% of the	attributed to the presence of terpenes and aromatic compounds. The extracts exhibited good antimicrobial activity against <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , and <i>Escherichia coli</i> , with <i>O. stricta</i> extracts being more active than <i>O. ficus-indica</i> .	

Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
		compounds found in the vegetative stage for <i>O. ficus-indica</i> .		
		In terms of total phenolic content: <i>Opuntia ficus-indica</i> : Vegetative: 16.3 mg GAE*/g extract Initial flowering: 13.4 mg GAE*/g extract Full flowering: 13.3 mg GAE*/g extract Post-flowering: 49.0 mg GAE*/g extract <i>Opuntia stricta</i> : Vegetative: 16.7 mg GAE*/g extract		

	Provenance	Extraction Procedure	Main Bioactive Com-	Main Conclusion	Ref
			pounds and Levels Found		
			Initial flowering: 15.3 mg GAE*/g extract Full flowering: 13.1 mg GAE*/g extract Post-flowering: 80.9 mg GAE*/g extract		
<i>Opuntia ficus-indica</i> (L) Miller	Flowers were collected during the post-flowering stage in June 2014, from wild populations located in Tunisia (latitude 34°46'29''N, longitude 10°39'73''E; elevation: 41 m), where the climate is semi-arid, characterized by a mean rainfall of 200 mm/year	Two flower extracts were evaluated: an aqueous extract (mucilage) and a methanolic extract (Soxhlet extraction). Mucilage extract was characterized in terms of monosaccharide composition by GC. Furthermore, antimicrobial activity was evaluated against	Mucilage composition found: Glucose: 32.41 mg/g extract Mannose: 11.46 mg/g extract Arabinose: 7.98 mg/g extract Xylose: 4.97 mg/g extract Galactose: 2.61 mg/g extract	The mucilage yield of the extraction found was 18.3% while 14.8% was reported for the Soxhlet extract. Glucose was the major component of the mucilage and originated from the plant flower's hemicellulose and cellulose. Both mucilage and methanolic extracts exhibited antimicrobial activity against both Gram-positive and Gram-negative foodborne bacteria, with superior effect against the former. The methanolic extract was	(Ammar, Bardaa, et al., 2015)

Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
	Gram-positive and Gram-negative food-borne bacteria.	Rhamnose: 1.99 mg/g extract Total sugar: 61.4 %	more efficient against the bacteria tested than mucilage, and the highest inhibition was found against <i>Listeria monocytogenes</i> . In terms of antioxidant properties, the mucilage and methanol extracts exhibited significant anti-radical activity, although the mucilage extract showed lower radical-scavenging activity than the methanolic extract.	
<i>Opuntia ficus-indica</i> (L) Miller	Flowers of <i>O. ficus-indica</i> were collected in Monasterace, Italy, in May 2007.	Dried flowers were subjected to maceration extraction process using methanol. Purification was performed by using Solid-Phase Extraction. The dried residue was analysed by <i>O. ficus-indica</i> flower methanol extract composition (mg/g): Quercetin 3-O-rutinoside: 7.09 Kaempferol 3-O-rutinoside: 4.00	Secondary metabolites belonging to the flavonol glycoside class were found in the cactus flowers. Total flavonoids of the flowers were 81.75 mg/g of fresh plant material, and Isorhamnetin 3-O-robinobioside was the major component representing 52.22%, followed by	(De Leo et al., 2010)

Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
	High Performance Liquid Chromatography coupled with Photo Diode Array and Electrospray Ionization Gas Chromatography (HPLC-PDA-ESI-MS) for qualitative investigation and by HPLC-PDA for quantitative analysis.	Quercetin 3-O-glucoside: 4.47 Isorhamnetin 3-O-robinobioside: 42.69 Isorhamnetin 3-O-galactoside: 9.79 Isorhamnetin 3-O-glucoside: 7.24 Kaempferol 3-O-arabinoside: 3.24	isorhamnetin 3-O-galactoside (11.98%). The flower extract has a pharmaceutical interest as it can be used for the treatment of depression, and its major component is associated with a testosterone 5 α -reductase inhibitor. This was the first report on the flower volatile composition of <i>O. ficus-indica</i> . There were no monoterpene hydrocarbons found, but oxygenated monoterpenes were at 16.5%, and sesquiterpene hydrocarbons were 18%, with Germacrene D as the major component.	
	Volatiles were analysed by GC and Gas Chromatography with Electron Impact Mass Spectrometry (GC-EIMS) system.	Composition of <i>O. ficus-indica</i> flower volatiles (%): (E)-3-Hexen-1-ol: 3.7 1-Hexanol: 12.3 Nonanal: 2.5 2-Ethyl hexyl acetate: 1.2		

Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
		Tetrahydrolavandulol: 5.5 1-Nonanol: 1.2 Pinocampheol: 1.7 Dihydrocitronellol: 2.6 Decanal: 8.2 Tridecane: 5.9 cis-2,3-Pinenediol: 1.9 η-Tetradecane: 9.1 β-Ylangene: 1.5 (E)-Geranyl acetone: 4.8 Allo-aromadendrene: 3.9 Germacrene D: 12.6 η –Pentadecane: 4.0 η –Hexadecane: 1.6		

	Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
				The yield of extraction found was 26%. Root methanolic extract exhibited remarkable content in phenolic and flavonoid compounds. In comparison to swallow root (<i>Decalepis hamiltonii</i>), <i>Opuntia</i> root extract presented almost the double phenolic compounds. Regarding the flavonoid content, <i>Opuntia</i> root extracts reported concentrations superior to those reported for the <i>Opuntia</i> fruit extracts, which are known for their antioxidant power. Extracts presented antiulcerogenic activities tested <i>in vivo</i> in rats. Phenolic and flavonoid wealth, radical scavenging activity, and reducing	
<i>Opuntia ficus-indica</i> f. <i>inermis</i>	The roots were collected from municipal areas of Gafsa, Tunisia.	Extraction from dried roots was performed by using methanol, under stirring. The solution was then centrifuged, and the supernatant was dried. Total phenolic compounds and flavonoid content were determined using UV/Vis spectroscopy.	Total phenolic: 57.56 mg GAE*/g extract Total flavonoids: 23.5 mg RE*/g extract		(Alimi et al., 2010)

Provenance	Extraction Procedure	Main Bioactive Compounds and Levels Found	Main Conclusion	Ref
			power have been implicated in the extract's antiulcer properties.	

*GAE (Gallic Acid Equivalent), RE (Rutin Equivalent).

It is noticeable that the flowers and roots of *Opuntia* spp. are rich in phenolic compounds, with proven antioxidant and antimicrobial activities (Alimi et al., 2010; Ammar, Bardaa, et al., 2015; Ammar et al., 2012). Once those parts of this perennial crop are generally neglected (i.e., lost in the cultivation process), there are opportunities to prepare such extracts to use either as food additives, with further purification in the pharmaceutical industry, or even as food supplements (Alimi et al., 2010; De Leo et al., 2010).

2.1.3 Applications in food products

2.1.3.1 Food applications of cladodes

Cladodes and their by-products can be used in a variety of industries (Figure 2.4). The most common uses for cladodes are for food and feed consumption (Ciriminna et al., 2019). As food, it is consumed fresh or processed into several products such as soups, salads, juices, or bakery products to produce cookies, bread, and biscuits (Msaddak et al., 2017; Ventura-Aguilar et al., 2017). Due to the presence of several compounds with bioactive properties and a high content of dietary fiber, cladodes are also widely applied in cosmetics, pharmaceuticals, and nutraceutical products. As an example, the incorporation of cladodes (up to 10% *w/w*) into durum wheat bread revealed an improvement in the antioxidant activity of bread without affecting the rheological properties (Sciacca et al., 2021). Fortification of pasta with cladodes extracts in substitution of water demonstrated to be useful by increasing the fiber content with antioxidant features and with satisfactory acceptance in sensory analysis (Attanzio et al., 2019). Furthermore, the use of cladodes powder (*Opuntia ficus-indica* f. *inermis*) as a substitute for wheat flour in cookies demonstrated an increase in dietary fiber and mineral content. The cookies produced contained a high level of fat, which makes the cookies highly susceptible to oxidation, but the use of cladodes powder showed a positive effect on reducing oxidation when compared with the control cookies, which were only produced with wheat flour (Msaddak et al., 2015). Cladodes were also added to maize flour to improve the nutritional and physicochemical properties of tortillas in Mexico. Once again, the substitution of maize flour with cladodes powder at 6%, improved the dietary fiber and mineral content (e.g. calcium) becoming a source for the intake of these nutrients (Ramírez-Moreno et al., 2015). In semi-arid regions, cladodes are commonly used for animal feed due to their richness in water and carbohydrates, necessary for their survival (A. M. Rodrigues et al., 2016). Cladodes were used as

whole or supplementary food e.g. sheep and goats (Mokoboki & Sebola, 2017; Rezende et al., 2020).

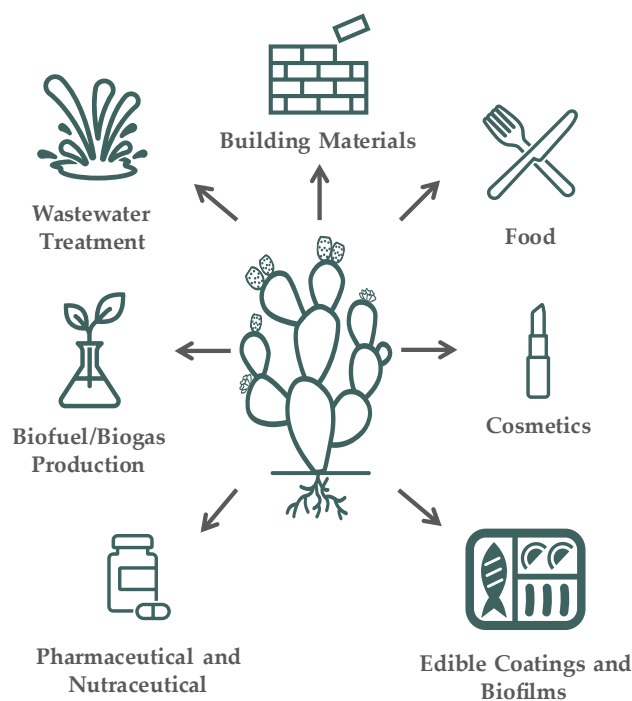


Figure 2.4 - Cladodes applications.

Mucilage and pectins are also used in food packaging applications as edible coatings and biobased films (Freitas et al., 2021; V. G. L. Souza et al., 2022). Some studies showed that *Opuntia ficus-indica* mucilage is effective as a coating material to extend the shelf-life of fresh strawberries (Del-Valle et al., 2005), kiwifruit slices (Allegra et al., 2016) and fig fruit (Allegra et al., 2017). Edible films produced from mucilage and pectin showed poor mechanical and physical properties, suggesting the need to incorporate other compounds to enhance those characteristics (Espino-Díaz et al., 2010; Gheribi & Khwaldia, 2019). The incorporation of reinforcements, such as nanocellulose, nanoclays and nanometal oxides, is an option, as it was observed with other biobased polymers (e.g. chitosan) (J. Pires et al., 2021; J. R. A. Pires et al., 2019). Furthermore, the presence of bioactive compounds on cladodes makes them useful to enhance bioactive characteristics when incorporated in films, e.g. starch-based films when compared to standalone films (De Farias et al., 2021). Several additives to improve cactus mucilage film's mechanical resistance have been studied, e.g. calcium and gelatin (Espino-Díaz et al., 2010; Lira-Vargas et al., 2014) or beeswax to reduce water vapor permeability (Lira-Vargas et al., 2014). The use of different plasticizers (glycerol, sorbitol, PEG-200, and PEG-400) was

also tested, showing that their structural features improve distinct interactions with mucilage polysaccharides (Gheribi et al., 2018). Incorporating essential oils or extracts rich in phenolic compounds, can add to the biobased films' antimicrobial and antioxidant activities, as was observed in other works with biobased polymers (V. G. L. Souza, Ferreira, Rodrigues, Pires, et al., 2019).

Although more studies are needed to improve the characteristics of the materials, cladodes mucilage and pectins demonstrate promising applications as an alternative to fossil-based plastics currently used in food industry.

2.1.3.2 Food applications of prickly pear fruits

The prickly pear and its by-products can be used in a variety of industries. Various products have been developed with cactus pear residues, such as yoghurts, snacks, and margarine.

In the food industry, one of the uses is the production of juices. To maintain stability and extend their shelf-life, heat treatment is commonly used. However, exposure to high temperatures can cause degradation of thermolabile compounds and modification of the organoleptic temperatures, so alternative technologies have been attempted such as the used of high pressure, pulsed electric fields (PEF) and ultrasound. The presence of phenolic compounds, vitamins and other bioactive compounds in the juices can be a complement for the consumer (Barba et al., 2017). In this sense, the use of PEF technology was applied to help in the inhibition of *S. cerevisiae* and along with pH reduction and the use of preservatives (sodium benzoate and potassium sorbate) in prickly pear juices (García-García et al., 2015). The combination of these factors leads to microbial reduction and preservation for 21 days at 25°C.

Prickly pear fruit is rich in several bioactive compounds so the addition of this fruit to other food products can provide or enhance several properties namely, antioxidant activity. That is the case of the incorporation of prickly pear into a gluten-free pasta from rice-field bean flour, which allowed the increment of phenolic compounds and antioxidant properties in a percentage of 15% (*w/w*) (Oniszczyk et al., 2020). This food product emerges as an alternative for celiac patients. Studies made on consumer preferences in Italy related with using *Opuntia ficus-indica* as an ingredient in new functional pasta, showed a significant respondent interest regarding the health benefits and the nutritional and environmental aspects of this type of functional pasta (Palmieri, Stefanoni, et al., 2021). However, the studies also reveal that the functional pasta should retain the organoleptic and physical properties of durum wheat-based

pasta(Palmieri, Suardi, et al., 2021). It was also demonstrated that the introduction of prickly pear peel powder (5% *w/w*) in cracker formulation could be a source of dietary fiber and bioactive compounds without affecting the quality of the product. The crackers presented an increment of around 8 % in terms of antioxidant activity and total dietary fiber went from 5.89 g/ 100g to 8.11 g/100g, when compared with the control (Elhassaneen, 2016).

Food supplements have been developed by using food by-products from *Opuntia*. Tablets were developed from *Opuntia ficus-indica* L. Mill fruits (green and red varieties) (Manzur-Valdespino et al., 2020). In this work, the formulation of tablets included the conjugation with microcrystalline cellulose with lactose and the addition of talcum powder and magnesium stearate. Each tablet presented a total weight between 0.7 and 1 g. In terms of dietary fiber, the tablets presented a content of 0.24 g, using green fruit and 0.15 g, using red fruits. Furthermore, the tablets showed good antioxidant activity with DPPH radical inhibition of 24% for the tablets made from red fruits and 20% for green fruits. According to this study, by-products of *O. ficus-indica* have a high potential for use in foods due to their high dietary fiber content and antioxidant activity, which can prevent free radical damage (Manzur-Valdespino et al., 2020).

The use of hydro-ethanolic extracts from prickly pear peels was tested as an alternative to vitamin E in the prevention of margarine oxidation (Chougui et al., 2015). The extracts produced were rich in phenolic compounds, with a content of 1512.58 mg GAE/100g of dry matter. Three different concentrations of the extracts were tested (50, 100 and 150 ppm). The use of prickly pear extract in margarine showed a positive effect on the reduction of oxidation when compared with the same product with vitamin E, even at the lowest concentration used (50 ppm). The margarine developed with 150 ppm of extract demonstrated a higher tendency to oxidation, which can be caused by the pro-oxidant effect of the phenolic compounds at high concentrations (Chougui et al., 2015).

Prickly pear extract was added to cooked beef burger patties, and its effect on quality parameters was evaluated (Parafati et al., 2021). The extract (5% *v/w*) was added directly or encapsulated in alginate beads. The encapsulation of bioactive compounds from prickly pear could be a vehicle for their preservation for a long time. The use of prickly pear extracts showed no adverse effects on the cooked burgers. In fact, the intrinsic antioxidant activity present in the extracts, especially the ones encapsulated in alginate not only enhances that property in the burgers but also avoids lipid oxidation when compared to other burgers in the study.

The seeds present in fruits also have the potential to be used in the food industry due to their richness in fatty acids, in particular, linoleic, palmitic, and stearic acids (Ciriminna et al., 2017).

2.1.3.3 Food applications of *Opuntia* spp. Flowers

The flowers from *Opuntia* spp. can also be used in food applications, but the most known uses are in decoctions and infusions made from dried flowers, which widely used in traditional medicine (Berrabah et al., 2019). It is reported that decoction and infusions from flowers of *Opuntia ficus-indica* are a source of minerals, namely K and Ca, and also a source of polyphenols, flavonoids and tannins (Ammar, Ennouri, Bouaziz, et al., 2015). The maceration of flowers from *Opuntia ficus-indica* was studied as a heat stabilizer of olive oil as well as its effect on the quality of the final product. The addition of 5% (*w/w*) of flowers to the olive oil leads to an increase in the phenolic compounds content, improving the stability in terms of oxidation (Ammar et al., 2017).

The knowledge of their characteristics, such as the hydration properties and oil holding capacity, is important as they may interfere with the functionality and nutritional quality of the food (Ammar et al., 2014). Flowers from *Opuntia ficus-indica* and *Opuntia stricta* harvested from a wild population located in Tunisia in the post-flowering stage were analyzed in terms of their oil holding capacity (OHC), and hydration properties through the determination of the swelling capacity (SWC), water solubility index (WSI) and water holding capacity (WHC). The hydration properties are related to the presence of soluble molecules (such as sugar, more sugar reflects superior WSI), to the polysaccharide content (correlated with SWC), and to the hydrophilic constituents (WHC). The OHC is an important parameter from an industrial point of view as it is correlated to the product's emulsifying capacity. The results varied depending on the specimen, and overall *O. ficus-indica* presented superior WSI, WHC and OHC, while the SWC was higher for *O. stricta*. The values found for SWC were similar to those reported for wheat and carrots but smaller than in cauliflower. Regarding the WHC, *Opuntia* spp. flowers presented values of the same magnitude as other dietary fiber concentrates (from by-products) and some commercial dietary fiber-rich supplements. OHC found were similar to those reported in the cladodes and of the same magnitude as other fruits, vegetables, and seaweeds (around 2 g/g), but lower than for cereal fibers (2-4 g/g) (Ammar et al., 2014).

Thus, *Opuntia* spp. flowers can be highlighted as an excellent alternative source of dietary fiber for human consumption, but also as functional ingredients in the food industry as

jellying agents to retard syneresis, modify the viscosity and texture of formulated food, or stabilize food emulsions (Ammar et al., 2014). Together with the nutraceutical and pharmacological approaches, the use of *Opuntia* spp. flowers can always be explored, adding some economic value to these valuable by-products.

2.1.4 Conclusions

Opuntia spp. is a crop that has been gaining attention throughout the years and has been increasingly studied. Due to its adaptability to adverse environments, it has a high potential to generate value-added products from its fruits and cladodes. Cladodes are a good source of dietary fiber and rich in water, so their consumption should be more considered in the human diet due to their health benefits. Fruits, due to their richness in sugars, can be easily used in the production of juices, jams, and marmalades. Recent studies have used innovative technology to produce food products with greater stability and safety. Moreover, cladodes and fruits are rich in several bioactive compounds and have a high potential to be used in several nutraceutical products. Betalains, which are present in fruits can also be used as food colorants as an alternative to the ones currently on the market and have a great potential to be used as a sensor in food packaging.

Nevertheless, *Opuntia* spp. by-products and their use in the food industry can be further investigated to better understand the potential uses of this crop in order to enhance its consume globally.

2.2 Bio-Based Sensors for Smart Food Packaging – Current Applications and Future Trends

2.2.1 Introduction

Fresh food products are easily degraded due to physical injuries that could result from handling, transportation, storage, or by intrinsic factors caused by chemical reactions, enzyme action, and microbial spoilage (Ghoshal, 2018; V. G. L. Souza et al., 2018). In this sense, the use of packaging is a strategic choice for the protection and conservation of food (Alizadeh-Sani et al., 2020) as it acts as a barrier to gases (oxygen, carbon dioxide), water vapor, and dust

(Ghaani et al., 2016; Halonen et al., 2020; V. G. L. Souza, Pires, Vieira, et al., 2019). Therefore, packaging must be able to provide four main functions: protection, containment (small items can be grouped together in one package), information transmission (how to use, transport, recycle, or dispose of the package or product), and marketing (encourage potential buyers to purchase the product) (Mohammadian et al., 2020; Robertson, 2016). It contributes to assuring the safety and quality of the food products for both suppliers and consumers, resulting in a reduction in food waste and more safety for the consumers (Mohanty & Swain, 2017; Trajkovska Petkoska et al., 2021). Moreover, from an economical point of view, food packaging allows the manufacturers to avoid economical losses caused by the damages that can occur during transportation, management, and storage of the food; and the packaging also plays an important role in the marketing strategy of the product as it is the communication channel between the consumer and the industry (Pilevar et al., 2019; V. G. L. Souza & Fernando, 2016; Videira-Quintela et al., 2021).

Besides their benefits, packaging generates a huge waste and causes environmental problems since most of them are produced with non-renewable and non-biodegradable petroleum-based feedstocks. These materials have excellent properties, low cost and processability, however, they are not biodegradable, and they contaminate soil, air, and water, representing one of the 21st-century global issues (Genovese et al., 2016; Mohanty & Swain, 2017; V. G. L. Souza et al., 2017). Recently, new packaging materials are being studied as an alternative to the traditional petroleum-based ones. This is the case of biopolymers, that are obtained from renewable resources, and some with feasible functional properties, e.g., antimicrobial properties, such as chitosan (V. G. L. Souza et al., 2020; X. Z. Tang et al., 2012).

Yet, packaging can also play an active role in the food preservation and may represent an information storefront on the state of the food being packaged (Roy & Rhim, 2020), thus helping to fight food waste and food deterioration. In this regard, active and intelligent packaging are being studied and developed and represent an important mission in the food packaging industry.

Active packaging consists on the use of materials that can release, emit, absorb or scavenge substances directly or indirectly to the food to maintain quality or delay its degradation (Biji et al., 2015; Vilela et al., 2018). This packaging technology uses active compounds with e.g., antioxidant or antimicrobial properties that are incorporated into a polymeric matrix, coatings, or in labels, pads, or sachets (Wilson et al., 2018). The active compounds applied (such as phenolic compounds) can be obtained from, e.g., plants, which includes industrial

crops such as kenaf, cardoon and hemp, algae, herbs, spices, fruits, or vegetables (Andrade et al., 2021; Barbosa et al., 2020; Nissen et al., 2010; Pascoal et al., 2015; V. G. L. Souza, Rodrigues, Ferreira, Pires, et al., 2019). Active packaging can be divided into three main systems: antioxidant (e.g., butylated hydroxytoluene, vitamin C, vitamin E) (Gómez-Estaca et al., 2014), antimicrobial (e.g., essential oils, peptides, phenolic compounds) (Ribeiro-Santos et al., 2017), scavengers or absorbers (e.g., oxygen scavengers, carbon dioxide absorbers or emitters, moisture control agents and ethylene absorbers or adsorbers) (Lee et al., 2015).

Intelligent packaging goes even further, by providing information to the consumer about the status of the packaged food using materials that monitor and interact with the packaging environment through an internal or external indicator (European Commission, 2009; Shivakumar et al., 2018). Intelligent packaging can also be used to monitor the effectiveness of active packaging (Ghoshal, 2018). Intelligent packaging can be divided in three principal systems: indicators, sensors and data carriers (Müller & Schmid, 2019).

Indicators determine the presence or absence of a substance, the extension of a reaction between different substances and the concentration of a specific substance. In general, indicators provide information through a visual change (Kalpana et al., 2019). The majority of the indicators are focused on the detection of time-temperature, freshness, gas formation, and provides a color change that can be easily identified by the consumer (Dobrucka & Przekop, 2019).

Data carriers have the function to guarantee traceability, automatization, and to protect against theft and counterfeit. Data carriers are a way to transmit information within the food supply chain and do not have the function to contact with the product (Chowdhury & Morey, 2019; Müller & Schmid, 2019). Data carriers most commonly used are barcode labels and Radio Frequency Identification (RFID) (Müller & Schmid, 2019).

Sensors are electronic devices that are used to detect, locate or quantify an alteration through a receptor that is translated into a signal, using a transducer, that reports a physical or chemical change (Kalpana et al., 2019; Sohail et al., 2018). Biosensors are one type of sensors related to biological reactions, where a bioreceptor recognizes a biochemical sign and the transducer convert it into a quantifiable electronic response (Biji et al., 2015; Chowdhury & Morey, 2019). Biosensors can detect pathogens, allergens, and other toxic compounds (Griesche & Baeumner, 2020).

Occasionally, in food packaging applications, the term “indicator” is often substituted by the term “sensor”. This result from the indicators “sensing” or monitoring action, expressed

through a visual change, although indicators do not have the same features of sensors (Kuswandi, 2017).

Smart packaging comprises both active and intelligent packaging systems altogether, with the purpose to provide a more accurate information about the food product conditions to the consumer, and also display a protective effect over the food product through the use of, e.g., antioxidant and antimicrobial agents (Figure 2.5) (S. Chen et al., 2020; Vanderroost et al., 2014; Young et al., 2020). Smart packaging can track a food product through the analysis and control of the internal or external environment, providing information to the entire food supply chain from manufacturers to consumers at any moment (Emanuel & Harloveleen, 2019). Despite intelligent and smart packaging being two distinct concepts, very often references to smart packaging are in fact to intelligent packaging (Vanderroost et al., 2014).

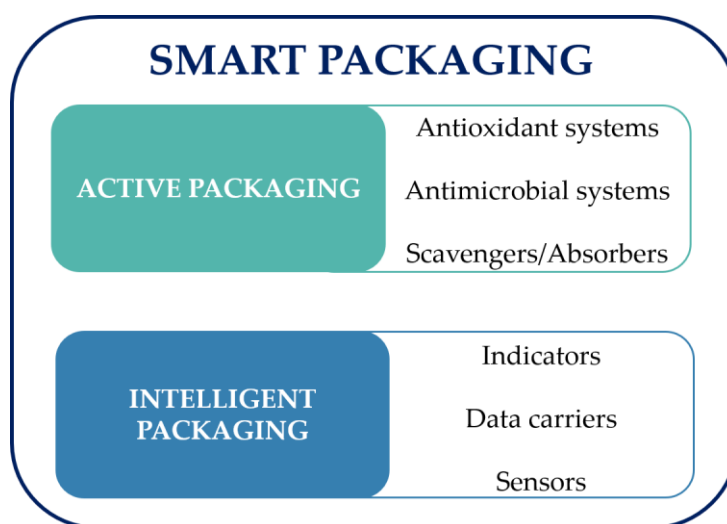


Figure 2.5 - Schematic illustration of active, intelligent, and smart packaging.

As biobased materials have emerged as alternatives to the use of traditional synthetic plastics, their application as indicators and sensors is also arising. For example, natural dyes and extracts that may substitute synthetic ones with the ability to change their color depending on physiological variations of the food materials (Alizadeh-Sani et al., 2020; Nešić et al., 2020). These pigments are generally incorporated into the polymeric matrix and change color, e.g., based on pH changes caused by microbial growth (Roy & Rhim, 2020). Thus, natural pigments can also constitute a more ecological and less toxic alternative to the already existing synthetic pigments that may cause harm to the consumers (Balbinot-Alfaro et al., 2019). Besides, the use of biobased compounds as indicators or sensors is also in line with the current European Union

policies that reinforce the need to increase the sustainability of the products by increasing their biodegradable and compostable character (European Commission, 2018).

Therefore, this work aims to approach some of the most recent research developments on bio-based sensors to be used as intelligent packaging for food products. The use of natural compounds that can act as indicators may provide to the consumer an accurate response about the quality of the food and represents an alternative to the use of hazardous compounds. In addition, some of the bio-based sensors may also act simultaneously on the products being packaged (e.g., through its antioxidant activity), and therefore, the global packaging system represents a smart packaging. This review focuses on the applications and performance of biosensors when in contact with perishable food products. Critical analysis of the most recent information available will be useful to identify future opportunities for the use of bio-based sensors in food packaging systems, helping to contribute to the development of more sustainable packaging.

2.2.2 Developments in Bio-Based Sensors

Developments on bio-based sensors (i.e., sensors that are using compounds obtained from natural products and using biopolymers) to be used as intelligent packaging for food products, are mainly focusing on freshness indicators.

Indicators have the function to provide information about changes occurring in a food product or in the surrounding environment, providing more accurate information about the status of a food product for the consumers (Sohail et al., 2018).

Freshness indicators are used in intelligent packaging to provide information about the quality decay of the food (Dobrucka & Przekop, 2019). These type of indicators are mostly based on a visual colorimetric response, caused by a chemical or microbiological change of the food product that reacts with the sensor indicating changes in the product, generally caused by a pH alteration (Poyatos-Racionero et al., 2018). The freshness sensor or indicator can be applied in the form of a label, tag, or visual indicator (Kuswandi, 2017). Freshness indicators can be divided into direct or indirect indicators. Direct indicators are the ones which action is based on the direct reaction with a specific metabolite or toxin produced, whereas the indirect ones are based on an indirect reaction caused by chemical or microbiological changes that modify the environment where the food is placed (Nemes et al., 2020; R. Sharma & Ghoshal, 2018).

To perform their function and help in the maintenance of food quality, freshness indicators are based on a color change of pigments that are added to the indicator and are reactive to pH changes. Synthetic dyes are commonly used such as methyl red, bromocresol purple, bromophenol blue, chlorophenol red have been proved to be able to react to pH changes when integrated into intelligent packaging systems (Bhargava et al., 2020; Dobrucka & Przekop, 2019). Although they are demonstrated to be effective, they are not fitted to be used for food applications due to their toxic, carcinogenic, and mutagenic characteristics that threaten not only the consumers' health but also the environment (Kurek et al., 2019).

The use of natural dyes instead of synthetic ones fits the consumption trend for sustainable and more natural healthy choices (Bhargava et al., 2020). Natural pigments extracted from plants and fruits are renewable, demonstrate low toxicity, and low environmental impacts (Kurek et al., 2019). To be used as a pH indicator it is mandatory to consider the stability and the pH response of the pigments, which depends on each type (Alizadeh-Sani et al., 2020; Castañeda-Ovando et al., 2009).

Recently, the use of natural color pigments in biopolymeric matrices in food packaging systems has emerged, fitting this trend of the awareness for sustainable choices (Alizadeh-Sani et al., 2020). Additionally, the majority of the natural pigments and biopolymeric matrices possesses bioactive properties not only acting as a pH indicator but also by providing active properties to the indicator and for the food product (Kalpana et al., 2019; Latos-Brozio & Masek, 2020).

2.2.3 Bio-based sensors - Applications

Microbial growth in food is a cause of food degradation leading to a loss of freshness by producing metabolites that can be detected by indicators present on the packaging. The presence or the formation of metabolites such as volatile nitrogen compounds, carbon dioxide, biogenic amines, ethanol, or sulfurous compounds are examples that can be detected through freshness indicators (Arvanitoyannis & Stratakis, 2012; Kalpana et al., 2019). Meat, fish, seafood, fruits, and vegetables are perishable products and assuring their quality is very important to protect the consumer from hazardous metabolites produced during its degradation process. Meat freshness decreases with microbial growth leading to the formation of several compounds like acetic acid, n-butyrate, and biogenic amines (R. Sharma & Ghoshal, 2018). The formation of these compounds compromises meat quality and so the freshness indicator can be used to detect them. In fish and seafood, spoilage is mainly indicated by the amount of total

volatile basic nitrogen (TVB-N) and the presence of biogenic amines resulting from the decarboxylation of amino acids due to the microbiological action (Prabhakar et al., 2020). Additionally, the production of vapors or gases such as ethanol or carbon dioxide (CO₂) produced by the action of microorganisms decays food quality and the metabolites can be detected by the freshness sensors (Yousefi et al., 2019).

Several studies have been arisen through the past few years concerning the development, evaluation, and application of biobased sensors using natural compounds in intelligent food packaging and some of them are reported and summarized in Table 2.5. In these studies, examples focused mainly on bio-sensors associated with biopolymers, but also some other synthetic polymers that are easily degraded were also added as examples. In the table, it was also indicated which was the action and function of the sensor and how they respond to the loss of freshness in the food. Most of the sensors are sensitive to a pH alteration caused by the release of volatile nitrogen compounds, and this change is consequently identified by a colorimetric response. Sensors are generally placed into the headspace of the food container, avoiding direct contact with food but close enough to detect media alterations and providing a response to food quality alterations. When these biosensors are associated with the biopolymers, they are usually incorporated in the polymeric structure and the modification in the color of the films indicates the food quality alterations of the product being packed. The information collected also clearly shows that extracts rich in pigmented chemical compounds, that change color with pH, have been intensively studied and used in these bio-sensors, especially anthocyanins. Additionally, most of the studies applied the bio-sensor in fish, meat, and seafood, most probably because their quality decay represent an important economic loss and also because, in the degradation process, the pH of the surrounding environment changes, this change being easily detected through the use of those pH-sensitive-biosensors. Interestingly, some of the compounds applied and tested in the sensor, not only present a pH-sensitive color but also have other bioactive properties, e.g., antimicrobial properties, and its presence in the polymeric matrix can also enhance the preservation activity of the packaging material. Yet, the incorporation of those compounds in the biopolymer can also change some of the mechanical, barrier and optical properties of the films (V. G. L. Souza et al., 2018; V. G. L. Souza, Pires, Rodrigues, et al., 2019). Consequently, in some applications, it might be necessary to reach a balance between the beneficial action of the pH bio-sensor activity and its adverse effects on some of the properties of pristine biopolymers (e.g. mechanical properties). Following the

information collected and summarized in Table 2.5, a wider description will be completed to clarify the significance of the studies.

Table 2.5 - Food application of bio-based pH sensors.

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
Gelatin-Gellan gum bilayer films	Mulberry fruits—antho- cyanins	Sensitivity of the films to volatile nitrogen com- pounds through pH change	• The use of ZnO nanoparticles im- proved the color stability of the films; • Bilayer films proved to be sensi- tive to ammonia; • Gellan gum-Mulberry anthocya- nins/Gellatin 2.0% film demonstrated vis- ible color changes linked with crucian spoilage	Crucian fish	(Yang et al., 2021)
Starch	Grape skin—anthocya- nins	Sensitivity of the films to volatile nitrogen com- pounds through pH change	• Anthocyanins caused changes in the mechanical properties; • Cassava starch sheets with the highest content in anthocyanins proved to be effective to act as a pH-sensor, showing color changes when the pH en- vironment changed.	Beef and fish	(Ve- dove et al., 2021)

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
Agarose	Red cabbage and rose—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	- The indicator sensor is reactive to an increase in ammonia production, causing conformational changes on anthocyanins and a color change was observed; - Under simulating conditions of ammonia production, the indicator turned from red to green; - Same results were observed when the indicator was in contact with buffalo meat.	Buffalo meat	(Shukla et al., 2016)
Chitin nano-fiber and methylcellulose	Red barberry—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	- Mechanical, optical, and physical-chemical properties of the films changed when anthocyanins were added to the chitin nanofiber/methylcellulose film; - A colorimetric pH-sensitive response was observed when the films were placed in contact with spoiled fish in containers.	Fish	(Sani et al., 2021)

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
Pectin	Red cabbage—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	- A color change (purple to yellow) was observed when exposed to different amines; - Same behavior was observed when the film was introduced in the headspace of the package containing spoiled food products.	Beef, chicken, shrimp and fish	(Dudnyk et al., 2018)
Chitosan, carboxymethyl cellulose	Blueberry and red grape skin pulp—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	- Extracts did not affect mechanical properties of the films produced; - Color changes occur in both films; - Color changes were more evident on carboxymethyl cellulose than on chitosan films.	Chicken meat	(Kurek et al., 2019)
Carboxymethyl cellulose sodium and <i>Artemisia sphaerocephala</i> Krasch. gum	Red cabbage—anthocyanins	pH and NH ₃	- Red cabbage extract influenced mechanical and optical properties; - Films changed from rose-bengal to aquamarine when the pH of buffer solutions went from acidic to alkaline conditions.	Not applied	(Liang et al., 2019)

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
			A more evident color was observed when in contact with NH₃ rich-atmosphere.		
K—carrageenan	<i>Lycium ruthenicum</i> Murr.—anthocyanins	Sensitivity of the films to volatile nitrogen compounds and to lactic acid production through pH change	- Films in contact with milk produced a color change from grayish purple to dark pink due to lactic acid production; (acidic conditions); - Films when exposed to shrimp caused a color change from grayish purple to yellow due to volatile amines production; (alkaline conditions).	Milk and Shrimp	(J. Liu et al., 2019)
Starch and gelatin	Red raddish—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	- A color response was observed in a pH range from 2 to 12; - As a response to volatile nitrogenous compounds produced from prawn and poultry meat spoilage, films changed from red to grey-purple.	Prawn and poultry meat	(Chayanich et al., 2020)
Methylcellulose	Jambolan fruit—anthocyanins	pH	A color response was observed in a pH range from 1–10;	Not applied	(da Silva Filipini)

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
			Films changed their color from red/pink under acidic conditions to purple/blue under alkaline conditions.		et al., 2020)
Chitosan and TiO ₂	Apple pomace—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	Films changed their color from red to light pink when the salmon was deteriorating.	Salmon	(Lan et al., 2021)
Composite of polyvinyl alcohol, microcrystalline cellulose and polyvinyl pyrrolidone	Red cabbage—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	- Foams with anthocyanins were successfully developed; - Foams changed their color when a pH change occurred; - Foams changed from purple to greenish-grey after 3 days in contact with prawn and chicken meat, aligned with food products degradation.	Prawn and chicken	(Zia et al., 2021)
Chitosan and Oxidized chitin nanocrystals	Black rice—anthocyanins	Sensitivity of the films to volatile nitrogen compounds through pH change	Films reacted to the contact with shrimp and promfeta, and a visible change of color was observed;	Shrimp and promfeta	(Wu et al., 2019)

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
			Films changed from purple to grey-blue color aligned with the food degradation		
Quaternary ammonium Chitosan-Polyvinyl alcohol	Cactus pears extract (CPE)—betalains	Sensitivity of the films to volatile nitrogen compounds through pH change	- Color of the films changed when CPE was added; - CPE addition affected mechanical, structural, antioxidant and antimicrobial properties of the films; - Films showed sensitivity to volatile nitrogen compounds present on the shrimp container.	Shrimp	(Yao et al., 2020)
Quaternary ammonium chitosan—Fish gelatin	Amaranth—betalains	Sensitivity of the films to volatile nitrogen compounds through pH change	- Physical and functional properties of the films were enhanced by amaranth extracts; - pH changes due to the increase in volatile nitrogenous compounds caused a color change from pink to yellow.	Shrimp	(Hu et al., 2020)
Starch-Polyvinyl alcohol	Red pitaya—betalains	Sensitivity of the films to volatile nitrogen	Physical and functional properties of the films were enhanced by red pitaya extracts;	Shrimp	(Qin et al., 2020)

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
		compounds through pH change	pH changes due to the increase in volatile nitrogenous compounds caused a color change from pink to yellow.		
Gelatin-Polyvinyl alcohol	Amaranth—betalains	Sensitivity of the films to volatile nitrogen compounds through pH change	- Physical and functional properties of the films were enhanced by Amaranth leaf extracts; - Films changed color from red to yellow following food products degradation.	Chicken and Fish	(Kanatt, 2020)
Cellulose	<i>Arnebia euchroma</i> -Naphthoquinone	Sensitivity of the films to volatile nitrogen compounds through pH change	- Films demonstrated a color response in a pH range from 5 to 12; - Films changed their color from rose-red to blue-violet when pH changes occurred.	Pork and shrimp	(Dong et al., 2020)
Cellulose paper	Gromwell (<i>Lithospermum erythrorhizon</i>)—Shikonin	Sensitivity of the films to volatile nitrogen compounds through pH change	Films changed from red to blue because of fish and pork degradation.	Fish and Pork	(Ezati et al., 2021)

Matrix	Sensor Material (Source/Type)	Function	Major Results	Food Application	Ref.
Agar—Polyvinyl alcohol	Curcumin	Sensitivity of the films to volatile nitrogen compounds through pH change	Films changed their color from, yellow to orange-red due to ammonia production from shrimp degradation.	Shrimp	(J. Zhang et al., 2021)
Tara gum and Polyvinyl alcohol	Curcumin	Sensitivity of the films to volatile nitrogen compounds through pH change	Films changed their color from, yellow to orange-red due to ammonia production from shrimp degradation.	Shrimp	(Ma et al., 2017)
Pectin and sulfur nanoparticles	Curcumin	Sensitivity of the films to volatile nitrogen compounds through pH change	- Addition of curcumin and sulfur nanoparticles improve thermal stability and UV light barrier. - A color change from yellow to orange was observed caused by shrimp degradation.	Shrimp	(Ezati & Rhim, 2020)
<i>Lallemantia iberica</i> seed gum	Curcumin	Sensitivity of the films to volatile nitrogen compounds through pH change	- Mechanical and barrier properties of the films were enhanced by Amaranth leaf extracts; - Films changed their color from, yellow to red due to ammonia production from shrimp degradation.	Shrimp	(Taghnia et al., 2021)

2.2.3.1 Anthocyanins Used as Bio-Based Sensors

Anthocyanins are natural water-soluble pigments present in several plants and fruits, they are classified as flavonoids and their antioxidant, anti-inflammatory, and anticarcinogenic properties are reported in the literature (Roy & Rhim, 2020). They are responsible for red, purple and blue color of a variety of plants and fruits and highly reactive to pH changes (their chemical structure changes with the changes in the pH, which result in different colors) (Castañeda-Ovando et al., 2009; Kurek et al., 2018)(Figure 2.6).

A pH-sensitive bilayer hydrogel film was developed using one layer made of gelatin and ZnO nanoparticles incorporated and the second layer made of gellan gum with anthocyanin extracts to monitor fish spoilage (Yang et al., 2021). Anthocyanins were extracted from mulberry fruits and their response to pH variations was studied. From pH 2 to 6, they show colors going from light pink to colorless. From pH 7 to 12, anthocyanins change their color from light yellow-green to orange color. The color stability of anthocyanins was improved by using ZnO nanoparticles on the upper layer of the film, acting as a UV-light barrier. Additionally, the bilayer film is suited for electrochemical writing. To monitor fish spoilage, crucian was placed in a box with a lid and a hole was made on it, and the bilayer film was placed covering the hole. Total volatile basic nitrogen (TVB-N) content and color changes were evaluated, showing that after 6 days refrigerated storage, the color changes occurred on the film, going from an initial pink to light green, and finally yellow as a result of the increase in the TVB-N content, which represents the degradation of the fish packaged. The color changes on the film are linked with the production of NH_3 , which could hydrolyze the hydroxyl group (OH^-) present on the hydrogel film and create alkaline conditions. Under these conditions, anthocyanins changed their structure from flavylium ion to a chalcone structure that is noticed by film color changes (Castañeda-Ovando et al., 2009). The use of nanoparticles improved the stability of the films and did not interfere with NH_3 -sensing properties. Additionally, the electrochemical writing ability of the films constitutes a green printing technique that allows the display of the information directly on the film, constituting an innovative way to present important information for the consumers. The films developed can act as an intelligent sensor on the detection of fish spoilage (Yang et al., 2021). However, no tests were done regarding their antimicrobial and antioxidant activity.

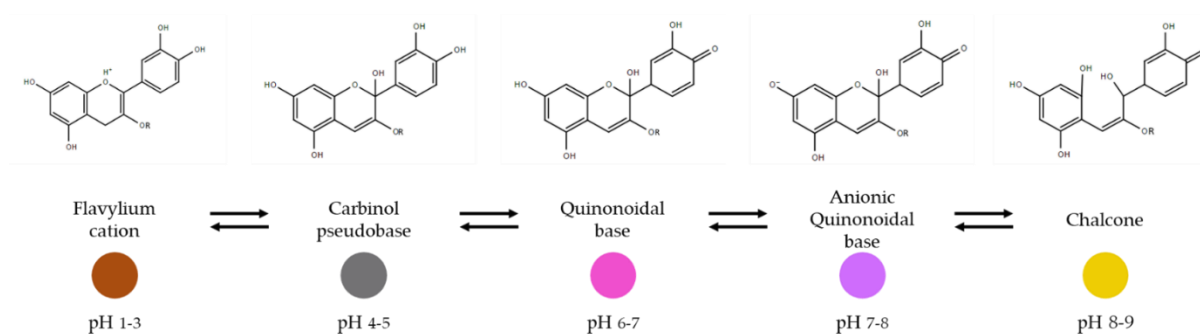


Figure 2.6 - Changes in anthocyanins structure due to pH changes, adapted from (Roy & Rhim, 2020).

The use of anthocyanins as a pH sensor to monitor beef and fish was studied by Vedove and collaborators (Vedove et al., 2021). Cassava starch sheets were produced by extrusion process with the introduction of anthocyanins during the process. Cassava starch sheets with different concentrations of anthocyanins (5, 10, and 20 mg per 100 g) were then placed on top of a falcon tube and samples of beef and fish were introduced in the tube, kept at 6 °C (beef) and 22 °C (fish), and the changes were evaluated for 3 days. The mechanical and physical properties of the cassava starch sheets were altered by the introduction of anthocyanins, resulting in an increase in swelling, reduction in flexibility and lower hydrophilicity compared to pristine cassava starch samples. As food degradation started, gas was produced (due to TVB-N production), pH increased, and the headspace environment became more alkaline. The gas production causes an hydrolyzation of the anthocyanins present on the cassava starch sheets and consequently, a slight color change occurred. The color change was more intense on the cassava starch sheets with a higher content of anthocyanins on both food products, nevertheless, authors reported that an improvement in the production and formulation is needed since a poor color change can confuse the consumers (Vedove et al., 2021). No tests were performed in order to evaluate the antimicrobial and antioxidant properties of the films. Anthocyanins extracted from red cabbage and roses were also studied to act as a pH indicator sensor to evaluate meat food spoilage (Shukla et al., 2016). Anthocyanins were mixed in an agarose solution and left to dry, and the performance of the indicator was evaluated firstly in vitro tests under ammonia production that simulates the meat degradation process, and then in situ in contact with buffalo meat. Once again, a color change occurs, going from red to green when the pH increases, a consequence of the production of volatile nitrogenous compounds that causes a conformational change of the structure of the anthocyanins (Wrolstad et al., 2005). Both tests demonstrated the potential of the film to be used as freshness indicator. However, no tests to access the antimicrobial and antioxidant activity were done. Other authors developed pH-sensitive films based on chitin nanofibers and methylcellulose with anthocyanins

extracted from barberry (*Berberis vulgaris* L.) fruits as sensing material (Sani et al., 2021). Films were produced by casting method and the anthocyanins extract was introduced in the chitin nanofiber and methylcellulose film solution. To assess the pH-sensitive films capacity and their response under high levels of ammonia, films were introduced on the fish packaging container and evaluated for 72 h. Once again, when the pH environment changed due to ammonia production, a color change from reddish to pale pink was observed indicating food degradation. However, these pH-sensitive films, were tested not only in terms of colorimetric response to the pH change, when facing an ammonia content increase, but also in terms of potential antibacterial and antioxidant activities. It is noteworthy that this is an important feature of smart packaging: the combination of intelligent properties conferred by the color changes provided by anthocyanins as a reaction to food degradation with active properties being important for food preservation. Indeed, films demonstrated to have a strong antioxidant capacity similar to a synthetic antioxidant (BHT). Nevertheless, this latter feature was only accessed on films and not on food products. Moreover, the effect of anthocyanins on the film matrix appeared to improve mechanical, optical, and physical-chemical properties.

Concerning nitrogenous volatile compounds, a sensor was developed consisting of a film of pectin with red cabbage extract rich in anthocyanins that were incorporated as an indicator (Dudnyk et al., 2018). The sensor was produced through casting method and the red cabbage extract was added to the filmogenic dispersion before it was left to dry. The use of plasticizers was determinant to maintain the integrity of the films. The effectiveness of the sensor was firstly tested by exposing the film to several amines and verified through UV-Vis spectroscopy, as amines causes a pH change to which anthocyanins are highly reactive changing films color from purple to orange or yellow (Halonen et al., 2020). Tests on different food products (beef, chicken shrimps and fish) were assessed by attaching the film to the headspace of the different food products containers. It was demonstrated a similar color change from purple to yellow that fits the results observed for the volatile nitrogenous compounds production and the increase in microbial growth. Films were made of edible components and demonstrated their potential to be used as freshness indicators in intelligent packaging. However, no tests were done regarding their antimicrobial and antioxidant activity.

Blueberry and red grape skin extracts were applied in two different matrices (chitosan and carboxymethyl cellulose) to monitor poultry meat freshness (Kurek et al., 2019). The addition of the extracts to the polymeric matrixes did not affect the mechanical properties of the films. Due to the different constitution of the polymeric matrixes, the use of anthocyanins rich

extracts caused different color changes when in contact with poultry meat. The films were placed directly on the poultry meat and when the food started to deteriorate a color change on the films was observed affected by a change in the pH caused by the production of amines, turning into alkaline conditions. Carboxymethyl cellulose (CMC) films changed their color from violet to pink when blueberry extract was used and from red to pink for red grape seed extract. In chitosan films, the color changes were less evident due to the difference between polymeric structures and rearrangements after the addition of anthocyanins, making the first polymer matrix more suitable to incorporate the extract and to be used as a pH sensor in poultry meat. In these tests, films were only essayed for its intelligent character and not for its active character.

Artemisia sphaerocephala Krasch. gum was used as a film-forming solution with the addition of carboxymethyl cellulose sodium as host-complex for red cabbage extract rich in anthocyanins (Liang et al., 2019). Thus, a film was prepared to act as a pH and NH₃ indicator. To improve anthocyanins stability (positively charged), carboxymethyl cellulose was added due to its attraction to positive and negative charges, avoiding anthocyanins migration (G. Jiang et al., 2020). Red cabbage extract influenced the mechanical and optical properties of the films. In contact with different buffer solutions, the color of the films changed from rose-bengal to aquamarine color and proved to be effective to act as a sensor for pH changes. Furthermore, NH₃ response was observed by a more pronounced color change. In this study, antimicrobial and antioxidant activities were not accessed.

Milk and shrimp freshness were evaluated through a pH sensor indicator film made of K—carrageenan and *Lycium ruthenicum* Murr. extract rich in anthocyanins (J. Liu et al., 2019). Different concentrations of extract (1.5, 2.5, 3.5, 4.5 wt%) were used and it was noted that an increase in extract concentration affected the film structure, optical, mechanical, and barrier properties negatively, so only the film with 2.5 wt% of the extract was used to monitor milk and shrimp freshness. A color change from grey to dark pink was observed when the film was in indirect contact with milk after 48 h (the film was placed on the top of the milk container). The color change occurred due to a decrease in the pH of the milk, caused by the production of lactic acid. On the other hand, a change in the pH from acidic to alkaline conditions caused by volatile amines produced by the shrimps turned the films from light grey to yellow in 72 h (J. Liu et al., 2019). Lower concentrations of extract end up being more suitable to be used to produce films, with fewer effects on the film structure. Besides the colorimetric response to pH changes, the films also demonstrated good antioxidant activity turning them suitable to be

used in smart packaging. However, in this study the active action of the packaging directly on food was not addressed.

Anthocyanins from red radish were used to develop a pH-sensitive film made of starch and gelatin (Chayavanich et al., 2020). The anthocyanins rich extract was introduced on the film-forming dispersion after the gelatin and starch and cast. Films showed a color response in a pH range from 2 to 12 when exposed to different solutions and demonstrated good mechanical properties. Films were attached to the top of Petri dishes to have contact with poultry meat and prawn environment during storage (0 °C, 4 °C, and 30 °C). At any temperature and for both food products, films changed their color from red to grey–purple, because of the production of volatile nitrogenous compounds that caused deprotonation of anthocyanins (Bhargava et al., 2020). The use of two biopolymers that interact with the anthocyanins avoided the pigment to be released into the water. Storage temperature was an important factor for anthocyanins stability once there is a preference for lower temperatures (Sinela et al., 2017). The antimicrobial and antioxidant activities were not evaluated in the study.

Jambolan skin extract is also a source of anthocyanins, was used as a sensing material to develop pH reactive films (da Silva Filipini et al., 2020). Jambolan is a fruit with low economic value and is highly perishable so the valorization of the skins for the recovery of anthocyanins for packaging use adds value for this particular product (Merz et al., 2020). The extracts were incorporated into the film-forming dispersion in different concentrations (10, 30, and 50% *v/v*), using methylcellulose as a matrix to develop films through the casting method. When exposed to different buffer solutions (pH range 1 to 10), all films showed a colorimetric response, changing their color from red and pink (acidic conditions) to purple and blue (alkaline conditions). Jambolan fruit extracts improved the mechanical and barrier properties of the films and demonstrated an increase in antioxidant activity (da Silva Filipini et al., 2020). Therefore, the films proved to be well suited to smart packaging due to their ability to provide a colorimetric response and also to demonstrate antioxidant ability, both by the action of anthocyanins. Further studies are needed to address the active action as a packaging material since it was only studied on standalone films.

Red apple pomace extract was incorporated into chitosan-based films with TiO₂ nanoparticles to develop intelligent packaging (Lan et al., 2021). Red apple pomace is rich in anthocyanins and a percentage of 5% *w/w* was used to develop films, through the casting method. TiO₂ was used to improve the stability and mechanical properties of the films (Oleyaei et al., 2016). Films were placed inside the top of a petri dish containing salmon and their colorimetric

pH response was evaluated during 48 h. Films modified their color from red to a light pink following the pH variations from 6 to 8 that occurred when the extracts were exposed to different buffer solutions. The use in simultaneous of nanoparticles and anthocyanins not only improved the mechanical properties and resulted in a film with pH-sensing properties, but also enabled the films to possess a strong antioxidant and antibacterial capacity with future potential application in smart packaging.

Black rice, a source of anthocyanins, was used as a sensing material used in a matrix composed of oxidized-chitin nanocrystals and chitosan to develop films (Wu et al., 2019). Films were prepared through the casting method and the anthocyanins extract was added in 0, 1, 3, and 5% *w/w* concentration. Films were placed on the surface of shrimp and promfet fish to evaluate its reaction as pH sensor. A color change was observed on the films with 3 and 5% *w/w* anthocyanins concentrations when exposed to food, which turned from an initial purple color to a grey-blue and brown, respectively. The total volatile basic nitrogen increased and was in solid accordance with the color changes observed, indicating food spoilage for both food samples. Furthermore, the films revealed a strong antioxidant capacity demonstrating a promising potential to be also applied as smart food packaging.

It is worth mentioning that, despite most of the advances are based on the development of films with sensing properties providing a color change, other materials are being developed with the same purpose. Regarding this, a porous foam pH indicator using polyvinyl alcohol, polyvinyl pyrrolidone, and reinforced with microcrystalline cellulose was attempted using anthocyanins from red cabbage as sensing material (Zia et al., 2021). In this work, a porous foam was developed using a freeze dryer and the resulting material was immersed on the anthocyanins extract. The resulting material was tested on prawns and chicken to evaluate its ability to work as a pH indicator. For both prawn and chicken, the indicator showed an initial purple color that faded into greenish-grey after 3 days of storage. The color changes followed the change of the pH environment that was initially acidic and became basic, showing the potential of the foams to be used in intelligent packaging as a colorimetric pH indicator for food spoilage. The assessment on antimicrobial and antioxidant activities was not done

Overall, anthocyanins are natural pigments, with antioxidant and antimicrobial properties and sensitivity to pH, so with interesting properties to construct a bio-based sensor for smart packaging systems (Koosha & Hamed, 2019; Mushtaq et al., 2018; Wu et al., 2020). Yet, it is of extreme importance to guarantee their stability to be used as indicators. Anthocyanins are highly sensitive to temperature alterations, which causes a loss of effectiveness of the

product, so it is important to maintain their stability (Cavalcanti et al., 2011). High temperatures leads to anthocyanins degradation, resulting in browning so lower temperatures are more desirable to maintain their stability (Sinela et al., 2017). Oxygen and light also contribute to anthocyanins degradation (Cavalcanti et al., 2011; Rein & Heinonen, 2004; Yang et al., 2021). To overcome this constraint, bilayer films can be produced where the anthocyanins can be incorporated in the inner-layer, in contact with food, and compounds such as ZnO can be added to an outer layer of the matrix to counteract light induced degradation (Cavalcanti et al., 2011; Rein & Heinonen, 2004; Yang et al., 2021). To develop a feasible film with sensing properties, the use of a proper biopolymer as the polymeric matrix for the incorporation of natural pigments is crucial (Yong & Liu, 2020). The interactions of the biopolymer with the sensing material causes a rearrangement on the final matrix and causes structural changes of anthocyanins that influence the final color of the films (Kurek et al., 2019). The color of the films enriched with anthocyanins also depends on the nature of biopolymers, with a preference for neutral biopolymers such as starch and tara gum, that interacts with the pigment through hydrogen bonds thus with low interference on the color of the pigments (Ma & Wang, 2016; Yong & Liu, 2020). In addition, the origin of the natural pigments is important to provide a visible color to the film, and to the color change promoted by the alterations of the food product, since anthocyanins from different origins have different colors and chemical structures (Kurek et al., 2018; Yong et al., 2019). Moreover, the concentration of anthocyanins incorporated is determinant to produce a successful film. An excessive amount can disrupt the structure of the film matrix caused by the aggregation of anthocyanins (Yong & Liu, 2020). Regarding sensor-pigments, some studies indicate that it is important to immobilize them in the polymer, avoiding its migration to the food products, so that its sensor activity could be maintained. Yong and Liu suggests the addition of hydrophobic compounds to the film matrix to prevent undesirable migration to the food products (Yong & Liu, 2020). Although pigments immobilization in the polymeric matrix will limit its activity, e.g., antioxidant or antimicrobial, still they can act as scavengers or absorbers or they can act directly to the area of the food that is in closed contact with the polymer. Additionally, mechanical and barrier properties of the films can be changed due to the incorporation of anthocyanins, and a balanced blend of reinforcements and plasticizers together with the pigments can help to maintain or enhance the food packaging efficiency (Cazón et al., 2017a). The incorporation of anthocyanins in a biodegradable film is considered a promising alternative to indicators and sensors that are produced using non-degradable sources.

2.2.3.2 Betalains used as bio-based sensors

Betalains are water-soluble nitrogen-containing pigments located in the vacuoles of some plants tissue (Melgar et al., 2017). Betalains exhibit antioxidant capacity acting as radical scavengers, showing positive effects in the inhibition of lipid peroxidation and heme decomposition at a very low concentration (del Socorro Santos-Díaz et al., 2017; Gengatharan et al., 2015). Their common biosynthetic precursor is betalamic acid and these pigments appear to be stable between a pH from 3 to 8 (Figure 2.7) (del Socorro Santos-Díaz et al., 2017; Melgar et al., 2017).

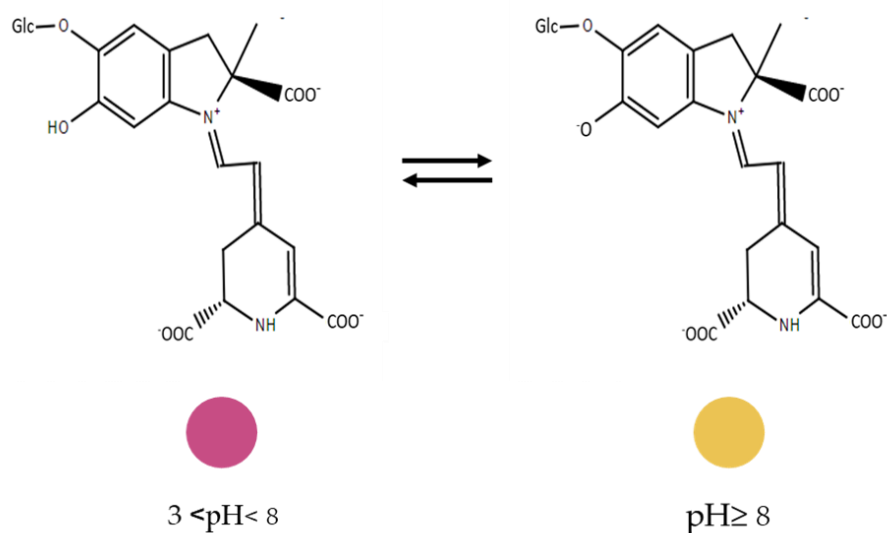


Figure 2.7 - Changes in betalains structure due to pH changes, adapted from (Alizadeh-Sani et al., 2020).

Yao and collaborators (Yao et al., 2020) developed quaternary ammonium chitosan and polyvinyl alcohol films (QAC-PVA) with the incorporation of betalains rich cactus pears extract (CPE) to act as a freshness indicator of fresh shrimp quality. Betalains are sensitive under alkaline conditions ($\text{pH} \geq 8$), turning from red-violet to yellow-orange with the pH alterations (Figure 2.7). CPE was added to QAC-PVA blend and then dried. Films were introduced into the headspace of the shrimp container to evaluate its activity. The addition of CPE (2 and 3% *wt%*) provided color changes of the films, moving from an original purple color to an orange when the freshness of the shrimp decreased at 24 h (Yao et al., 2020). Additionally, the addition of CPE to the films enhanced the UV-Vis light barrier, the elongation at break, antioxidant and antimicrobial properties. Films showed potential to be used in smart packaging due to their

intelligent and active features, although its activity was only evaluated on the films and not on the food.

Another pH sensor for ammonia detection was reported, using amaranth as the source of betalains and a blend of quaternary ammonium chitosan and fish gelatin to form a film and evaluate the freshness of shrimp (Hu et al., 2020). Betalains were extracted from amaranth and introduced in different concentrations (5, 10, and 15 *wt%*) in the filmogenic solution. The films were placed on the inner cover of the shrimp container and a color change from pink to yellow was observed, due to the betalains reaction to pH changes caused by the increase in volatile nitrogenous compounds. Moreover, the physical and functional properties of the films were studied demonstrating that the use of amaranth extract enhanced most of them. It was demonstrated that an increase in betalains concentration improved both the antioxidant and antimicrobial capacity of the films, however those activities were not accessed *in situ*. Those films demonstrated promising results to be used in smart packaging.

The same authors developed polyvinyl alcohol and starch films (2:1 starch:polyvinyl alcohol) incorporated with betalains extracted from red pitaya peel (Qin et al., 2020). Betalains were extracted and incorporated into the film solution on different concentrations (0, 0.25, 0.50, 1 *wt%* starch basis). The inclusion of betalains extracts improved the film's mechanical, water vapor, and light barrier properties when compared with the control films of pristine polymers. The films containing the betalains-rich extract were placed in the headspace of a petri dish containing fresh shrimps to test their freshness at 20 °C and for 48 h. Initially, the films possessed a pink color that was maintained for 24 h, but after, they turned into yellow, demonstrating its intelligent application. Moreover, the antioxidant and antimicrobial properties of the films were studied, indicating that the addition of this pigment improves those functionalities. Films with 1% of the betalain-rich extract were the most promising to be applied as a freshness indicator and due to their antioxidant and antimicrobial properties they have shown potential to be applied in smart packaging systems, which was not assessed *in situ*.

Kanatt and collaborators (Kanatt, 2020) developed active and intelligent films made of gelatin and polyvinyl alcohol with the incorporation of Amaranth leaf extracts mainly rich in betalains. The extracts were incorporated into the film-forming solution and films were prepared by the casting method. The addition of the extracts enhanced the physical and functional properties of the films. To be applied on fish and chicken, pouches were made through heat sealing, so in this case, the film was in contact with the food product. Films changed their color from red to yellow following the chicken and fish degradation. That color change was in

accordance with the TVB-N content, pH, microbiological quality, and oxidative rancidity evaluated on the food products. Overall, the films demonstrated the potential to be used as smart packaging.

Betalains are gaining more attention due to their potential to be used as natural sensors in food packaging. Betalains stability is affected by extrinsic factors that include oxygen, temperature, and light (Rodriguez-Amaya, 2019). To obtain the maximum potential as a colorimetric indicator it is important to maintain the functionality of the pigment. Pigment concentrations used to develop films must be carefully evaluated otherwise, they could affect the mechanical and physical properties of the films due to agglomeration of the pigments when used in excess (Kanatt, 2020). Betalains when introduced in films tend to improve their physical, barrier and antioxidant properties (Castro-Enríquez et al., 2020). Opacity is one of the parameters that are positively affected by the addition of these types of pigments by acting as a light barrier thus it may prevent food oxidative degradation (Akhtar et al., 2012; Gutiérrez et al., 2016). Since betalains are more pH-stable than anthocyanins, their use in smart packaging constitutes a very promising alternative.

2.2.3.3 Curcumin Used as Bio-Based Sensor

Curcumin is a polyphenol extracted from the roots of turmeric and exhibits antioxidant, antimicrobial, anti-inflammatory activity and presents a strong yellow–orange color (Balbinot-Alfaro et al., 2019; Bhargava et al., 2020). From a pH range of 1 to 7, it presents yellow coloration and above a pH of 8, curcumin color changes to red–orange (Balbinot-Alfaro et al., 2019) (Figure 2.8).

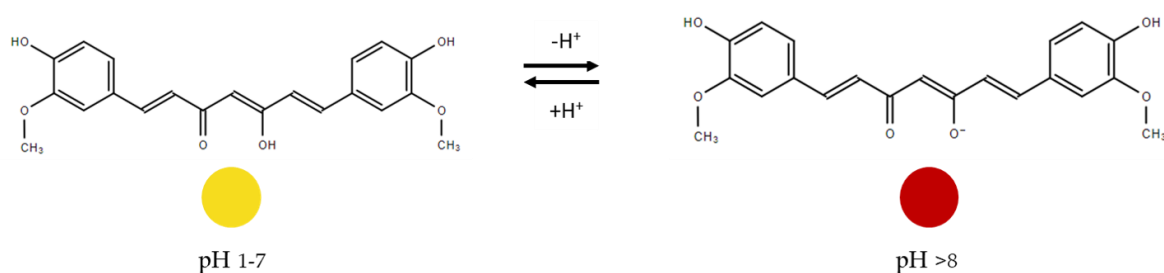


Figure 2.8 - Changes in curcumin structure due to pH changes, adapted from (Halonen et al., 2020).

Curcumin was used as a sensing material to develop a film with tara gum and polyvinyl alcohol (Ma et al., 2017). Different concentrations (0, 0.1, 0.3, and 0.5% *w*t%) of curcumin were added to the filmogenic dispersion, and films were developed through the casting

method. Curcumin addition caused a slight decrease in the barrier properties of the films. To evaluate the sensing ability of the films to act as intelligent packaging, films were placed on the inside of a petri dish containing fresh shrimp, and the changes in color were studied at ambient temperature. At the initial time, films showed a yellow color and at the end of the experience presented an orange–red color. This was due to pH changes of the shrimps, caused by their degradation and resulting in the increment of the total basic volatile nitrogen content through ammonia production. The antioxidant and antimicrobial properties were not evaluated in this study. The film showed potential to be used in intelligent packaging.

In another study, agar-PVA films with curcumin provided a color change from yellow to orange–red when the shrimp freshness decline (J. Zhang et al., 2021). Hydrogel films were prepared by the casting method by dissolving the curcumin (0, 1, 2, and 3% *w/w*) on the agar and polyvinyl alcohol mixing solution. Additionally, a print label was developed through electrochemical writing. The ammonia sensitivity of the curcumin films was assessed using only the 2% *w/w* curcumin film, by placing it inside the shrimp container. In the presence of ammonia, pH increased and films could change their color, indicating shrimp degradation thus demonstrating potential to be used as an indicator in intelligent packaging. The antioxidant and antimicrobial properties were not accessed in this study. Moreover, the electrochemical writing on the films was accomplished adding value to the indicator, acting as a new possibility to provide information about the product.

Pectin-based films incorporated with curcumin and sulfur nanoparticles were developed to produce a film to monitor shrimp freshness during storage (Ezati & Rhim, 2020). Films were prepared through the casting method. The addition of curcumin and sulfur nanoparticles improved thermal stability and UV-light barrier properties. Films were placed on the cover of the shrimp container and kept under 25 °C for 36 h to evaluate the colorimetric response to pH changes. Films changed from yellow to orange, caused by the changes on pH due to the production of volatile nitrogen compounds revealing shrimp degradation and, therefore, showing potential to be applied as intelligent packaging. Furthermore, films showed strong antibacterial and antioxidant properties to be applied as active packaging. However the effect of those parameters was not accessed directly on food products. This solution showed potential application in smart packaging

Curcumin and *Lallemantia iberica* seed gum, as the matrix, were used to develop films to monitor shrimp freshness (Taghinia et al., 2021). Films were prepared by the casting method using tween 80 as emulsifier. Films showed improvement in the water vapor barrier and also

the mechanical properties when curcumin was added. Shrimps were covered with the films and stored at 25 °C and after 5 days, their color changed from an initial yellow to red color. These results followed the increase in TVB-N measured on the shrimps that lead to a pH change, causing the color changes. Moreover, the films containing curcumin revealed to have antimicrobial and antioxidant properties, thus demonstrating potential to be used also as active packaging. Yet, those effects were not tested directly on the preservation of food products. Overall, the film could be applied in smart packaging, either to monitor and to delay seafood degradation.

Curcumin possesses antimicrobial, and antioxidant activity, and it has the potential to participate as an active agent to extend certain food products shelf life (Tyagi et al., 2015). When added to films their mechanical properties are not affected (at least with the quantities that have been studied) and its hydrophobic nature contributes to increase film's hydrophobic properties (Ma et al., 2017). In addition, the thermostability of curcumin may also improve the stability of the film (N. Luo et al., 2012). The combination of its active properties with its indicator properties (color change with pH change) demonstrates clearly the great potential of the use of curcumin on films as a bio-based sensor for smart packaging.

Other natural pigments have been studied to be used as sensors in food packaging. Naphthoquinones are pigments that are sensitive to acid and basic environments so have the potential to be used as indicators (M. H. Cho et al., 1999; V. P. Papageorgiou et al., 1999). Moreover, they can improve the moisture resistance of the films due to their hydrophobic character (V. Papageorgiou et al., 2006).

Naphthoquinone dye and cellulose extracted from *Arnebia euchroma* were used to develop a pH indicator for food spoilage (Dong et al., 2020). Cellulose pulp was used to make a cellulose-based film, and naphthoquinone dye solution was introduced through impregnation on the film. Films were placed in the headspace of Petri dishes containing pork and shrimp samples. The increment of the total volatile basic nitrogen, as an important indicator for food spoilage (Kuswandi, 2017), followed by a pH change, induced a colorimetric response of the films. Films demonstrated a response in a pH range from 5 to 12. A color change from rose-red (fresh) to purple (gentle spoilage) and finally to blue-violet (total spoilage) represented the different phases of food spoilage for both products. Neither antioxidant nor antimicrobial activities were evaluated on the films.

A similar pH sensor based on naphthoquinone (shikonin) and cellulose was also developed (Ezati et al., 2021) and a similar method was applied as aforementioned to develop

the color sensing film. Shikonin, a red naphthoquinone pigment, was extracted from gromwell root (*Lithospermum erythrorhizon*) to be used as color sensor (Ezati et al., 2021). Film application on fish and pork packages showed a similar outcome, revealing a color change from red to blue as consequence of the pH increase in the environment due to the production of TVB-N. These authors also accessed the antioxidant activity of the films produced, revealing that shikonin enhanced this parameter on the paper demonstrating their potential to be used not only as intelligent packaging, but also as active packaging. In this regard, tests of that activity on food must be attempted to verify if there are beneficial effects on extending shelf-life. Moreover, both studies demonstrated that naphthoquinones are color stable under acidic conditions.

2.2.3.4 Other potential Applications of Bio-based sensors

Although there is a lack of information about smart packaging systems for fruits and vegetables there have been some recent advances in the field. Fruits and vegetables are highly perishable, a consequence of the respiration process (CO₂ release) and the ethylene production, which promotes faster degradation. Ethylene is a plant hormone that participates in the acceleration of the ripening of the fruits (Jedermann et al., 2014). Scavenging and monitoring ethylene content, as well as reducing its amount in the packages atmosphere can provide valuable information and is important to avoid faster degradation of the perishables (N. Iqbal et al., 2017). There are ripeness sensors commercially available as is the case of ripeSense™, a sensor that reacts to aromas emitted by the ripening of the fruit, changing its color from an initial red to yellow (*RipeSense*, n.d.). The color changes indicate the fruits current ripeness level, allowing consumers to make a choice based on their preferences. A chemo resistive sensor was also developed to detect concentrations of ethylene at ppm levels using single-walled carbon nanotubes and a Cu(I) complex, that binds with the ethylene molecules, changing the resistance of the sensor which is then measured by the transducer (Esser et al., 2012). More recently, a polydiacetylene-based sensor for ethylene detection was developed, based on a colorimetric response (Nguyen et al., 2020). The sensor mechanism is based on the reaction between polydiacetylene and Lawesson reagent through the substitution of carboxylic groups with thiol, which when exposed to ethylene changed from blue to red following the increase in the ethylene concentration. Developing a bio-based sensor that could act in a similar manner would contribute to a more sustainable solution to pack fruits and vegetables.

Carbon dioxide (CO₂) is an important food additive and food packaging quality indicator. When food starts to deteriorate, the CO₂ levels start to increase mostly due to

microorganisms growth (Puligundla et al., 2012). Additionally, CO₂ is used in modified atmosphere packaging to prevent microbial growth (Bhargava et al., 2020). A CO₂ indicator was developed based on the colorimetric response of a blend of lysine, ϵ -polylysine and anthocyanins (Saliu & Della Pergola, 2018). This blend was introduced in an ethylcellulose solution to form a label and the effectiveness was assessed under simulated conditions and poultry meat. The use of ethylcellulose confers to the coating material a permeability to CO₂ and impermeability to protons, avoiding pH-sensitive results. The CO₂ detection by the sensor is directly caused by the alteration of the amino acid, and the formation of carbamic acid that causes a pH alteration and induces a colorimetric response by the anthocyanins (Alshamrani et al., 2016). The CO₂ indicator label, under cold storage conditions, demonstrated to be reactive to CO₂ exposure, changing from a blue to a purple color when the CO₂ concentration increased, and demonstrating the potential to be used in monitoring food freshness. Oxygen is a disadvantageous trigger for food degradation, and in that sense, modified atmosphere packaging (MAP) is used to limit the interactions of the food products with the surrounding environment. Typically, it consists in low O₂ concentration (0–2%) and high CO₂ concentration (20–80%) (Yousefi et al., 2019). Ageless Eye™ is a commercially available redox indicator based on the color change of methylene blue to colorless when the dye is oxidized due to the increase in O₂ levels when the package is compromised, acting as a leak indicator (Alam et al., 2021; Banerjee et al., 2016; Chemical, n.d.). This type of solutions could be explored, modified, and tested in order to construct a bio-based sensor with potential to be applied in intelligent packaging with the same functionalities.

The use of nanosensors has a great interest in smart packaging due to their potential ability to detect, identify, and quantify pathogens, toxins, allergens, and other chemical markers (C. Sharma et al., 2017). When introduced in food packaging they can be useful to monitor food freshness and help in the determination of a more accurate shelf-life (Primožič et al., 2021). Several nanosensors have been developed and used in a wide range of applications, and a more detailed overview is given in lately published reviews (Alahi & Mukhopadhyay, 2017; Griesche & Baeumner, 2020; Shafiq et al., 2020). However, and although some nanosensors have showed efficiency in detecting certain contaminants, their application in food packaging has not yet been applied.

Temperature is another determinant factor related to food spoilage that impacts food shelf life (Yousefi et al., 2019). A critical temperature indicator based on a solvent melting point was developed, combining radio frequency identification with carbon nanotubes films (Lorite

et al., 2017). This indicator is based on critical temperature. When this temperature is reached, the solvent flows through a capillary, soaks the carbon nanotube film and increases its resistance which is detected by the RFID. This type of sensor can be potentially useful to be used during storage through all the supply chain. A similar solution, more bio-based, could also be developed, in order to provide a structure that would be of interest by the food distribution chains, turning their business more sustainable.

2.2.4 Conclusions

Food packaging is an important factor to preserve and maintain the quality of food products. The use of intelligent packaging constitutes an innovative path to provide more accurate information not only for the suppliers but most importantly for the consumers. Indeed, these new packaging systems, can be designed to inform the quality of the products being packed and allow to identify the degree of food deterioration, with benefits in terms of food safety and quality. Intelligent food packaging is also a promising area for the development of new and bio-based packaging systems fitting the actual global consumers' demands and the awareness for the environmental issues caused by petroleum-based materials.

In this review, a focus was made on bio-based freshness indicators and their applications. These bio-based solutions provide information about the degree of freshness of a product and have been mostly employed in perishable products like meat, fish, and seafood. Most of the indicators tested used natural colorants and biopolymers (polysaccharides or proteins) and the freshness sensors developed were based on the pigments color change linked with a pH change. Once the components used can be obtained from biomass and biobased by-products, their development and application also contribute to a circular economy and a bioeconomy concept, in line with the Sustainable Development Goals of the United Nations and with the European Green Deal set by the European Commission.

The pigments anthocyanins, curcumin, and betalains, generally extracted from fruits and plants and their wastes, were the most studied options to develop the bio-based sensors. These pigments have also antimicrobial and antioxidant properties, and when integrated with a package they can also provide some activity to the biopolymer, helping to increase the shelf-life of the products being packed. Therefore, these bio-based sensors represent a potential application in smart food packaging systems. Despite their positive effect to be used as pH sensors, these pigments show some instability to temperature, O₂, and light. So more studies are

needed to improve the efficiency and stability of the indicators in the polymeric matrix. Encapsulation of the pigments can immobilize the compound inside solid particles or liquid vesicles to maintain stability, structure, and also to regulate the release of the pigment (Castro-Enríquez et al., 2020; Cavalcanti et al., 2011). The use of nanoparticles, such as ZnO nanoparticles, as UV-light blocking agents is also an effective mechanism to protect and immobilize pigments (Sothornvit, 2019; Yang et al., 2021). Nanoclays, such as montmorillonite (MMT), commonly used with reinforcement purposes, also show promising results as pigments stabilizers (Ribeiro et al., 2018; Sothornvit, 2019). Moreover, the combination of two different pigments to increase the sensor's stability and the variety of color changes can be a novel strategy. For example, by mixing alcohol-soluble pigments and water-soluble pigments the possibilities to use as indicators are enhanced, becoming suitable for both water-soluble and fat-soluble foods (Roy & Rhim, 2020). The use of bilayers is also a strategy that can be applied to help stabilizing the pigments.

It would be also interesting to study a combination of these pigments with other active compounds (such as essential oils that can be obtained from a variety of aromatic plants or industrial crops), in the biocomposites, and to test them in situ, to certain types of food, to understand its potential as smart food packaging. Besides, there is a need to develop bio-based sensors that can be applied in fruits and vegetables, since these products are highly perishable and represent an economical loss and environmental problem. Additionally, bio-based packaging systems that can detect leaks in packages or that can detect pathogens, toxins, allergens, and other chemical markers, or that can detect how long a temperature breach has lasted across a cold chain, would definitely be welcomed by the food supply industries, once the amount of food waste produced could be reduced and the sustainability of the chain could be improved.

The use of biopolymers and natural pigments for intelligent and smart food packaging systems still shows some constraints and more studies are needed to better understand how the bio-based sensors interact with the food products. Even though most of the indicators developed do not contact directly with the food product, thus some migration and toxicological studies are needed.

Another key point is that, despite the innovation and development of new intelligent and smart packaging materials bio-based, there are still some limitations and, according to literature and market research, no suitable solutions are currently commercially available. One main reason is the increase in the cost of the packaging and the fact that existing solutions are

not compatible with digital platforms. As most of the current bio-based sensors provide a visual response, it would be also interesting to develop systems that can provide a different response, e.g., information that can be emitted through a signal, readable, and transmitted through app notifications or LED (light-emitting diode) alarm messages. Therefore, highly sensitive, eco-friendly, low cost paper-based electrical sensors could be developed to spread the use of intelligent packaging (Barandun et al., 2019). This would contribute to minimize food waste and to provide value to by-products from lignocellulosic industrial crops such as giant reed or miscanthus (Fernando et al., 2004, 2016).

QUALITY PRESERVATION AND SHELF-LIFE EXTENSION OF PRICKLY PEAR (*OPUNTIA FICUS-INDICA* L. MILL) USING EDIBLE COATINGS

This chapter presents a study carried out at the beginning of the Ph.D. project to evaluate the potential of polysaccharide-based edible coatings for the preservation of prickly pear fruits. Two varieties of prickly pear, orange and red, were coated with four different polysaccharide formulations, and their shelf life was assessed. The main goal of this comparison was to better understand the performance of the different coatings, in particular pectin. This was especially relevant considering one of the main goals of this Ph.D. which aimed to extract pectin from *Opuntia* cladodes, and its potential application in the preservation of prickly pear fruits.

The results of this work provided valuable insights into the role of edible coatings in fruit preservation and led to the following publication, which is presented in this chapter as it was published:

Rodrigues C, Polesca C, Bicalho I, Souza VGL, Coelho I, Fernando AL. Quality Preservation and Shelf-Life Extension of Prickly Pear (*Opuntia ficus-indica* L. Mill) Using Edible Coatings. *Foods*. 2025; 14(2):161. <https://doi.org/10.3390/foods14020161>

3.1 Introduction

Opuntia ficus-indica L. Mill (OFI) is a native plant from Mexico belonging to the *Cactaceae* family and is currently well-established in several countries of the Mediterranean region such as Morocco, Tunisia, Italy, Spain, and Portugal (Aruwa et al., 2018; Ciriminna et al., 2019), as well as in Mexico and other countries of Latin America and Africa (Arba et al., 2017; Díaz-Delgado et al., 2022). OFI is known for its large pads (cladodes) but mostly for its fruits, denominated as cactus pears or prickly pears. The fruits are succulent, colorful, spiny, and rich in a variety of nutrients such as sugars, minerals, fibers, and bioactive compounds (e.g., ascorbic acid, betalains, flavonoids, phenols) (Cruz-Bravo et al., 2019; El-Mostafa et al., 2014). The recent growth in demand for this fruit is associated with an increase in consumers' healthier lifestyle choices (Alsubhi et al., 2023). The prickly pears are generally consumed as fresh fruits (in nature) or as minimally processed products (Liguori & Inglese, 2015).

Despite such positive characteristics, the prickly pear is seasonal (harvested during the summer) and very perishable due to its high metabolic activity and possible physical damage that can occur during harvesting and transportation (D'Aquino et al., 2017; D'Aquino, Satta, et al., 2019). Thus, the window time from harvesting to consumption is noticeably short, and post-harvesting preserving methods should be applied to extend its shelf life to enable its availability for prolonged periods (Díaz-Delgado et al., 2024).

The seasonality of prickly pears raises the necessity of studying the maximum storage duration of whole fruits to ensure that their nutritional and sanitary qualities remain uncompromised before consumption or minimal processing (Guerrero-Beltrán & Ochoa-Velasco, 2018). The prickly pear is classified as a non-climacteric fruit, and the most common post-harvesting technique used for preservation is refrigerated storage with temperatures between 5 to 8 °C for 2–5 weeks (Andreu-Coll et al., 2021; Guerrero-Beltrán & Ochoa-Velasco, 2018; Martins, Ribeiro, Miranda, et al., 2023). During refrigeration storage, the fruits are very susceptible to suffering chilling injury when temperatures lower than 5 °C are reached, diminishing their shelf life and causing surface pitting and browning (Heyes, 2018; Martins, Ribeiro, Miranda, et al., 2023; Yahia & Sáenz, 2011). As an alternative or as a refrigeration complement, other postharvest treatments such as chemical treatments, intermittent warming, controlled atmosphere, film wrapping, or edible coatings have been used to maintain cactus pears' quality and prolong their shelf life (Blancas-Benitez et al., 2022; D'Aquino, Inglese, et al., 2019; Ochoa-Velasco & Guerrero-Beltrán, 2014).

Edible coating is, by definition, a thin layer of edible material applied to the surface of foods in addition to or as a substitution for commonly used coating materials (Pham et al., 2023). It must also be acceptable to consumers as retaining the product's original taste, texture, and appearance (Ncama et al., 2018; Pham et al., 2023). Edible coatings are obtained by the application of the coating suspension on the surface of the fruit by dipping the fruit into the suspension or by spraying the coating on the fruit's surface (Palma, Mangia, et al., 2018). The coating acts as a semi-permeable barrier that aims to protect the fruits against water loss, spoilage, and color and texture changes and to reduce the respiration rate (Cruz-Monterrosa et al., 2023; Guerreiro et al., 2016). Edible coatings are generally made from eco-friendly sources that are food-grade and help in the preservation of food characteristics and safety, due to their intrinsic properties or by the addition of other components into the matrix such as bioactive compounds (Carrillo-Lomelí et al., 2024; Juikar & Warkar, 2023; S. Sharma et al., 2024). To improve the potential of coating effectiveness, some components can be added to the coating solution such as plasticizers and cross-linking agents as well as UV-blocking agents (Pedreiro et al., 2021; X. Tang & Yan, 2017). Coating adhesion capacity is a crucial characteristic influenced by the surface properties of the food, the specific objectives of the coating, and the application technique used (Ncama et al., 2018). The most common technique for coating application is dipping. The dipping method depends directly on various elements, namely coating solution characteristics (e.g., density, viscosity, and surface tension), the time for immersion, the dipping velocity, and the drying conditions (Kocira et al., 2021; Zhao et al., 2021). The dipping method can have some disadvantages, including irregular thick coatings that may affect fruit respiration, potential contamination of the coating solution, surface food damage, and high consumption of coating solution per food mass, which challenges scalability (Ncama et al., 2018). The effectiveness of a coating can be evaluated by monitoring the physicochemical, biochemical, and microbiological properties of the coated food throughout the storage period (Carrillo-Lomelí et al., 2024). The most commonly used edible coatings are polysaccharides such as alginate, starch, chitosan, and pectin (Pedreiro et al., 2021). Polysaccharides' coatings are characterized for having good barrier properties to gases (carbon dioxide and oxygen) but they possess high water vapor permeability and low water solubility leading to a higher swelling which directly affects the moisture loss of the fruits (Mannozi et al., 2017; Ncama et al., 2018; Pedreiro et al., 2021; Zhao et al., 2021). Each of them possesses particular characteristics and applications. Alginate is a linear polysaccharide extracted from brown seaweeds, and it is used as a coating due to its thickening properties, gel-forming capacity, emulsion stabilizing,

and high availability (Hassan et al., 2018; Mannozi et al., 2017). Alginate edible coatings have been demonstrated to be effective in the preservation of strawberries (Peretto et al., 2017), fresh-cut Fuji apples (Rojas-Graü et al., 2007), rose apples (Duong et al., 2022), mangoes (Rastegar et al., 2019), blueberries (Mannozi et al., 2017), and sweet cherries (Chiabrando & Giacalone, 2015). Starch is an abundant polysaccharide that can be extracted from potatoes, corn, and beans and is composed essentially of amylose and amylopectin (Cazón et al., 2017b). Starch-based edible coatings are used due to their good mechanical properties, high availability, and low cost (Wróblewska-Krepsztul et al., 2018). Their use on plums (Thakur et al., 2018), bananas (Thakur et al., 2019), fresh-cut pineapples (Bierhals et al., 2011), Red Crimson grapes (Fakhouri et al., 2015), and mandarin oranges (Wigati et al., 2023) was proven to contribute to extending the fruits' shelf life. Chitosan (poly- β -(1,4)-2-amino-2-deoxy-D-glucose) is the second most abundant polysaccharide in nature, obtained from the N-deacetylation of chitin, present in the crustacean shells (Darder et al., 2003; V. G. L. Souza, Rodrigues, et al., 2018b). It is worth mentioning chitosan's intrinsic antimicrobial activity, which together with its good gel and film-forming capacity, highlights this polysaccharide as a good matrix to preserve food products (Jiao et al., 2019; V. G. L. Souza, Rodrigues, et al., 2018b; Won et al., 2018). Its use as an edible coating contributed to the conservation of cherry tomatoes (Won et al., 2018), peaches (Jiao et al., 2019), sweet cherries (Tokatlı & Demirdöven, 2020), apples (Khalifa et al., 2016), and strawberries (Khalifa et al., 2016; Pagliarulo et al., 2016). Pectin is a complex polysaccharide and is usually extracted from citrus peels and is widely used to enhance viscosity and gel-forming capacity (Vanitha & Khan, 2020). Studies of the use of pectin edible coatings in fresh-cut peaches (Ayala-Zavala et al., 2013), limes (Maftoonazad & Ramaswamy, 2019), fresh-cut persimmons (Sanchís et al., 2017), strawberries (Muñoz-Labrador et al., 2018), and plums (Panahirad et al., 2020; Thakur et al., 2018) demonstrate that is a favorable method not only to extend fruits' shelf life but also to maintain their nutritional value.

Chitosan, as a cationic polymer, forms stable films or coatings through its inherent intermolecular interactions, such as hydrogen bonding and electrostatic forces. Starch, on the other hand, creates films via hydrogen bonding and gelatinization processes during its structural transition in the presence of water and heat. In contrast, anionic polymers like sodium alginate and pectin require cross-linking agents to form stable film networks. These agents, typically divalent cations such as Ca^{2+} , help create cohesive and durable coatings. Calcium chloride is widely used as a cross-linking agent in food applications due to its recognized

safety and effectiveness in enhancing the structural and functional properties of edible films (Y. Zhang et al., 2024).

Some previous studies applied coatings to prickly pear preservation, focusing on the use of natural compounds to extend the fruits' shelf life. As an example, the use of 1% chitosan in a 1% (*v/v*) solution of acetic acid was demonstrated to be effective in delaying the weight loss and integrity of the fruits. Moreover, a 1% chitosan coating in 1% (*v/v*) and 2.5% (*v/v*) acetic acid solutions led to the maintenance of bioactive compounds present in prickly pears (Ochoa-Velasco & Guerrero-Beltrán, 2014). Additionally, a concentration of 3% cassava starch as a coating solution for prickly pears was efficient in maintaining fruit quality for 25 days (Nunes et al., 2019).

In this context, the objective of this study was to evaluate the effect of four edible coatings (alginate, starch, chitosan, and pectin) on the physicochemical, bioactive, textural, and microbiological properties of two cactus pear varieties (orange and red) for 6 weeks. The weight loss, moisture, pH, titratable acidity, total soluble solids, color, and textural properties of the fruits, coated and uncoated, and kept under refrigeration (5 ± 2 °C) for 6 weeks were monitored. The effect of the coatings on the bioactive properties (total phenolic compounds, antioxidant activity, ascorbic acid, and betalain content) was also evaluated. Finally, the total mesophilic aerobic microorganisms, yeasts, and mold growth were measured to evaluate the effects of the different coatings on the microbiological quality of prickly pear peel and pulp. Since it is a seasonal fruit, the main motivation for this study is to understand the extension of cactus pear shelf life by using these edible coatings in order to meet the industry's and consumers' demands. Even though there are several studies pointing to the use of polysaccharide coatings to extend prickly pear shelf life, an extensive evaluation and comparison among various coatings has not yet been carried out. Once the exact efficacy of the coatings depends on the unique surface properties, respiration rates, and physicochemical characteristics of the tested fruits, this work serves as a valuable reference on the effectiveness of the different formulations.

3.2 Materials and Methods

3.2.1 Materials and Reagents

3.2.1.1 Biomass Material

OFI prickly pears (orange and red varieties) were harvested in the period of September 2019 and kindly provided by the *Pepe Aromas* company, located in Vale Pereiro, Évora, Portugal. The fruits were harvested and brushed and the spikes were removed in the company facilities, and then they were brought in cardboard boxes under refrigeration to the NOVA School of Science and Technology facilities. In the laboratory, prickly pears were washed with water and left to dry for further use. From a total of 400 fruits (200 from each variety) the sample selection was based on the size and appearance and 62 prickly pears from each variety were selected to proceed with the experiment.

3.2.1.2 Reagents

Chitosan with high molecular weight (31–37 kDa) and with a deacetylation degree of 75% and pectin from citrus peel (galacturonic acid of $\geq 74\%$ of dried basis) were used as a polymeric matrix and purchased from Sigma-Aldrich (St. Louis, MO, USA). Corn starch and sodium alginate were also used as polymeric matrices supplied from Sosa (Barcelona, Spain). All chemical reagents were of analytical reagent grade and were used as purchased. Glacial acetic acid ($\geq 99.8\%$ purity) and gallic acid (99% purity) were purchased from Alfa Aesar (Karlsruhe, Germany), while Folin-Ciocalteu reagent, sodium carbonate anhydrous (99.5% purity), sodium hydroxide ($\geq 99\%$ purity), and sodium chloride (99.5% purity) were obtained from Panreac (Barcelona, Spain). Calcium chloride ($\geq 97\%$ purity) was purchased from Honeywell (Charlotte, NC, USA). Ethanol absolute ($\geq 99.8\%$ purity), 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by Sigma-Aldrich (USA). The 2,6-dichlorophenol-indophenol was purchased from Merck (Darmstadt, Germany). All microbiological reagents were purchased from Biokar (Allonne, France): Dichloran Rose Bengale Chloramphenicol (DRBC), tryptone, and plate count agar (PCA). All the water used was purified using a Milli-Q system (Millipore, St. Louis, MO, USA).

3.2.2 Coatings

3.2.2.1 Preparation of Coatings Solution

Coating solutions were prepared by dissolving alginate (2% *w/v*), pectin (2% *w/v*), and starch (2% *w/v*) in distilled water under controlled stirring and heating (70 °C for alginate and pectin and 100 °C for starch) until the mixtures were homogenized. Then, the solutions were cooled to room temperature. The chitosan coating solution was prepared by dissolving 2% (*w/v*) in 1% (*v/v*) of glacial acetic acid solution under continuous stirring overnight at room temperature. The concentration used for the coating solution was standardized and applied based on previous experiments and literature (Chiumarelli et al., 2010; Oms-Oliu et al., 2008; Rojas-Graü et al., 2007; V. G. L. Souza et al., 2022; V. G. L. Souza, Pires, Vieira, et al., 2019).

3.2.2.2 Fruit Coatings

Previously washed fruits were submerged for 2 min in the coating solution. Afterward, the fruits were placed on a grid to drain and dry. In the case of fruits coated with sodium alginate and pectin solutions, fruits were drained and then dipped (2 min) in a calcium chloride (2% *w/v*) solution for cross-linking of the coating material. The fruits were then placed on a grid to drain and dry. Fruits were stored in petri dishes, under refrigeration (5 ± 2 °C) for 6 weeks. As indicated previously, anionic polymers such as sodium alginate and pectin require cross-linking agents to form stable film networks. For this reason, calcium chloride was used as a cross-linking agent.

3.2.3 Prickly Pear Evaluation

The prickly pears' peel and pulp were characterized every week for 6 weeks. In this study, we intentionally extended the storage duration to 6 weeks, to assess the effect of edible coatings on prickly pears beyond the typical maximum storage time (2–5 weeks) to provide insights into their potential for extending the shelf life of the fruits. The following methods were applied.

3.2.3.1 Physical and Chemical

3.2.3.1.1 Moisture Content and Weight Loss

Moisture content was evaluated according to the AOAC (Association of Official Analytical Chemists) method (AOAC, 1990). Weight loss was determined gravimetrically by the

difference of the initial weight (0 days), and the fruit weight at weeks 1, 2, 3, 4, 5, and 6. The results were calculated according to Equation (1):

$$\text{Weight loss (\%)} = \left(\frac{a - b}{a} \right) \times 100 \quad (1)$$

where a is the initial weight and b is the final weight for each week.

3.2.3.1.2 pH, Titratable Acidity, and Total Soluble Solids

The pH was measured by using a potentiometer HI 2210 (Hanna Instruments, Woonsocket, RI, USA). Titratable acidity was determined according to the AOAC (Association of Official Analytical Chemists) method (AOAC, 1990). The analysis was performed by mixing 5 g of sample with deionized water and determined by titration with NaOH 0.02 N. The results were calculated according to Equation (2) and expressed as %(w/w) of g of citric acid equivalent.

$$\text{Titrateable acidity} = \left(\frac{v_{\text{NaOH}} \times \text{meq}_{\text{citric acid}} \times N_{\text{NaOH}}}{W \times 1000} \right) \times 100 \quad (2)$$

where v_{NaOH} is the volume of NaOH used in the titration, $\text{meq}_{\text{citric acid}}$ is the equivalent value for citric acid (70.7 g), N_{NaOH} is the normality of NaOH, and the W is the weight (g) of the sample used.

Total soluble solids were determined by refractometry using an analogic and manual refractometer HI 96801 (Hanna Instruments, USA) and expressed in °Brix.

3.2.3.2 Color and Textural Properties

3.2.3.2.1 Color

Color was measured by the determination of the CIE (International Commission on Illumination)– $L^*a^*b^*$ coordinates using a CR 410 colorimeter (Minolta Co., Tokyo, Japan). L^* represents lightness, where $L = 0$ (black) to $L = 100$ (white), a^* represents redness from –(green) to +(red), and b^* from –(blue) to +(yellow). The $L^*a^*b^*$ coordinates allowed calculation of the hue angle (h°) according to the following equation (Equation (3)) (Mohammadi et al., 2008):

$$h^\circ = \arctan^{-1} \left(\frac{b^*}{a^*} \right), \text{ if } a^* > 0; \arctan^{-1} \left(\frac{b^*}{a^*} \right) + 180, \text{ if } a^* < 0 \quad (3)$$

3.2.3.2.1 Textural Properties

The mechanical properties of prickly pear were evaluated by uniaxial compression test using a texturometer CT3 4500 (Ametek Brookfield, Middleborough, MA, USA). Stress at

failure values of the samples were determined using a 50.8 mm diameter cylindrical with a rounded edge 20 mm long probe at a compression speed of 1 mm/s until it penetrates 6 mm in the cubic sample. The fruits were peeled and two samples of 20 × 20 × 20 mm of each fruit were collected from the equatorial region (center) of the prickly pear interior (Ochoa-Velasco & Guerrero-Beltrán, 2014).

3.2.3.3 Bioactive Properties

To access the bioactive properties, 10 g of prickly pear pulp was mixed with 100 mL of deionized water for 30 min under constant stirring. After that time, the samples were filtered and used in the analysis described below.

3.2.3.3.1 Ascorbic acid Content

The ascorbic acid content was determined by the 2,6-dichlorophenol indophenol titrimetric method according to AOAC method (AOAC, 1990).

3.2.3.3.2 Total Phenolic Content (TPC)

Total phenolic content was determined according to the Folin–Ciocalteu method (Singleton et al., 1999) with slight modifications. Briefly, 1 mL of diluted prickly pear pulp was mixed with 3 mL of Milli-Q water and 0.25 mL of Folin–Ciocalteu reagent. After 5 min of incubation at room temperature, 0.75 mL of sodium carbonate solution 20% (*w/v*) was added, stirred, and stored in the dark for 60 min. The absorbance of the mixture was measured in a UV-Vis spectrophotometer E-1000UV (Peak Instruments, Westford, MA, USA) at 760 nm. Gallic acid was used to elaborate a calibration curve with solutions from 0–120 mg/L. TPC was expressed in mg of gallic acid equivalent (GAE)/100 g.

3.2.3.3.3 Antioxidant Activity

Antioxidant activity was measured by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (Brand-Williams et al., 1995). Briefly, 1 mL of the diluted prickly pear pulp was mixed with 3 mL of 60 µmol/L DPPH ethanolic solution, then the mixture was kept in the dark for 20 min. The absorbance was measured at 517 nm in a UV-Vis spectrophotometer E-1000UV (Peak Instruments, USA). The results were calculated as the Trolox equivalent antioxidant capacity (TEAC), using a calibration curve and expressed in mg of Trolox equivalent/mL.

3.2.3.3.4 Betalain Content

For betalain content (BC) determination, aqueous extracts from pulp were performed. Betaxanthins (yellow pigments) and betacyanins (red pigment) content were calculated as described in Equation (4) (Castellanos-Santiago & Yahia, 2008; Stintzing et al., 2005):

$$BC \text{ content (mg/L)} = \left(\frac{Abs \times DF \times MW \times 1000}{\epsilon \times l} \right) \quad (4)$$

where *Abs* is the absorbance measured, *DF* is the dilution factor (1:100), *MW* is the molecular weight (g/mol), *l* de path length (1 cm), and ϵ the molar extinction coefficient (L/(mol/cm)).

For betaxanthin and betacyanin quantification, the betanin and indicaxanthin molecular weights and molar extinction coefficients were: betanin (*MW* = 308 g/mol; ϵ = 48,000 L/(mol.cm)) and indicaxanthin (*MW* = 550 g/mol; ϵ = 60,000 L/(mol.cm)). The absorbance of betaxanthins and betacyanins was measured at λ = 480 nm and λ = 535 nm, respectively, in a UV-Vis spectrophotometer E-1000UV (Peak Instruments, USA).

3.2.3.4 Microbiological Growth

Total mesophilic aerobic microorganism (TMAM) and yeast and mold growth were measured to evaluate the microbiological quality of prickly pear peel and pulp, according to ISO 4833-1:2013 (ISO, 2013) and ISO 21527-1:2008 (ISO, 2008), respectively. TMAM counts were performed in PCA after incubation at 30 °C for 72 h. Mold and yeast counts were performed in DRBC after incubation at 25 °C for 128 h. The results were expressed as log CFU (colony forming units)/g of sample.

3.2.3.5 Statistical Analysis

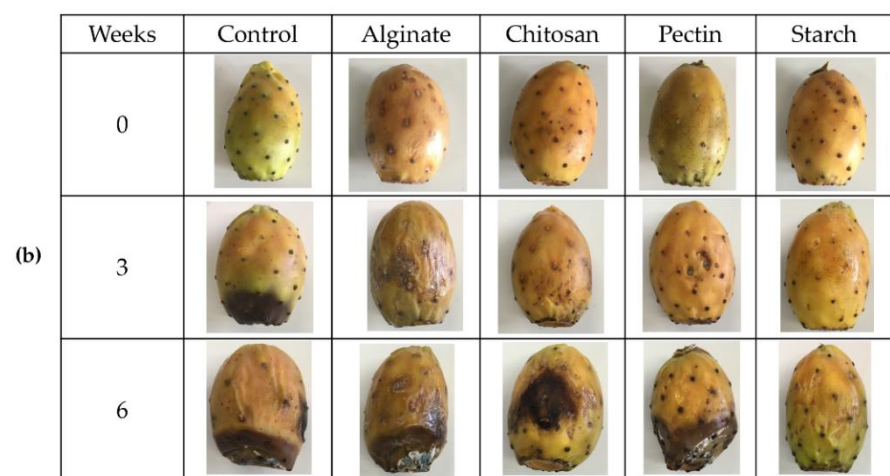
All experiments were conducted using a completely randomized design with three replications. Statistical analysis of the data was performed through a one-way analysis of variance (ANOVA) using Software OriginLab (version 8.5) (Northampton, MA, USA), and when the ANOVA was significant ($p < 0.05$), differences among mean values were processed by the Tukey test. Significance was defined at $p < 0.05$.

3.3 Results and Discussion

The visual aspect of both red and orange prickly pear fruits, with and without coatings, during the six-week study, is presented in Figure 3.1.



Red Peel



Orange Peel

Figure 3.1 - Visual aspect of red (a) and orange (b) prickly pears at initial time, 3, and 6 weeks of the experiment

3.3.1 Weight Loss

The weight loss percentages of red and orange prickly pears over the weeks are displayed in Figure 3.2. Fruit weight loss is related to water loss derived from metabolic processes of respiration and transpiration that occur in fruits during storage (Ferreira et al., 2020; Rastegar et al., 2019). Coatings can help in reducing weight loss by lowering respiration rate, improving the fruits' appearance, and extending their shelf life (Pham et al., 2023).

Polysaccharide edible coatings have good barrier properties to gases but high water vapor permeability, which can affect weight loss (Kocira et al., 2021). In general, an increase in weight loss during the weeks was verified for all the fruits studied. The use of coatings significantly reduced the weight loss of prickly pears when compared with controls ($p < 0.05$), at the end of the 6 weeks. However, no significant differences were observed between control and pectin-coated fruits in the orange variety at that time. The most successful coatings in the reduction of weight loss were starch (8.2%) in red fruits and chitosan (9.9%) in orange fruits. These results were in agreement with Batista Silva et al., (2018) who reported that a decrease in weight loss was also observed in chitosan-coated guava when compared with the control (Batista Silva et al., 2018). Starch as a coating material was shown to be effective in reducing weight loss in guava (G. V. da S. Pereira et al., 2021) and in Red Crimson grapes (Fakhouri et al., 2015) when compared to uncoated fruits. Regarding fresh-cut prickly pears, similar behavior was observed by Liguori et al., showing an increase in weight loss for uncoated fresh-cut prickly pears compared to mucilage-coated fruits kept under similar conditions for 9 days (Liguori et al., 2021). Even though polysaccharide edible coatings show high water vapor permeability, the solvent used in the coating solution can influence that property and particularly in chitosan, the use of acetic acid, as in this study, can help in the reduction of water vapor permeability, which has a positive influence on weight loss (Cazón et al., 2017b) .

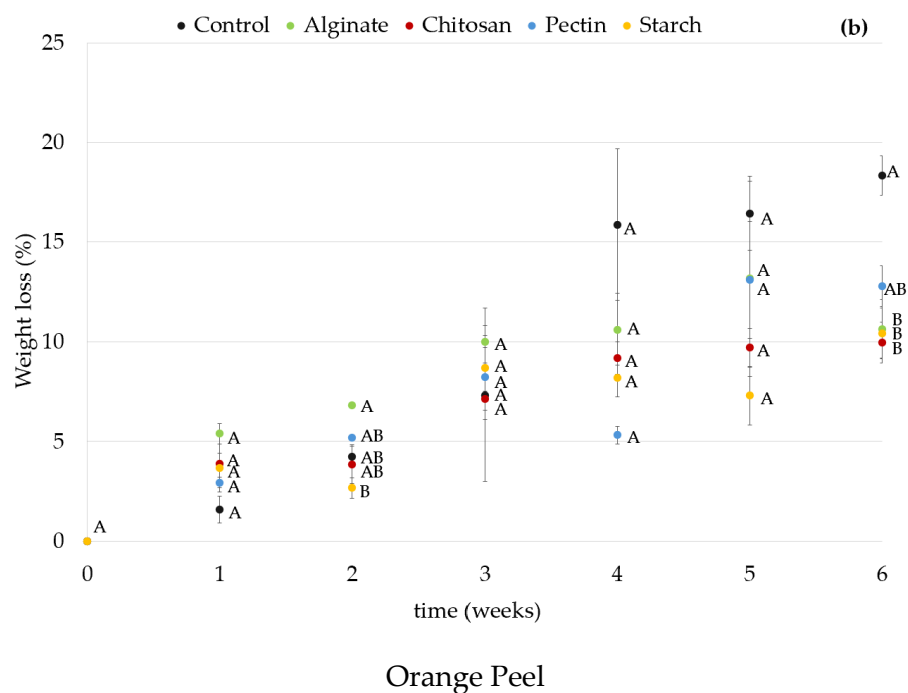
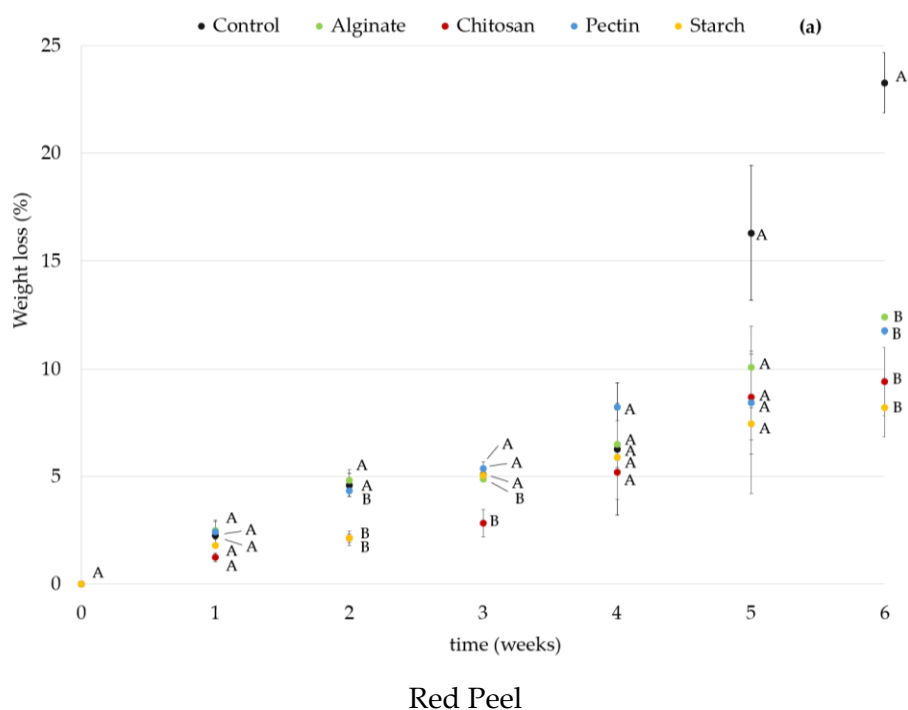


Figure 3.2 - Weight loss (%) results of red (a) and orange (b) prickly pear during storage time. (A–B): Different upper case letters indicate significant differences ($p < 0.05$) between coatings at the same time of the experiment.

3.3.2 Moisture Content, pH, Titratable Acidity, and Total Soluble Solids

The results obtained on the moisture content, pH, titratable acidity, and total soluble solids are presented in Table 3.1 and Table 3.2 for the red and orange varieties, respectively.

Table 3.1 - Physicochemical results of the coated and uncoated red prickly pear pulp fruits during storage time

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Moisture (%)	0	86.7 ± 2.2 ^{Aa}	86.7 ± 2.2 ^{Aa}	86.7 ± 2.2 ^{Aa}	86.7 ± 2.2 ^{Aa}	86.7 ± 2.2 ^{Aa}
	1	82.2 ± 7.2 ^{Aa}	82.0 ± 4.8 ^{Aa}	85.0 ± 2.9 ^{Aa}	84.1 ± 1.1 ^{Aa}	84.7 ± 1.8 ^{Aa}
	2	81.1 ± 2.4 ^{Aa}	81.5 ± 2.0 ^{Aa}	83.0 ± 0.9 ^{Aa}	80.6 ± 0.4 ^{Aa}	80.7 ± 1.6 ^{Aa}
	3	79.4 ± 0.8 ^{Aa}	79.8 ± 1.0 ^{Aa}	81.9 ± 1.8 ^{Aa}	79.8 ± 1.5 ^{Aa}	80.3 ± 2.9 ^{Aa}
	4	78.7 ± 1.0 ^{Aa}	79.4 ± 0.0 ^{Aa}	80.3 ± 1.1 ^{Aa}	78.1 ± 0.5 ^{Aa}	80.2 ± 2.8 ^{Aa}
	5	77.8 ± 1.6 ^{Aa}	78.8 ± 0.9 ^{Aa}	80.3 ± 1.8 ^{Aa}	76.4 ± 0.2 ^{Aa}	78.8 ± 0.0 ^{Aa}
	6	77.5 ± 0.2 ^{Aa}	78.6 ± 2.5 ^{Aa}	78.9 ± 1.7 ^{Aa}	74.1 ± 9.8 ^{Aa}	78.1 ± 1.6 ^{Aa}
pH	0	6.15 ± 0.10 ^{Aa}	6.15 ± 0.10 ^{Aa}	6.15 ± 0.10 ^{Aa}	6.15 ± 0.10 ^{Aa}	6.15 ± 0.10 ^{Aa}
	1	6.41 ± 0.04 ^{Aa}	6.14 ± 0.16 ^{Aa}	6.31 ± 0.04 ^{Aa}	6.36 ± 0.05 ^{Aa}	6.28 ± 0.01 ^{Aa}
	2	6.31 ± 0.01 ^{Aa}	6.26 ± 0.00 ^{ABa}	6.18 ± 0.03 ^{Ba}	6.29 ± 0.02 ^{Aa}	6.23 ± 0.02 ^{ABa}
	3	6.21 ± 0.04 ^{Aa}	6.20 ± 0.00 ^{Aa}	6.17 ± 0.17 ^{Aa}	6.29 ± 0.04 ^{Aa}	6.45 ± 0.08 ^{Aa}
	4	6.11 ± 0.15 ^{Aa}	6.19 ± 0.03 ^{Aa}	6.21 ± 0.11 ^{Aa}	6.15 ± 0.18 ^{Aa}	6.17 ± 0.01 ^{Aa}
	5	6.36 ± 0.05 ^{Aa}	6.17 ± 0.29 ^{Aa}	6.65 ± 0.02 ^{Aa}	6.20 ± 0.12 ^{Aa}	6.31 ± 0.11 ^{Aa}
	6	5.96 ± 0.09 ^{ABa}	5.53 ± 0.10 ^{Ba}	6.14 ± 0.13 ^{ABa}	6.08 ± 0.16 ^{ABa}	6.39 ± 0.04 ^{Aa}
TSS (°Brix)	0	13.2 ± 0.7 ^{Aa}	13.2 ± 0.7 ^{Aa}	13.2 ± 0.7 ^{Aa}	13.2 ± 0.7 ^{Aa}	13.2 ± 0.7 ^{Aa}
	1	13.0 ± 2.0 ^{Aa}	14.6 ± 0.4 ^{Aa}	12.8 ± 1.8 ^{Aa}	14.9 ± 0.8 ^{Aa}	14.8 ± 0.1 ^{Aa}
	2	14.7 ± 0.0 ^{Aa}	16.5 ± 0.4 ^{Aa}	12.7 ± 0.5 ^{Aa}	14.0 ± 2.1 ^{Aa}	11.9 ± 1.8 ^{Aa}
	3	14.9 ± 0.8 ^{Aa}	11.9 ± 0.6 ^{Aa}	12.5 ± 2.0 ^{Aa}	15.8 ± 0.4 ^{Aa}	15.0 ± 0.1 ^{Aa}
	4	11.7 ± 1.2 ^{Aa}	12.3 ± 1.4 ^{Aa}	12.4 ± 0.8 ^{Aa}	10.7 ± 1.1 ^{Aa}	12.1 ± 0.8 ^{Aa}
	5	13.5 ± 1.3 ^{Aa}	14.3 ± 1.5 ^{Aa}	11.2 ± 0.9 ^{Aa}	14.0 ± 2.6 ^{Aa}	12.1 ± 3.4 ^{Aa}
	6	11.6 ± 1.6 ^{Aa}	13.1 ± 0.0 ^{Aa}	12.7 ± 1.1 ^{Aa}	13.0 ± 1.5 ^{Aa}	13.2 ± 0.9 ^{Aa}
Total titratable acidity (% citric acid (wt/wt))	0	0.024 ± 0.002 ^{Ab}	0.024 ± 0.002 ^{Ab}	0.024 ± 0.002 ^{Ab}	0.024 ± 0.002 ^{Ac}	0.024 ± 0.002 ^{Aa}
	1	0.059 ± 0.004 ^{Aa}	0.066 ± 0.010 ^{Aab}	0.071 ± 0.008 ^{Aa}	0.058 ± 0.002 ^{Aab}	0.069 ± 0.010 ^{Aa}
	2	0.052 ± 0.002 ^{Aab}	0.061 ± 0.004 ^{Aab}	0.054 ± 0.003 ^{Aab}	0.053 ± 0.006 ^{Aab}	0.063 ± 0.007 ^{Aa}
	3	0.045 ± 0.005 ^{Aab}	0.048 ± 0.006 ^{Aab}	0.052 ± 0.010 ^{Aab}	0.053 ± 0.002 ^{Aab}	0.041 ± 0.005 ^{Aa}
	4	0.048 ± 0.002 ^{Aab}	0.051 ± 0.009 ^{Aab}	0.056 ± 0.001 ^{Aa}	0.052 ± 0.001 ^{Ab}	0.054 ± 0.008 ^{Aa}
	5	0.059 ± 0.008 ^{Aa}	0.068 ± 0.020 ^{Aab}	0.044 ± 0.006 ^{Aab}	0.062 ± 0.008 ^{Aab}	0.061 ± 0.026 ^{Aa}
	6	0.061 ± 0.018 ^{Aa}	0.083 ± 0.022 ^{Aa}	0.052 ± 0.014 ^{Aab}	0.069 ± 0.002 ^{Aa}	0.057 ± 0.004 ^{Aa}

(a-c): Within each parameter, values in the same column not sharing lower case superscript letters indicate statistically significant differences among weeks ($p < 0.05$); (A,B): Within each

parameter, values in the same row not sharing upper case superscript letters indicate statistically significant differences among coatings ($p < 0.05$).

Fruits usually have a high moisture content, making them highly perishable and susceptible to microbial contamination (Iturralde-García et al., 2022). Thus, the use of edible coatings can be helpful in maintaining their shelf life by playing a role as a barrier against moisture losses, gas exchanges, and mechanical damages, to mention a few (Blancas-Benitez et al., 2022). The initial moisture content of both prickly pear varieties is approximately 84%, which is similar to the values reported by Martins et al. (2023) (Martins, Ribeiro, Miranda, et al., 2023). The moisture content of the red prickly pear (Table 3.1) did not statistically change ($p > 0.05$) over the storage period assessed; however, comparing day 6 to day 0, it is noticeable that all fruits presented a tendency of a gradual decrease in this attribute, reflecting the water loss in fruit. Despite slight variations in the moisture content, there were no statistically significant differences ($p > 0.05$) between the control and the coated samples, independently of the coating. From the beginning of the experiment until the end, fruits lost about 9–10% of their water, and this behavior was transversal to all coated fruits and controls, showing that the use of coatings was not impactful for the preservation of water content. In the orange variety (Table 3.2), similar behavior was observed for both coated and uncoated fruits. Despite there being no significant differences ($p > 0.05$), the control fruits exhibited the most rapid and marked moisture loss from an initial 82.2% to 59.8% after the sixth week of storage, while the coated fruit showed final moisture of approximately 77–78%, showing that coatings had a positive effect on retaining water in orange prickly pears, which corroborates with the findings in weight loss. Comparable results were reported by Andreu-Coll et al. (2021), showing that moisture loss was more predominant in uncoated prickly pears than in alginate-coated orange prickly pears, during 10 days of storage at 4 °C (Andreu-Coll et al., 2021). Overall, a loss in the moisture content was observed for both varieties, but the losses were lower in red prickly pear and coatings did not show a high positive effect on moisture retention.

Prickly pear is considered a neutral fruit in terms of pH (5.3–7.1) and the values obtained throughout the experiment were within that range for both varieties (Liguori et al., 2021; Martins, Ribeiro, Miranda, et al., 2023; Piga, 2004). The pH values of the coated and control red fruits varied moderately throughout the storage period. On week 2, significant differences were observed ($p < 0.05$), with chitosan-coated fruits showing the lowest results (6.18) and the control the highest (6.31). In the sixth week, alginate-coated fruits showed a significant

decrease (5.53) ($p < 0.05$) when compared to the remaining coated fruits and control. Amongst all the coatings, chitosan seemed to help maintain the pH stability of the red prickly pears as well as starch. These results could be due to the intrinsic properties of chitosan that reduce microbial or enzymatic activity (Zhao et al., 2021). In the orange variety, the pH values of the fruits had slight variations during the storage period with no statistically significant differences ($p > 0.05$). These observations were also noted by Panza et al. (2022) who found no differences in pH between uncoated and alginate-coated red prickly pears after 10 days of storage (Panza et al., 2022). In general, the pH levels of both red and orange prickly pears remained stable during storage, with slight decreases over time.

Table 3.2 - Physicochemical results of the coated and uncoated orange prickly pear pulp fruits during storage time.

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Moisture (%)	0	82.2 ± 0.1 ^{Aa}	82.2 ± 0.1 ^{Aa}	82.2 ± 0.1 ^{Aa}	82.2 ± 0.1 ^{Aa}	82.2 ± 0.1 ^{Aa}
	1	81.2 ± 0.4 ^{Aa}	82.2 ± 0.1 ^{Aa}	80.9 ± 1.9 ^{Aa}	82.2 ± 0.1 ^{Aa}	82.2 ± 0.1 ^{Aa}
	2	79.0 ± 0.7 ^{Aa}	81.7 ± 1.1 ^{Aa}	80.1 ± 1.5 ^{Aa}	82.0 ± 1.8 ^{Aa}	81.7 ± 2.8 ^{Aa}
	3	77.4 ± 2.8 ^{Aa}	79.8 ± 1.1 ^{Aa}	79.9 ± 0.1 ^{Aa}	80.8 ± 1.4 ^{Aa}	80.5 ± 2.7 ^{Aa}
	4	73.7 ± 2.6 ^{Aa}	77.8 ± 2.8 ^{Aa}	79.5 ± 0.1 ^{Aa}	80.1 ± 1.4 ^{Aa}	79.7 ± 1.4 ^{Aa}
	5	69.0 ± 9.9 ^{Aa}	77.6 ± 3.0 ^{Aa}	78.9 ± 0.3 ^{Aa}	78.4 ± 0.3 ^{Aa}	79.0 ± 0.0 ^{Aa}
	6	59.8 ± 22.4 ^{Aa}	77.1 ± 0.1 ^{Aa}	78.6 ± 0.2 ^{Aa}	77.1 ± 0.7 ^{Aa}	77.7 ± 0.7 ^{Aa}
pH	0	6.48 ± 0.21 ^{Aa}	6.48 ± 0.21 ^{Aa}	6.48 ± 0.21 ^{Aa}	6.48 ± 0.21 ^{Aa}	6.48 ± 0.21 ^{Aa}
	1	6.54 ± 0.04 ^{Aa}	6.38 ± 0.01 ^{Aa}	6.36 ± 0.07 ^{Aa}	6.39 ± 0.06 ^{Aa}	6.42 ± 0.00 ^{Aa}
	2	6.33 ± 0.03 ^{Aa}	6.36 ± 0.02 ^{Aa}	6.35 ± 0.06 ^{Aa}	6.30 ± 0.02 ^{Aa}	6.37 ± 0.08 ^{Aa}
	3	6.48 ± 0.05 ^{Aa}	6.46 ± 0.04 ^{Aa}	6.44 ± 0.05 ^{Aa}	6.45 ± 0.01 ^{Aa}	6.04 ± 0.64 ^{Aa}
	4	6.34 ± 0.06 ^{Aa}	6.47 ± 0.01 ^{Aa}	6.25 ± 0.04 ^{Aa}	6.51 ± 0.11 ^{Aa}	6.40 ± 0.10 ^{Aa}
	5	6.44 ± 0.02 ^{Aa}	6.40 ± 0.02 ^{Aa}	6.08 ± 0.18 ^{Aa}	6.01 ± 0.21 ^{Aa}	6.33 ± 0.21 ^{Aa}
	6	6.43 ± 0.05 ^{Aa}	6.36 ± 0.20 ^{Aa}	5.98 ± 0.22 ^{Aa}	6.36 ± 0.09 ^{Aa}	6.45 ± 0.05 ^{Aa}
TSS (°Brix)	0	13.5 ± 1.2 ^{Aa}	13.5 ± 1.2 ^{Aa}	13.5 ± 1.2 ^{Aa}	13.5 ± 1.2 ^{Aa}	13.5 ± 1.2 ^{Aa}
	1	10.1 ± 1.3 ^{Aa}	13.3 ± 0.1 ^{Aa}	12.6 ± 0.6 ^{Aa}	10.4 ± 0.0 ^{Aa}	13.9 ± 0.7 ^{Aa}
	2	10.2 ± 0.2 ^{Aa}	13.6 ± 1.5 ^{Aa}	10.5 ± 0.3 ^{Aa}	12.0 ± 0.7 ^{Aa}	11.8 ± 1.5 ^{Aa}
	3	11.6 ± 1.6 ^{Aa}	13.5 ± 0.3 ^{Aa}	13.1 ± 0.6 ^{Aa}	14.2 ± 0.1 ^{Aa}	12.3 ± 0.9 ^{Aa}
	4	16.8 ± 1.3 ^{Aa}	14.2 ± 1.1 ^{Aa}	13.4 ± 0.6 ^{Aa}	11.4 ± 0.9 ^{Aa}	13.2 ± 2.3 ^{Aa}
	5	13.7 ± 0.8 ^{Aa}	12.6 ± 0.1 ^{Aa}	13.1 ± 0.7 ^{Aa}	13.0 ± 0.9 ^{Aa}	11.9 ± 1.4 ^{Aa}
	6	12.7 ± 1.6 ^{Aa}	12.0 ± 0.9 ^{Aa}	11.3 ± 1.1 ^{Aa}	12.9 ± 0.4 ^{Aa}	11.6 ± 1.6 ^{Aa}

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Total titratable acidity (% citric acid (wt/wt))	0	0.025 ± 0.002 ^{Ac}	0.025 ± 0.002 ^{Ac}	0.025 ± 0.002 ^{Ab}	0.025 ± 0.002 ^{Ab}	0.025 ± 0.002 ^{Aa}
	1	0.038 ± 0.002 ^{Bbc}	0.050 ± 0.003 ^{Aab}	0.043 ± 0.003 ^{ABab}	0.047 ± 0.001 ^{ABa}	0.052 ± 0.005 ^{Aa}
	2	0.040 ± 0.003 ^{Aab}	0.039 ± 0.003 ^{Abc}	0.048 ± 0.006 ^{Aab}	0.047 ± 0.004 ^{Aa}	0.047 ± 0.000 ^{Aa}
	3	0.042 ± 0.007 ^{Aab}	0.046 ± 0.003 ^{Aab}	0.053 ± 0.006 ^{Aab}	0.047 ± 0.002 ^{Aa}	0.056 ± 0.031 ^{Aa}
	4	0.052 ± 0.001 ^{Aab}	0.044 ± 0.007 ^{Aab}	0.058 ± 0.012 ^{Aab}	0.051 ± 0.008 ^{Aa}	0.047 ± 0.006 ^{Aa}
	5	0.045 ± 0.000 ^{Aab}	0.055 ± 0.002 ^{Aab}	0.058 ± 0.014 ^{Aab}	0.064 ± 0.010 ^{Aa}	0.052 ± 0.014 ^{Aa}
	6	0.054 ± 0.004 ^{Aa}	0.058 ± 0.006 ^{Aa}	0.066 ± 0.014 ^{Aa}	0.055 ± 0.006 ^{Aa}	0.048 ± 0.004 ^{Aa}

Prickly pear has usually a total soluble solid content of 12–17 °Brix (Piga, 2004; C. Rodrigues et al., 2023). The content of soluble solids increase is related to fruit ripening and is also responsible for the sweet taste of fruits (Pham et al., 2023). The initial °Brix values obtained, 13.2 °Brix (red prickly pear) and 13.5 °Brix (orange prickly pear) are similar to the values reported in the literature (Lekhuleni et al., 2021; Trindade et al., 2023). The total soluble solids (TSS) content, measured in °Brix, showed some variations during the storage period. By week 6, all treatments maintained TSS values within a similar range to the initial values, with no statistically significant differences ($p > 0.05$) in both varieties. However, a notable increase in TSS was observed in control and coated red fruits, especially in the alginate-coated red fruits at week 2, which could be related to the concentration of sugars as water content decreased (Allegra et al., 2022). This temporary increase may suggest that alginate-coated fruits underwent more rapid dehydration early in the storage period compared to the other treatments, although this effect stabilized by week 6. Similar behavior was observed in grapes coated with alginate during 12 days of storage (W. F. C. de Souza et al., 2021). In the orange fruits, coated fruits showed more stable values, especially the alginate- and chitosan-coated fruits, maintaining a value of TSS of 13 °Brix over the time of the experiment. While there were no significant differences among coatings at most storage times ($p > 0.05$), the control showed a high variability, indicating that coatings may contribute to stabilizing TSS levels. The use of edible coatings in fruits can contribute to retard the alterations in TSS by affecting the respiratory and metabolic processes by retarding them (Nair et al., 2020). Moreover, the alterations of this parameter can also be related to distinct stages of maturity of the fruits and the senescence phenomenon that occurred during the storage period (W. F. C. de Souza et al., 2021).

Total titratable acidity is an important parameter directly related to the acids present in food. In food, organic acids represent a great part of the acids present and have a high influence on

flavor, color, and microbial activity, amongst others (Tyl & Sadler, 2017). The total titratable acidity at the initial time for both varieties is coincident with the values reported in the literature for fresh fruits (FAO, 2013; Lekhuleni et al., 2021). In the red fruits, total titratable acidity remained relatively stable throughout the time, showing some fluctuations at specific time points. Alginate-coated fruits showed statistically significant differences ($p < 0.05$), especially between week 0 and week 6 when this coating showed the highest value of TTA (0.08% of citric acid (wt/wt)), which is in agreement with the pH value at that time of the experiment. In contrast, chitosan-coated fruits maintained lower total acidity values throughout the weeks, even though significant differences were observed ($p < 0.05$) likely due to the intrinsic antimicrobial and antifungal properties of this polysaccharide which potentially helped on the inhibition of antimicrobial and antifungal activity and thus limited acid production (Elsabee & Abdou, 2013; Kumarihami et al., 2022). At week 6, no significant differences ($p > 0.05$) were observed between coated and uncoated fruits. In the orange fruits, values increased slightly for all coated and uncoated fruits over the time of the experiment. Some fluctuations were observed right in the first week of the experiment when significant differences ($p < 0.05$) were observed, especially between uncoated fruits and starch-coated fruits. At the end of the storage period, chitosan-coated red fruits presented the highest value of TTA (0.07% citric acid (wt/wt)) and alginate-coated the lowest (0.05% citric acid (wt/wt)) amongst the coated and uncoated fruits but with no significant differences ($p > 0.05$) between them. Alginate coating was helpful in retarding the acidity loss in plums for 35 days at 2 °C (Valero et al., 2013). The use of cassava starch as a coating in prickly pear cv. Gigante demonstrated positive effects on delaying the total titratable acidity increment when compared to uncoated fruits (Nunes et al., 2019). The increase in TTA over time was observed in both varieties, but the red variety showed more pronounced differences between coatings. The orange variety, however, showed a slight but more uniform increase in TTA across all coatings. The increase of acidity common to both varieties may be related to the low respiratory activity at the end of storage time and highly related to fruit senescence, which could indicate the accumulation of organic acids (Nunes et al., 2019).

3.3.3 Color and Textural Properties

The color and textural properties of red and orange coated and uncoated prickly pears are presented in Figure 3.3 and Figure 3.4, respectively.

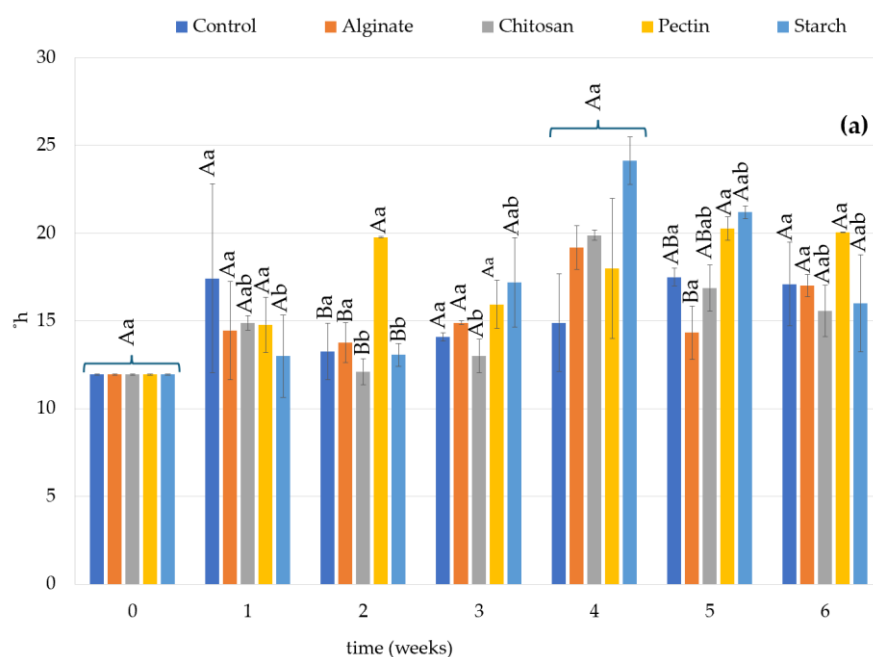
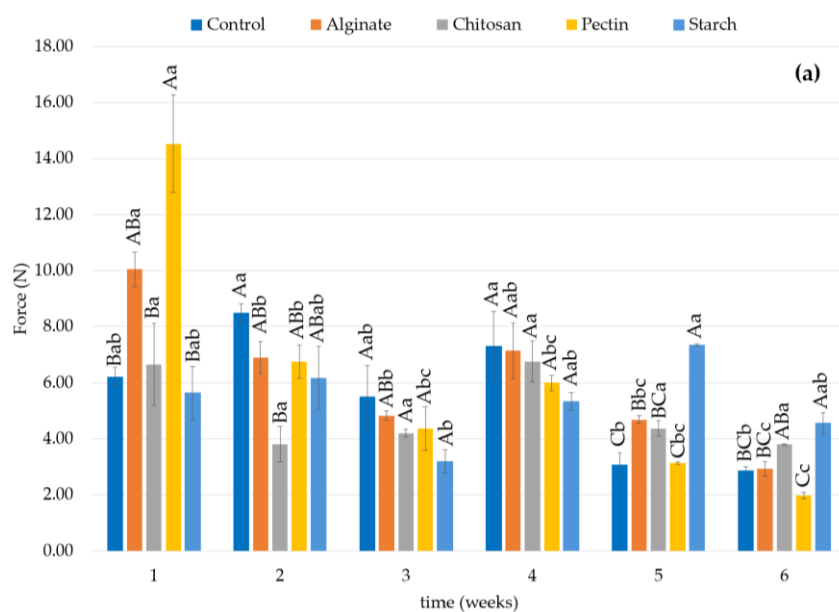


Figure 3.3 - Hue angle (h°) results of red (a) and orange (b) prickly pear pulp during storage time. (A,B): Different capital letters indicate statistically significant differences ($p < 0.05$) between treatments at the same time of the experiment. (a-c): Different lower case letters indicate statistically significant differences for the same sample over time ($p < 0.05$).

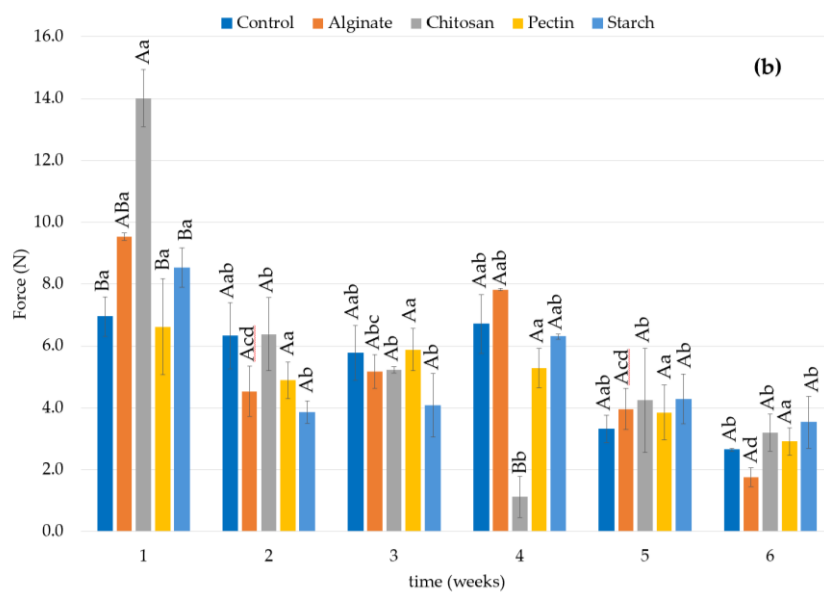
For the red prickly pears, the hue angle had some variations over time but, progressively changed from red to red-orange color (Figure 3.3a). At the beginning of the experiment, a value of h° of around 12 was obtained, slightly lower than the value of 16.26 reported by Lekhuleni et al. (2021) (Lekhuleni et al., 2021). Chitosan- and starch-coated fruits showed significant differences over time ($p > 0.05$) while the other coated and uncoated fruits showed no difference ($p < 0.05$). Chitosan and starch contributed positively to the maintenance of the fruits' color, while pectin led to the highest variation in hue angle value at the end of the experiment. The use of chitosan coating also had a positive effect on hue maintenance in guavas (Batista Silva et al., 2018), and starch had the same effect in bananas (Thakur et al., 2019). The change in color from red to reddish-orange can be related to browning phenomena that possibly caused the degradation of betalains (Table 3.3) and could also be affected by storage conditions (Barrett et al., 2010).

Hue angle is related to ripening phenomena and for orange prickly pears decreases with the progression of ripening, as color changes from yellowish-orange to red-orange. The initial h° value obtained for the orange prickly pear was around 93.50, which is similar to the value reported by Eroglu et al. (2022) for the same variety of fruit (Eroglu et al., 2022). The hue angle decreased with storage weeks irrespective of the coating (Figure 3.3b). The change in hue angle was significant and rapid in coated and non-coated fruits until week 2 and less rapid afterward.

In the last week of storage, orange prickly pear fruits had hue angle values in this decreasing order: pectin > alginate > chitosan > starch > control. Therefore, coatings inhibit respiration and ethylene production delaying senescence by creating a modified atmosphere around the fruit surface. The same was reported for tomatoes (Shakir et al., 2022), guavas (Formiga et al., 2019), mangos (Sousa et al., 2021), and persimmons (Saleem et al., 2022). The decrease in hue angle was also noted in another study with *Opuntia ficus-indica* (Andreu-Coll et al., 2021) and *Opuntia albicarpa* (Ochoa-Velasco & Guerrero-Beltrán, 2016) under cold storage. Firmness is a key property in the quality characteristics of fruits and vegetables (Ochoa-Velasco & Guerrero-Beltrán, 2016). Overall, the firmness of all fruits, despite the variety (orange or red) and presence of coating (Figure 3.4), reduced over the refrigerated storage time, however with statistical significance only for some of the treatments.



Red Pulp



Orange Pulp

Figure 3.4 - Firmness results (N) of red (a) and orange (b) prickly pear pulp during storage time (A–C): Different capital letters indicate statistically significant differences ($p < 0.05$) between treatments at the same time of the experiment (a–c): Different lower case letters indicate statistically significant differences for the same sample over time ($p < 0.05$).

Differences in the fruits coated with different biopolymers were observed since week 1 of storage ($p < 0.05$), with superior firmness in the red fruits coated with pectin or alginate, while in the orange fruits, chitosan or alginate stood out. At the end of the refrigerated storage, at week 6, for the red prickly pears, only pectin- and alginate-coated fruits presented statistical difference from the initial firmness ($p < 0.05$), and within coatings, fruits protected with starch

presented the highest firmness. In orange fruits, only pectin maintained the firmness from week 1 to week 6 ($p > 0.05$), and no differences were observed between coatings. Similar behavior was observed in prickly pears minimally processed and coated with Guar and Xanthan (Mohamed et al., 2013). The authors reported a delay in the loss of fruit firmness in the initial storage days; however, after 9 days, this difference was not statistically significant, and control and coated fruits presented the same firmness. During the ripening process, a decline in firmness is expected (Ochoa-Velasco & Guerrero-Beltrán, 2016). The loss of firmness of prickly pears can be associated with the breakdown of its cell wall structure, along with enzymatic activities involving pectinmethylesterase, polygalacturonase, and cellulase, which affect pectic substances (Ochoa-Velasco & Guerrero-Beltrán, 2014). The presence of coatings may have influenced this biochemical metabolism as it retards the gas exchange (O_2 , CO_2 and H_2O) through the membrane formed (coating) (C. Rodrigues et al., 2021a) .

3.3.4 Bioactive Compounds

Bioactive properties are displayed in Table 3.3 and Table 3.4 for the red and orange varieties, respectively, showing the results of total phenolic compounds, antioxidant activity, ascorbic acid content, and betalains (indicaxanthin and betanin). In general, these parameters were significantly affected by storage time and coating applied.

Phenolic compounds are a group of secondary metabolites that are produced by plants and food and play an important role in protection from environmental factors such as UV light [14,29]. The initial value of total phenolic compounds for red and orange prickly pears was 772 mg GAE/100 g and 588 mg GAE/100 g, respectively. These values are within the same range as the ones reported by Valero-Galván et al. (2021) (Valero-Galván et al., 2021) . Total phenolic compound (TPC) content varied throughout storage time. There were no statistically significant differences between coatings during the entire experiment for either red or orange varieties and control fruits ($p > 0.05$). Alginate-coated red fruits showed a significant reduction ($p < 0.05$) from week 0 to week 6 (543 mg GAE/100 g). In week 5, pectin-coated orange fruits exhibited a rise in TPC (878 mg GAE/100 g) and showed significant differences ($p < 0.05$) when compared to the remaining weeks of the study. Overall, the red variety showed a higher TPC over the weeks, particularly with starch among the coatings. Thus, these results indicate that coatings helped in the mitigation of the immediate loss of phenolic compound content, their long-term efficacy varied, but no coating highly influenced the preservation of phenolic compounds. Similar behavior in coated fruits was also reported in strawberries using

hydroxyethyl cellulose/sodium alginate/asparagus waste extract (C. Liu et al., 2021). Moreover, cold storage conditions are reported to influence the phenolic compound content and help with the maintenance and/or increase of their content when compared to room temperature (Andreu-Coll et al., 2021; Anorve Morga et al., 2006; Cruz-Bravo et al., 2019) .

Antioxidant activity is directly related to the presence of antioxidant compounds such as phenols and ascorbic acid that can act as a protection against reactive oxygen species (ROS). Through radical scavenging, the antioxidants can help in the degradation of fruits and vegetables (Pedreiro et al., 2021). Overall, a decrease in antioxidant activity was observed over the weeks for both red and orange fruits. For red fruits, antioxidant activity revealed a significant decline in all treatments after the beginning of the experiment ($p < 0.05$), with the control showing the most rapid loss. Alginate-coated red fruits' antioxidant activity was significantly reduced by week 6 (459 mg eq Trolox/mL) compared to week 0, but lower than with chitosan (560 mg eq Trolox/mL) and starch (602 mg eq Trolox/mL) and superior to the control (385 mg eq Trolox/mL), suggesting chitosan and starch are more effective in delaying the degradation of bioactive antioxidant compounds present in the fruits. The statistical differences between coatings became more evident by week 5 when starch showed the higher antioxidant activity to be statistically superior ($p < 0.05$), while the other coatings showed lower antioxidant capacity. In orange fruits, the antioxidant activity declined in all treatments after the beginning, with the control dropping drastically by week 1 ($p < 0.05$). Alginate- and starch-coated fruits showed higher antioxidant activity in the first two weeks, but by the end of the experiment, antioxidant activity was almost entirely depleted in all samples (less than 10% of the initial activity). In fact, at the end of 6 weeks, starch-coated fruits were the ones with higher antioxidant activity (460 mg eq Trolox/mL) showing no statistically significant differences when compared with the other coated fruits and the control ($p > 0.05$). Thus, starch appeared to be the best coating in preserving antioxidant activity in red and orange fruits even though rapid antioxidant degradation occurred. These results are not supported by other authors who report that edible coatings can help in the maintenance of antioxidant properties in coated fruits (W. F. C. de Souza et al., 2021; Tahir et al., 2019) ; however, they corroborate with the trends observed for the contents in betalains and ascorbic acid, as discussed below.

Table 3.3 - Bioactive properties results of the coated and uncoated red prickly pear pulp fruits during storage time.

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
TPC (mg GAE/100 g)	0	772 ± 0 ^{Aa}	772 ± 0 ^{Aab}	772 ± 0 ^{Aa}	772 ± 0 ^{Aa}	772 ± 0 ^{Aa}
	1	702 ± 84 ^{Aa}	754 ± 65 ^{Aab}	754 ± 119 ^{Aa}	764 ± 5 ^{Aa}	886 ± 84 ^{Aa}
	2	591 ± 11 ^{Aa}	640 ± 5 ^{Aab}	637 ± 100 ^{Aa}	605 ± 95 ^{Aa}	770 ± 130 ^{Aa}
	3	759 ± 86 ^{Aa}	624 ± 92 ^{Aab}	621 ± 138 ^{Aa}	697 ± 46 ^{Aa}	635 ± 38 ^{Aa}
	4	924 ± 343 ^{Aa}	824 ± 38 ^{Aa}	683 ± 119 ^{Aa}	586 ± 70 ^{Aa}	764 ± 151 ^{Aa}
	5	672 ± 16 ^{Aa}	664 ± 35 ^{Aab}	478 ± 5 ^{Aa}	726 ± 76 ^{Aa}	713 ± 122 ^{Aa}
	6	635 ± 97 ^{Aa}	543 ± 43 ^{Ab}	602 ± 27 ^{Aa}	554 ± 43 ^{Aa}	648 ± 154 ^{Aa}
Antioxidant activity (mg eq Trolox/100 mg)	0	2394 ± 526 ^{Aa}	2394 ± 526 ^{Aa}	2394 ± 526 ^{Aa}	2394 ± 526 ^{Aa}	2394 ± 526 ^{Aa}
	1	1193 ± 433 ^{Aa}	1543 ± 83 ^{Aab}	1227 ± 67 ^{Aab}	1527 ± 217 ^{Aab}	1618 ± 208 ^{Aa}
	2	1327 ± 67 ^{Aa}	1768 ± 75 ^{Aab}	1293 ± 50 ^{Aab}	1410 ± 400 ^{Aab}	1843 ± 100 ^{Aa}
	3	1893 ± 300 ^{Aa}	1660 ± 83 ^{Aab}	1735 ± 92 ^{Aab}	1710 ± 100 ^{Aab}	1560 ± 250 ^{Aa}
	4	1393 ± 750 ^{Aa}	1335 ± 158 ^{Aab}	1035 ± 158 ^{Aab}	618 ± 92 ^{Ab}	1293 ± 633 ^{Aa}
	5	243 ± 0 ^{Ba}	527 ± 367 ^{ABb}	518 ± 375 ^{ABb}	410 ± 117 ^{ABb}	1627 ± 50 ^{Aa}
	6	385 ± 225 ^{Aa}	459 ± 263 ^{Aab}	560 ± 267 ^{Ab}	302 ± 92 ^{Ab}	602 ± 408 ^{Aa}
Ascorbic acid content ([Ascorbic acid] mg/kg)	0	3280 ± 0 ^{Aa}	3280 ± 0 ^{Aa}	3280 ± 0 ^{Aa}	3280 ± 0 ^{Aa}	3280 ± 0 ^{Aa}
	1	3200 ± 800 ^{Aa}	3600 ± 400 ^{Aa}	3200 ± 0 ^{Aa}	2800 ± 400 ^{Aab}	3600 ± 400 ^{Aa}
	2	1600 ± 0 ^{Aab}	1400 ± 200 ^{Ab}	1400 ± 200 ^{Abc}	1600 ± 0 ^{Ac}	1400 ± 200 ^{Ab}
	3	1600 ± 0 ^{Aab}	1600 ± 0 ^{Ab}	1600 ± 0 ^{Abc}	1600 ± 0 ^{Ac}	1600 ± 0 ^{Ab}
	4	1600 ± 0 ^{Cab}	1600 ± 0 ^{Db}	1200 ± 0 ^{Ec}	1600 ± 0 ^{Bc}	1600 ± 0 ^{Ab}
	5	1200 ± 0 ^{Ab}	1600 ± 0 ^{Ab}	1600 ± 0 ^{Abc}	1400 ± 200 ^{Ac}	1400 ± 200 ^{Ab}
	6	1400 ± 200 ^{Ab}	1600 ± 0 ^{Ab}	1800 ± 200 ^{Ab}	1800 ± 200 ^{Abc}	1800 ± 0 ^{Ab}
Betainins–Indicaxanthin (mg/100 g)	0	32.2 ± 0.0 ^{Aa}	32.2 ± 0.0 ^{Aa}	32.2 ± 0.0 ^{Aa}	32.2 ± 0.0 ^{Aa}	32.2 ± 0.0 ^{Aa}
	1	30.8 ± 3.8 ^{Aa}	31.4 ± 3.8 ^{Aa}	33.0 ± 0.3 ^{Aa}	30.8 ± 5.1 ^{Aa}	34.3 ± 3.5 ^{Aa}
	2	13.5 ± 3.2 ^{Aa}	14.1 ± 3.2 ^{Aab}	22.1 ± 2.2 ^{Aa}	13.8 ± 4.8 ^{Aa}	22.8 ± 1.6 ^{Aa}
	3	31.8 ± 9.9 ^{Aa}	29.5 ± 3.9 ^{Aab}	36.3 ± 8.0 ^{Aa}	26.0 ± 6.1 ^{Aa}	26.6 ± 4.2 ^{Aa}
	4	23.1 ± 6.4 ^{Aa}	29.8 ± 4.2 ^{Aa}	20.5 ± 5.1 ^{Aa}	13.2 ± 2.9 ^{Aa}	18.6 ± 7.7 ^{Aa}
	5	21.5 ± 0.3 ^{Aa}	16.4 ± 4.8 ^{Aab}	24.7 ± 3.5 ^{Aa}	24.1 ± 0.3 ^{Aa}	25.7 ± 0.6 ^{Aa}
	6	20.2 ± 6.7 ^{Aa}	10.6 ± 1.0 ^{Ab}	22.5 ± 0.6 ^{Aa}	11.6 ± 1.9 ^{Aa}	27.3 ± 16.4 ^{Aa}

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Betalains– Betanin (mg/100 g)	0	53.3 ± 0.0 ^{Aa}	53.3 ± 0.0 ^{Aa}	53.3 ± 0.0 ^{Aa}	53.3 ± 0.0 ^{Aa}	53.3 ± 0.0 ^{Aa}
	1	48.6 ± 14.7 ^{Aa}	53.6 ± 11.5 ^{Aa}	51.8 ± 0.5 ^{Aab}	52.3 ± 11.0 ^{Aa}	60.5 ± 13.8 ^{Aa}
	2	22.0 ± 5.5 ^{Aa}	21.1 ± 6.4 ^{Aa}	37.6 ± 1.8 ^{Ab}	13.8 ± 7.3 ^{Ab}	40.8 ± 7.8 ^{Aa}
	3	58.2 ± 17.0 ^{Aa}	50.0 ± 6.9 ^{Aa}	65.1 ± 8.3 ^{Aa}	43.1 ± 9.2 ^{Aab}	43.1 ± 3.7 ^{Aa}
	4	34.8 ± 11.9 ^{Aa}	47.7 ± 9.2 ^{Aa}	26.1 ± 6.0 ^{Ab}	17.4 ± 3.7 ^{Aab}	23.8 ± 8.2 ^{Aa}
	5	31.6 ± 1.4 ^{Aa}	24.8 ± 9.2 ^{Aa}	37.6 ± 1.8 ^{Ab}	38.0 ± 5.0 ^{Aab}	36.2 ± 2.3 ^{Aa}
	6	28.9 ± 14.2 ^{Aa}	16.0 ± 0.5 ^{Aa}	33.0 ± 7.3 ^{Ab}	14.7 ± 1.8 ^{Ab}	47.7 ± 33.0 ^{Aa}

(a–c): Within each parameter, values in the same column not sharing lower case superscript letters indicate statistically significant differences among weeks ($p < 0.05$); (A–E): Within each parameter, values in the same row not sharing upper case superscript letters indicate statistically significant differences among coatings ($p < 0.05$).

Ascorbic acid or vitamin C is present in fruits and plays an important role in contributing to the antioxidant capacity (Liguori et al., 2021; C. Rodrigues et al., 2023). The red and orange fresh fruits presented a value of ascorbic acid of 3280 mg/kg and 1094 mg/kg, respectively. These values are similar to values reported for prickly pears by other authors, with the value obtained for the red fruit being slightly higher (Valero-Galván et al., 2021). Regarding the red variety, a significant decrease in ascorbic acid content was observed after the first week across all treatments ($p < 0.05$), with control fruits showing the steepest reductions. Significant differences between coatings were also noted at week 4 when chitosan-coated samples presented a lower ascorbic acid content (1200 mg/kg), statistically different from other coatings. At week 6, starch (1800 mg/kg) and chitosan (1800 mg/kg) maintained significantly higher ascorbic acid content compared to the control (1400 mg/kg). In the orange variety, ascorbic acid levels remained relatively stable over time in all treatments, with no significant decline ($p > 0.05$). Alginate-, chitosan-, and starch-coated samples showed slightly higher retention of ascorbic acid by week 6 (1600 mg/kg), but the differences were not statistically significant when compared to the control ($p > 0.05$). At the end of 6 weeks, pectin-coated fruits demonstrated the lower value of ascorbic acid. Overall, ascorbic acid content was better preserved by coatings in the red variety, particularly with starch as a coating at the end of 6 weeks. The use of a pre-gelatinized corn starch/cellulose nanofiber/basil essential oil as a coating to preserve ascorbic acid content was also reported in mandarin oranges showing to be effective in the maintenance of this parameter after 12 days of storage (Wigati et al., 2023). Allegra et al. (2022)

suggest that the use of an edible coating led to prickly pear protection from oxidation, leading to gas exchange reduction between the fruit and the environment (Allegra et al., 2022) . The orange variety showed more stability over time even though the values were lower than the other variety, but no coating was particularly emphasized to preserve ascorbic acid

Betalains are plant pigments that exhibit functional properties namely antioxidant capacity and in which prickly pears constitute a great source (Carreón-Hidalgo et al., 2022; C. Rodrigues et al., 2021a) . Betalains can be divided into two main groups, namely betacyanins (where betanin is included), of red-purple color, and betaxanthins (where indicaxanthin is included) of the yellow-orange color (Calva-Estrada et al., 2022; Carreón-Hidalgo et al., 2022) . Indicaxanthin values for red and orange prickly pears were 32.2 mg/100 g and 20.9 mg/100 g, respectively, and are consistent with values reported in the literature (Valero-Galván et al., 2021) . In both red and orange prickly pear fruits, indicaxanthins did not exhibit significant differences ($p > 0.05$) between the different coatings and control throughout the study. In red fruits, it was possible to observe significant differences in alginate ($p < 0.05$) with a reduction at week 6 (10.6 mg/100 g) with a lower value when compared with the initial value (32.2 mg/100 g). In orange fruits at week 5, alginate and starch exhibited higher levels of indicaxanthin, but the differences were not statistically significant. At the end of the experiment, alginate and pectin-coated fruits showed lower values in indicaxanthin. In the red variety, starch can be suggested as the coating to help with the preservation of indicaxanthin when compared to the other coatings. The use of coatings was shown to be effective in the preservation of indicaxanthin in prickly pears coated with mucilage after 9 days of storage when compared with uncoated fruits (Liguori et al., 2021). The results suggest that in the orange variety, coatings did not provide any major benefit in the preservation of indicaxanthin content.

Table 3.4 - Bioactive properties results for the coated and uncoated orange prickly pear pulp fruits during storage time

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
TPC (mg GAE/100 g)	0	588 ± 0 ^{Aa}	588 ± 0 ^{Aa}	588 ± 0 ^{Aa}	588 ± 0 ^{Aab}	588 ± 0 ^{Aa}
	1	497 ± 57 ^{Aa}	626 ± 51 ^{Aa}	610 ± 24 ^{Aa}	616 ± 89 ^{Aab}	589 ± 19 ^{Aa}
	2	654 ± 62 ^{Aa}	535 ± 35 ^{Aa}	548 ± 16 ^{Aa}	621 ± 3 ^{Aab}	583 ± 57 ^{Aa}
	3	589 ± 41 ^{Aa}	708 ± 14 ^{Aa}	783 ± 197 ^{Aa}	648 ± 51 ^{Aab}	575 ± 81 ^{Aa}
	4	516 ± 59 ^{Aa}	467 ± 184 ^{Aa}	667 ± 27 ^{Aa}	505 ± 0 ^{Ab}	443 ± 68 ^{Aa}
	5	686 ± 176 ^{Aa}	537 ± 59 ^{Aa}	667 ± 38 ^{Aa}	878 ± 108 ^{Aa}	610 ± 46 ^{Aa}
	6	564 ± 54 ^{Aa}	486 ± 51 ^{Aa}	662 ± 43 ^{Aa}	567 ± 24 ^{Aab}	635 ± 114 ^{Aa}
Antioxidant activity (mg eq Trolox/100 mg)	0	4445 ± 54 ^{Aa}	4445 ± 54 ^{Aa}	4445 ± 54 ^{Aa}	4445 ± 54 ^{Aa}	4445 ± 54 ^{Aa}
	1	685 ± 25 ^{Abc}	910 ± 100 ^{Abc}	777 ± 17 ^{Abc}	727 ± 67 ^{Ac}	852 ± 92 ^{Ac}
	2	1543 ± 117 ^{Ab}	1352 ± 142 ^{ABb}	977 ± 50 ^{Bb}	1277 ± 17 ^{ABb}	1435 ± 75 ^{ABb}
	3	760 ± 83 ^{Abc}	602 ± 8 ^{Accl}	552 ± 58 ^{Accl}	560 ± 133 ^{Accl}	377 ± 50 ^{Ad}
	4	893 ± 417 ^{Abc}	502 ± 75 ^{Accl}	393 ± 100 ^{Ad}	177 ± 33 ^{Ade}	402 ± 58 ^{Accl}
	5	110 ± 0 ^{Bc}	185 ± 58 ^{ABd}	685 ± 58 ^{Abcd}	193 ± 67 ^{ABde}	335 ± 158 ^{ABd}
	6	180 ± 8 ^{Abc}	138 ± 138 ^{Ad}	368 ± 25 ^{Ad}	352 ± 0 ^{Ae}	460 ± 350 ^{Ad}
Ascorbic acid content ([Ascorbic acid] mg/kg)	0	1094 ± 58 ^{Aa}	1094 ± 58 ^{Aa}	1094 ± 58 ^{Aa}	1094 ± 58 ^{Aa}	1094 ± 58 ^{Aa}
	1	1200 ± 0 ^{Aa}	1400 ± 200 ^{Aa}	1400 ± 200 ^{Aa}	1200 ± 0 ^{Aa}	1400 ± 200 ^{Aa}
	2	800 ± 0 ^{Aa}	1200 ± 400 ^{Aa}	1600 ± 0 ^{Aa}	1600 ± 0 ^{Aa}	1200 ± 400 ^{Aa}
	3	1200 ± 400 ^{Aa}	1400 ± 200 ^{Aa}	1400 ± 200 ^{Aa}	1600 ± 0 ^{Aa}	1600 ± 0 ^{Aa}
	4	1600 ± 0 ^{Aa}	1600 ± 0 ^{Ba}	1200 ± 0 ^{Ea}	1200 ± 0 ^{Da}	1200 ± 0 ^{Ca}
	5	1200 ± 0 ^{Aa}	1600 ± 0 ^{Aa}	1800 ± 0 ^{Aa}	1800 ± 200 ^{Aa}	1600 ± 0 ^{Aa}
	6	1600 ± 0 ^{Aa}	1600 ± 0 ^{Aa}	1600 ± 0 ^{Aa}	1200 ± 400 ^{Aa}	1600 ± 0 ^{Aa}
Betainins–Indicaxanthin (mg/100 g)	0	20.9 ± 6.7 ^{Aa}	20.9 ± 6.7 ^{Aa}	20.9 ± 6.7 ^{Aa}	20.9 ± 6.7 ^{Aa}	20.9 ± 6.7 ^{Aa}
	1	15.7 ± 3.5 ^{Aa}	29.8 ± 11.9 ^{Aa}	15.7 ± 2.2 ^{Aa}	20.2 ± 4.2 ^{Aa}	28.2 ± 3.2 ^{Aa}
	2	24.4 ± 3.8 ^{Aa}	21.2 ± 5.8 ^{Aa}	30.8 ± 10.3 ^{Aa}	21.2 ± 8.3 ^{Aa}	34.3 ± 13.2 ^{Aa}
	3	29.2 ± 1.6 ^{Aa}	23.7 ± 6.4 ^{Aa}	22.5 ± 1.3 ^A	23.7 ± 1.3 ^A	22.5 ± 6.4 ^{Aa}
	4	24.7 ± 5.5 ^{Aa}	23.1 ± 5.8 ^{Aa}	31.8 ± 7.4 ^{Aa}	24.4 ± 5.8 ^{Aa}	23.4 ± 9.3 ^{Aa}
	5	18.9 ± 0.3 ^{Aa}	33.7 ± 17.0 ^{Aa}	18.3 ± 5.5 ^{Aa}	20.9 ± 1.6 ^{Aa}	32.7 ± 3.9 ^{Aa}
	6	20.2 ± 1.4 ^{Aa}	12.5 ± 1.4 ^{Aa}	16.4 ± 4.1 ^{Aa}	11.2 ± 3.2 ^{Aa}	19.3 ± 6.4 ^{Aa}

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Betaines– Betanin (mg/100 g)	0	12.83 ± 1.83 ^{Aa}	12.83 ± 1.83 ^{Aa}	12.83 ± 1.83 ^{Aa}	12.83 ± 1.83 ^{Aa}	12.83 ± 1.83 ^{Aa}
	1	2.75 ± 0.92 ^{Ab}	4.12 ± 1.38 ^{Ab}	1.37 ± 1.37 ^{Ab}	3.21 ± 1.38 ^{Ab}	1.37 ± 0.46 ^{Ab}
	2	0.46 ± 0.46 ^{Ab}	1.38 ± 0.46 ^{Ab}	1.38 ± 0.46 ^{Ab}	1.38 ± 0.46 ^{Ab}	1.83 ± 0.92 ^{Ab}
	3	3.67 ± 0.00 ^{Ab}	3.21 ± 0.46 ^{Ab}	1.83 ± 1.83 ^{Ab}	2.75 ± 0.00 ^{Ab}	2.59 ± 0.46 ^{Ab}
	4	5.04 ± 0.46 ^{ABb}	4.12 ± 0.46 ^{Bb}	6.42 ± 0.00 ^{Aab}	4.58 ± 0.00 ^{ABb}	5.04 ± 0.46 ^{ABb}
	5	2.75 ± 0.92 ^{Ab}	3.21 ± 1.38 ^{Ab}	0.92 ± 0.92 ^{Ab}	1.83 ± 0.00 ^{Ab}	1.83 ± 0.92 ^{Ab}
	6	4.13 ± 0.46 ^{Ab}	3.67 ± 0.00 ^{Ab}	4.58 ± 0.92 ^{Ab}	3.21 ± 0.46 ^{Ab}	3.67 ± 0.00 ^{Ab}

(a–e): Within each parameter, values in the same column not sharing lower case superscript letters indicate statistically significant differences among weeks ($p < 0.05$); (A–E): Within each parameter, values in the same row not sharing upper case superscript letters indicate statistically significant differences among coatings ($p < 0.05$).

Betanin content in fresh fruits was 53.3 mg/100 g and 12.8 mg/100 g for red and orange varieties, respectively, being similar to values described by other authors (Valero-Galván et al., 2021). Betanin content in red prickly pears was maintained relatively well, showing no statistically significant differences among coatings and control within the same time of experience. The same behavior was observed in orange fruits except for week 4, with significant differences ($p < 0.05$) between alginate- and chitosan-coated fruits. In red fruits, significant declines were observed by week 6, particularly in alginate- and pectin-coated samples ($p < 0.05$). Chitosan-coated samples had higher betanin retention (33 mg/100 g) and starch (47.7 mg/100 g) by week 6 compared to alginate (16.0 mg/100 g) and pectin (14.7 mg/100 g), making chitosan and starch potentially more effective coatings for betanin preservation. Despite starch-coated fruits showing higher values in betanin at the end of the sixth week of the experiment, it was not statistically significant when compared to the beginning ($p > 0.05$). Thus, chitosan appears to be the coating to be more effective in retaining betanin content. The use of mucilage from prickly pear as coating material of prickly pears was effective in the preservation of betanin content in prickly pears after 9 days of storage (Liguori et al., 2021). In orange fruits, betanin content dropped significantly after the start in all the coated and uncoated fruits ($p < 0.05$). At the end of the experiment, betanin content had stabilized at lower concentrations, with no significant differences between coatings ($p > 0.05$) showing that no coating was effective in maintaining betanin levels. Moreover, storage temperature, antioxidant compounds,

and fruit maturity stage can produce effects on betalain content, leading to losses (Palma, Continella, et al., 2018) .

3.3.5 Microbiological Growth

Microbiological growth is an important parameter in terms of food safety since it is one of the major causes of food loss leading to economic losses (Blancas-Benitez et al., 2022; Severo et al., 2021) . In this experiment, mesophiles, yeast, and mold counts were evaluated through time in both peel and pulp for both varieties. For both varieties, there were some variations throughout the experiment, but it was observed that values of microbiological growth were lower in pulp when compared to peel. These general results not only emphasize the intrinsic protection function of the peel in fruits, but the use of coatings also played a significant role in avoiding higher contamination.

Mesophiles contamination in red prickly pear peel is presented in Table 3.5. Significant variations ($p < 0.05$) were noted between coatings during the entire experiment but not in the last week. At week 6, alginate- and pectin-coated fruits showed the lowest results for mesophile contamination but with no significant differences. The effects of alginate and pectin coatings were also demonstrated in blueberries (Campos et al., 2011; Mannozi et al., 2017) .

Yeast and mold contamination in red prickly pear peels ($p < 0.05$) showed significant differences only in week 4. At the end of the experiment, both coated and control fruits showed values of yeast and mold contamination within the same range and starch-coated fruits were the ones that presented lower values. Starch coating proved to be potentially effective on delaying contamination of strawberries (Campos et al., 2011) .

Table 3.5 - Microbiological content results of the coated and uncoated red prickly pear peel fruits during storage time.

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Mesophiles (log CFU/g)	0	3.98 ± 0.26 ^{Aa}	3.98 ± 0.26 ^{Aa}	3.98 ± 0.26 ^{Aa}	3.98 ± 0.26 ^{Aa}	3.98 ± 0.26 ^{Aa}
	1	3.45 ± 0.20 ^{Aa}	3.76 ± 0.35 ^{Aa}	3.76 ± 0.25 ^{Aa}	3.76 ± 0.33 ^{Aab}	4.60 ± 0.70 ^{Aa}
	2	3.18 ± 0.38 ^{Aa}	3.16 ± 0.73 ^{Aa}	3.83 ± 0.65 ^{Aa}	2.45 ± 0.20 ^{Abc}	2.43 ± 0.48 ^{Aa}
	3	3.69 ± 0.23 ^{Aa}	4.37 ± 0.42 ^{Aa}	3.72 ± 1.06 ^{Aa}	2.55 ± 0.27 ^{Abc}	2.99 ± 0.03 ^{Aa}
	4	3.80 ± 0.75 ^{Aa}	4.66 ± 0.65 ^{Aa}	3.72 ± 0.72 ^{Aa}	2.12 ± 0.09 ^{Ac}	2.12 ± 0.39 ^{Aa}
	5	2.90 ± 0.22 ^{ABa}	3.66 ± 0.59 ^{ABa}	2.40 ± 0.16 ^{Ba}	3.21 ± 0.03 ^{ABabc}	4.51 ± 0.41 ^{Aa}
	6	3.29 ± 1.34 ^{Aa}	2.66 ± 0.41 ^{Aa}	3.68 ± 0.08 ^{Aa}	2.93 ± 0.37 ^{Aabc}	3.26 ± 0.53 ^{Aa}

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Yeasts and Molds (log CFU/g)	0	2.01 ± 0.02 ^{Aa}	2.01 ± 0.02 ^{Aa}	2.01 ± 0.02 ^{Aa}	2.01 ± 0.02 ^{Aa}	2.01 ± 0.02 ^{Aa}
	1	3.04 ± 0.09 ^{Aa}	3.04 ± 0.39 ^{Aa}	3.56 ± 0.65 ^{Aa}	3.13 ± 0.00 ^{Aa}	3.75 ± 0.62 ^{Aa}
	2	3.40 ± 0.12 ^{Aa}	3.19 ± 0.06 ^{Aa}	3.83 ± 0.84 ^{Aa}	2.73 ± 0.30 ^{Aa}	2.30 ± 0.35 ^{Aa}
	3	3.10 ± 0.44 ^{Aa}	3.79 ± 0.12 ^{Aa}	4.09 ± 0.88 ^{Aa}	3.41 ± 0.03 ^{Aa}	3.44 ± 0.29 ^{Aa}
	4	2.76 ± 0.33 ^{ABa}	3.83 ± 0.23 ^{Aa}	1.65 ± 0.00 ^{Ba}	2.33 ± 0.68 ^{ABa}	1.89 ± 0.24 ^{ABa}
	5	3.17 ± 0.05 ^{Aa}	3.45 ± 0.00 ^{Aa}	2.28 ± 0.63 ^{Aa}	3.43 ± 0.11 ^{Aa}	3.85 ± 0.37 ^{Aa}
	6	3.62 ± 1.49 ^{Aa}	3.70 ± 0.97 ^{Aa}	3.43 ± 0.37 ^{Aa}	3.17 ± 0.21 ^{Aa}	2.16 ± 0.50 ^{Aa}

^(a-c): Within each parameter, values in the same column not sharing lower case superscript letters indicate statistically significant differences among weeks ($p < 0.05$); ^(A,B): Within each parameter, values in the same row not sharing upper case superscript letters indicate statistically significant differences among coatings ($p < 0.05$).

In red prickly pear pulp (Table 3.6), the contamination of mesophiles had some variations throughout the 6 weeks of the experiment. Significant differences were observed between coatings in weeks 1, 2, and 4. At the end of the experiment, even though no statistically significant differences ($p > 0.05$) were observed, control fruits showed the highest mesophilic contamination compared to coated fruits, and alginate- and chitosan-coated fruits showed the least contamination at the end, as was also demonstrated in kiwi fruits and sweet cherries (Tokatlı & Demirdöven, 2020; Vivek & Subbarao, 2018). Prickly pears showed no contamination in yeasts and molds at the beginning of the experiment, and those values increased over time. In the sixth week, all coatings showed a positive effect in protecting the fruits against contamination despite no significant differences found between them with chitosan showing the lower value. Chitosan demonstrated to be effective against yeasts and molds in fresh-cut kiwi fruit (Vivek & Subbarao, 2018).

Table 3.6 - Microbiological content results of the coated and uncoated red prickly pear pulp fruits during storage time.

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Mesophiles (log CFU/g)	0	2.00 ± 0.27 ^{Aa}	2.00 ± 0.27 ^{Aab}	2.00 ± 0.27 ^{Aa}	2.00 ± 0.27 ^{Aa}	2.00 ± 0.27 ^{Aab}
	1	2.06 ± 0.07 ^{Aa}	1.26 ± 0.00 ^{ABb}	0.95 ± 0.21 ^{Ba}	1.21 ± 0.28 ^{ABa}	0.84 ± 0.11 ^{Bb}
	2	3.43 ± 0.00 ^{Aa}	2.14 ± 0.70 ^{ABab}	1.74 ± 0.00 ^{ABa}	0.96 ± 0.00 ^{Ba}	1.59 ± 0.33 ^{ABab}
	3	1.20 ± 0.24 ^{Aa}	2.06 ± 1.10 ^{Aab}	2.11 ± 0.25 ^{Aa}	1.74 ± 0.18 ^{Aa}	3.56 ± 0.94 ^{Aa}
	4	1.46 ± 0.50 ^{Ba}	4.48 ± 0.00 ^{Aa}	1.95 ± 0.34 ^{Ba}	1.65 ± 0.39 ^{Ba}	0.95 ± 0.00 ^{Bb}
	5	2.89 ± 0.53 ^{Aa}	2.80 ± 0.36 ^{Aab}	1.26 ± 0.00 ^{Aa}	2.41 ± 0.75 ^{Aa}	1.59 ± 0.33 ^{Aab}
	6	2.69 ± 1.43 ^{Aa}	1.20 ± 0.24 ^{Ab}	1.20 ± 0.24 ^{Aa}	1.26 ± 0.30 ^{Aa}	1.74 ± 0.00 ^{Aab}
Yeasts and Molds (log CFU/g)	0	<1 ^{Aa}	<1 ^{Ab}	<1 ^{Ab}	<1 ^{Ab}	<1 ^{Aa}
	1	2.50 ± 0.00 ^{Ba}	<1 ^{Db}	<1 ^{Db}	2.66 ± 0.00 ^{Aa}	1.66 ± 0.00 ^{Cc}
	2	3.53 ± 0.24 ^{Aa}	1.96 ± 0.30 ^{Bab}	2.26 ± 0.00 ^{ABa}	2.16 ± 0.35 ^{Ba}	2.20 ± 0.06 ^{Bbc}
	3	1.66 ± 0.00 ^{Aa}	2.83 ± 0.69 ^{Aa}	1.96 ± 0.60 ^{Aa}	2.16 ± 0.20 ^{Aa}	3.27 ± 0.50 ^{Aa}
	4	2.98 ± 0.25 ^{Aa}	2.31 ± 0.05 ^{ABab}	1.66 ± 0.00 ^{Bab}	1.96 ± 0.00 ^{ABa}	2.98 ± 0.45 ^{Aab}
	5	2.78 ± 0.52 ^{Aa}	2.67 ± 0.41 ^{Aab}	1.84 ± 0.11 ^{Aa}	2.61 ± 0.00 ^{Aa}	1.66 ± 0.00 ^{Abc}
	6	3.16 ± 1.50 ^{Aa}	2.56 ± 0.00 ^{Aab}	1.96 ± 0.00 ^{Aa}	2.13 ± 0.00 ^{Aa}	2.56 ± 0.00 ^{Aab}

(a-c): Within each parameter, values in the same column not sharing lower case superscript letters indicate statistically significant differences among weeks ($p < 0.05$); (A-D): Within each parameter, values in the same row not sharing upper case superscript letters indicate statistically significant differences among coatings ($p < 0.05$).

In the peel of orange prickly pears, some variations throughout the weeks had occurred ($p < 0.05$) (Table 3.7). In the sixth week, control fruits had a higher value of mesophiles when compared to coated fruits, even though no statistically significant differences were observed. Chitosan, pectin, and starch were the coatings that led to lower values of antimicrobial contamination in the peel of orange prickly pears. Pectin coating was also effective against mesophilic bacteria when applied to blueberries (Mannozi et al., 2017). The intrinsic antimicrobial properties of chitosan demonstrated in this study were also reported in sweet cherries (Tokatl & Demirdöven, 2020). In terms of yeast and mold contamination, control fruits had an increase over time, showing statistically significant differences ($p < 0.05$), as did the coated fruits. Even though at the end of 6 weeks there were no significant differences observed, coatings

apparently protected fruits against yeast and molds, especially chitosan. Chitosan coating was also effective against yeast and mold in sweet cherries (Tokatlı & Demirdöven, 2020) .

Table 3.7 - Microbiological content results of the coated and uncoated orange prickly pear peel fruits during storage time.

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Mesophiles (log CFU/g)	0	3.69 ± 0.79 ^{Aa}	3.69 ± 0.79 ^{Aa}	3.69 ± 0.79 ^{Aa}	3.69 ± 0.79 ^{Aa}	3.69 ± 0.79 ^{Aa}
	1	5.26 ± 0.00 ^{Aa}	3.80 ± 0.55 ^{ABa}	2.45 ± 0.21 ^{Ba}	2.95 ± 0.00 ^{Ba}	2.95 ± 0.00 ^{Ba}
	2	3.04 ± 0.24 ^{Aa}	2.92 ± 0.18 ^{Aa}	2.72 ± 0.35 ^{Aa}	2.65 ± 0.00 ^{Aa}	2.45 ± 0.20 ^{Aa}
	3	3.55 ± 0.39 ^{Aa}	2.62 ± 0.25 ^{ABa}	1.97 ± 0.17 ^{Ba}	3.73 ± 0.00 ^{Aa}	3.01 ± 0.05 ^{ABa}
	4	4.01 ± 0.10 ^{Aa}	3.10 ± 0.63 ^{Aa}	2.31 ± 1.35 ^{Aa}	3.77 ± 0.12 ^{Aa}	2.83 ± 0.30 ^{Aa}
	5	3.58 ± 0.21 ^{ABa}	4.34 ± 0.07 ^{Aa}	3.21 ± 0.00 ^{ABa}	2.74 ± 0.49 ^{Ba}	2.73 ± 0.00 ^{Ba}
	6	5.28 ± 0.61 ^{Aa}	3.54 ± 0.43 ^{Aa}	3.14 ± 0.01 ^{Aa}	3.00 ± 0.74 ^{Aa}	3.11 ± 0.31 ^{Aa}
Yeasts and Molds (log CFU/g)	0	1.61 ± 0.05 ^{Ab}	1.61 ± 0.05 ^{Ab}	1.61 ± 0.05 ^{Ac^d}	1.61 ± 0.05 ^{Ac}	1.61 ± 0.05 ^{Ac}
	1	3.52 ± 0.09 ^{ABab}	3.35 ± 0.00 ^{Bab}	1.21 ± 0.00 ^{Cd}	3.94 ± 0.00 ^{Aa}	3.15 ± 0.20 ^{Bab}
	2	3.30 ± 0.07 ^{Aab}	2.75 ± 0.62 ^{ABab}	1.56 ± 0.00 ^{Bd}	3.53 ± 0.20 ^{Aab}	3.39 ± 0.03 ^{Aab}
	3	3.78 ± 0.07 ^{Aa}	3.75 ± 0.06 ^{Aa}	2.73 ± 0.00 ^{Aa}	3.39 ± 0.39 ^{Aab}	3.47 ± 0.10 ^{Aab}
	4	3.78 ± 0.70 ^{Aa}	3.65 ± 0.20 ^{Aa}	2.12 ± 0.24 ^{Abc}	2.52 ± 0.09 ^{Abc}	2.65 ± 0.15 ^{Ab}
	5	3.33 ± 0.17 ^{Aab}	3.81 ± 0.23 ^{Aa}	2.13 ± 0.00 ^{Bb}	3.03 ± 0.23 ^{ABab}	3.23 ± 0.06 ^{Aab}
	6	5.07 ± 0.66 ^{Aa}	4.39 ± 0.65 ^{Aa}	2.47 ± 0.03 ^{Aab}	3.34 ± 0.43 ^{Aab}	3.54 ± 0.29 ^{Aa}

(a-d): Within each parameter, values in the same column not sharing lower case superscript letters indicate statistically significant differences among weeks ($p < 0.05$); (A-C): Within each parameter, values in the same row not sharing upper case superscript letters indicate statistically significant differences among coatings ($p < 0.05$).

In the pulp of orange prickly pears, the values in terms of mesophiles (Table 3.8) had some variations ($p < 0.05$) between coatings at weeks 2 and 3, but not for the rest of the weeks. By the end, starch-coated fruits showed lower values of mesophiles than the rest of the coated fruits and the control, which was also demonstrated in strawberries (Campos et al., 2011) .

No significant statistical differences ($p > 0.05$) were observed in orange pulp regarding yeast and mold contamination at the end of the experiment (Table 3.8). Despite that, uncoated fruits showed the highest values of yeast and mold, indicating the possible protective effects of coatings. Chitosan- and starch-coated fruits had less contamination in the sixth week. Starch

demonstrated the same tendency in strawberries (Campos et al., 2011) , and chitosan in sweet cherries (Tokatlı & Demirdöven, 2020) .

Table 3.8 - Microbiological content results of the coated and uncoated orange prickly pear pulp fruits during storage time.

Parameter	Week	Control	Alginate	Chitosan	Pectin	Starch
Mesophiles (log CFU/g)	0	2.67 ± 0.14 ^{Aab}	2.67 ± 0.14 ^{Aa}	2.67 ± 0.14 ^{Aa}	2.67 ± 0.14 ^{Aa}	2.67 ± 0.14 ^{Aa}
	1	0.95 ± 0.00 ^{Ab}	1.69 ± 0.26 ^{Aa}	1.56 ± 0.30 ^{Ab}	1.41 ± 0.15 ^{Aab}	0.95 ± 0.00 ^{Ab}
	2	2.21 ± 0.00 ^{Cab}	2.24 ± 0.00 ^{Ba}	1.80 ± 0.00 ^{Db}	0.96 ± 0.00 ^{Eb}	2.37 ± 0.00 ^{Aab}
	3	2.55 ± 0.17 ^{Aab}	1.97 ± 0.17 ^{ABa}	1.55 ± 0.11 ^{BCb}	0.96 ± 0.00 ^{Cab}	1.94 ± 0.13 ^{ABab}
	4	2.00 ± 0.00 ^{Aab}	1.67 ± 0.24 ^{Aa}	1.44 ± 0.00 ^{Ab}	1.31 ± 0.35 ^{Aab}	1.58 ± 0.33 ^{Aab}
	5	3.01 ± 0.93 ^{Aa}	1.35 ± 0.09 ^{Aa}	1.44 ± 0.00 ^{Ab}	0.96 ± 0.00 ^{Aab}	1.46 ± 0.50 ^{Aab}
	6	2.50 ± 0.00 ^{Aab}	2.42 ± 1.16 ^{Aa}	2.09 ± 0.13 ^{Aab}	2.62 ± 0.71 ^{Aa}	1.83 ± 0.27 ^{Aab}
Yeasts and Molds (log CFU/g)	0	<1 ^{Aa}	<1 ^{Ac}	<1 ^{Ab}	<1 ^{Ab}	<1 ^{Ab}
	1	2.13 ± 0.00 ^{Aa}	<1 ^{Ac}	<1 ^{Ab}	1.66 ± 0.00 ^{Aab}	2.16 ± 0.48 ^{Aa}
	2	2.36 ± 0.00 ^{ABa}	2.26 ± 0.00 ^{Bb}	<1 ^{Cb}	2.86 ± 0.00 ^{Aa}	2.16 ± 0.20 ^{Ba}
	3	2.46 ± 0.20 ^{Aa}	2.01 ± 0.35 ^{Ab}	2.44 ± 0.44 ^{Aa}	2.56 ± 0.00 ^{Aab}	2.13 ± 0.00 ^{Aa}
	4	2.26 ± 0.00 ^{Aa}	1.96 ± 0.30 ^{Abc}	2.23 ± 0.27 ^{Aa}	2.05 ± 0.09 ^{Aab}	2.13 ± 0.00 ^{Aa}
	5	2.70 ± 1.04 ^{Aa}	2.26 ± 0.00 ^{Ab}	1.66 ± 0.00 ^{Aab}	1.66 ± 0.00 ^{Aab}	<1 ^{Ab}
	6	2.83 ± 0.70 ^{Aa}	3.41 ± 0.00 ^{Aa}	1.66 ± 0.00 ^{Aab}	2.42 ± 0.76 ^{Aab}	1.96 ± 0.00 ^{Aab}

(a-c): Within each parameter, values in the same column not sharing lower case superscript letters indicate statistically significant differences among weeks ($p < 0.05$); (A-E): Within each parameter, values in the same row not sharing upper case superscript letters indicate statistically significant differences among coatings ($p < 0.05$).

3.4 Conclusions

Coatings had a positive effect on the preservation of quality and shelf life of prickly pears kept under refrigeration for 6 weeks, with some differences among the red and orange varieties. The most successful coatings in the reduction of weight loss were starch in red prickly pears with a reduction of around 8% and chitosan in orange prickly pears with a reduction of around 10%, when compared with the control of both prickly pears that lost 23% and 18%, respectively. These coatings also contributed positively to the maintenance of the prickly

pears' color. In terms of texture, red prickly pears coated with pectin or alginate exhibited superior firmness, while orange prickly pears benefited most from chitosan or alginate coatings. Regarding bioactivity, starch was the best coating in preserving antioxidant activity in red prickly pears, while in orange prickly pears, the coatings did not produce a significant effect. Starch was also the most effective in maintaining ascorbic acid content, particularly in the red prickly pear. Starch also contributed to the preservation of indicaxanthin and betanin in red prickly pears that presented at the end of 6 weeks values of 27.3 mg/100 g and 47.7 mg/100 g, respectively, when compared to the initial values of 32.2 mg/100 g and 53.3 mg/100 g showing good retention of these bioactive compounds. In orange prickly pears, the starch-coated fruits also demonstrated superior efficacy in retaining both indicaxanthin and betanin content. Regarding microbiological quality, although all coated and control prickly pears showed similar levels of yeasts and molds, starch-coated fruits displayed lower contamination levels. In red prickly pears, alginate and pectin coatings were most effective at reducing anti-microbial contamination on the peel, and on the pulp alginate and chitosan showed lower levels of contamination (1.20 log CFU/g). Overall, starch and chitosan coatings showed the best results on the reservation of physicochemical, bioactive, and microbiological properties of prickly pears to meet consumers' demand for fresh fruit. Even though this study focuses specifically on prickly pears, the fundamental principles behind the interaction between coating materials and fruit surfaces have broad relevance. Edible coatings create a barrier that minimizes moisture loss, gas exchange, and microbial growth, which are transversal challenges in fruit preservation. However, the effectiveness of these coatings varies depending on each fruit's unique surface properties, respiration rates, and physicochemical characteristics. This research provides a valuable reference for extending the methodology application to other fruits, additional studies tailored to specific fruit types must be addressed to optimize formulations and validate their effectiveness.

The results of this study demonstrate the promising potential of coatings for preserving prickly pears, but several constraints must be addressed in future studies. A study of the impact of these coatings under different temperature conditions must be addressed. Additionally, the combination with other preservation methods, such as modified atmosphere, and the study of coating formulation in order to improve their features such as UV protection or antioxidant capacity, would be highly valuable for extending the shelf life of prickly pears even further. Future studies could also include the assessment of sensory qualities, such as taste and texture, to fully understand the effect of coatings on prickly pear quality from the

consumer's perspective. In addition, studies should investigate the scalability of these coatings for commercial use, taking into account the cost-effectiveness and practicality of applying these coatings in larger-scale operations. These areas of research would contribute to a more comprehensive understanding of how coatings can be used to enhance the shelf life and quality of prickly pears and similar fruits, while also addressing broader concerns related to sustainability and commercial viability.

PECTIN EXTRACTION FROM *OPUNTIA* SPP. CLADODES: PROCESS OPTIMIZATION AND CHARACTERIZATION

This chapter addresses one of the objectives defined for this Ph.D. work, which focused on the characterization and extraction of pectin/mucilage from *Opuntia ficus-indica* cladodes. To achieve this, cladodes were first analyzed in terms of their physicochemical composition. An extraction process was then developed and optimized using experimental design in order to determine the most suitable conditions for pectin recovery. The extracted pectin was subsequently characterized, and its potential application in film formation was explored. The results of this work will be submitted to a scientific peer-reviewed journal (*Sustainable Food Technology*) under the title *Pectin Extraction from Opuntia spp. Cladodes: Process Optimization and Characterization*.

4.1 Introduction

Opuntia ficus indica (OFI) is a plant that belongs to the *Cactaceae* family, genus *Opuntia*, that is well adapted to arid and semi-arid lands and widespread around the world (e.g. Mexico and Mediterranean area) (Ahmed et al., 2021; Ciriminna et al., 2019; Missaoui et al., 2020; Scalis et al., 2016). The fruits, known as cactus pear or prickly pear, have gained further attention due to their high nutritional content and health beneficial effects, due to their antioxidant, anti-cancer and anti-inflammatory properties (El-Mostafa et al., 2014; C. Rodrigues et al., 2023). Cladodes, the stems, are mostly used for human consumption but also animal feed (Ciriminna et al., 2019; Msaddak et al., 2017), and they are rich in water, carbohydrates and fiber (namely,

cellulose, hemicellulose, pectin, lignin and mucilage) (del Socorro Santos-Díaz et al., 2017; Ginestra et al., 2009; Hernández-Urbiola et al., 2011; Stintzing & Carle, 2005). The chemical composition of cladodes depends on several factors such as maturity stage, environmental conditions, harvest season, post-harvest treatment and the type of species (Astello-García et al., 2015). Cladodes also find application in several industries such as cosmetics, pharmaceuticals and nutraceuticals (Attanzio et al., 2019; Sciacca et al., 2021). Cladodes have also been applied as adsorbent materials in the textile industry, as flocculant agents in water and wastewater treatment, as additives in building construction and have been valorized for energy, such as the production of hydrochars and biogas (Gallegos-Villela et al., 2021; Louati et al., 2018; Michelin et al., 2020; Nharingo et al., 2015; Timpanaro et al., 2021; Trindade et al., 2021; Volpe et al., 2018).

Moreover, the cactus needs to be pruned annually, generating an enormous amount of cactus stems/cladodes, which are a waste product/by-product capable of numerous applications, such as those aforementioned (Bacchetta et al., 2024; Del-Valle et al., 2005). Thus, pruning cladodes residues is a significant source of low-cost biomass for mucilage/pectin extraction, and their exploitation would in part help to solve the problem of disposal/waste materials. The industrial exploitation of this crop generates by-products between 20% and 45% of the fresh weight of cladodes and fruits, respectively (Barba et al., 2017; Bensadón et al., 2010). For instance, in Mexico, the biggest OFI producer, around 40,000 tons of nopal waste are lost annually (de Farias et al., 2023). Mucilage from OFI is a mixture of heterogenous polysaccharides with high molecular weight constituted mostly by arabinose, galactose, galacturonic acid, rhamnose and xylose (León-Martínez et al., 2010; Sáenz et al., 2004). Due to its elastic flow characteristic and ability to form a molecular network, OFI mucilage can be used in the food industry as edible films/coatings (de Farias et al., 2023; Gheribi et al., 2018; C. Rodrigues et al., 2025). The mucilage structure consists of two distinct water-soluble fractions: pectin, which exhibits gelling properties in the presence of Ca^{2+} , and mucilage, that has no gelling properties (C. Rodrigues et al., 2023). Pectin is a complex polysaccharide mainly constituted by α -D-galacturonic acids (Wang et al., 2021), commonly used in the food industry as gelling and stabilizing agent, e.g. in the formulation of jellies and jams (Aragona et al., 2018; del Socorro Santos-Díaz et al., 2017; Lefsih et al., 2016).

Pectin extraction is commonly performed under acidic conditions with high temperatures (60-100°C) (Freitas et al., 2021; Roy et al., 2023). High temperatures facilitate the solvent diffusion into the plant matrix helping the extraction and also allowing the polymer's

solubilization. However, the use of high temperatures can cause pectin depolymerization, ultimately reducing the extraction yield (Sayah et al., 2016). An acidic pH is fundamental for the extraction process since it promotes the hydrolysis of protopectin (Bayar et al., 2017). The use of strong acids as solvents, such as sulfuric acid and hydrochloric acid, is commonly employed but their use is associated with high costs and significant environmental impacts (Roy et al., 2023). To overcome this challenge, mild acids, including citric acid, malic acid, and acetic acid are valuable alternatives to use as solvents in pectin extraction. Despite these mild acids exhibit lower hydrolyzing efficiency compared to strong acids, they are considered food-grade making them more suitable for food applications (Chandel et al., 2022; Roy et al., 2023). Moreover, several other factors are of extreme importance to obtain the best results for pectin extraction such as pH, time, agitation, surface area and solid-liquid ratio (Freitas et al., 2020; Kumar et al., 2023).

Some other techniques have been employed to enhance pectin extraction and the most innovative and green technologies include enzymatic extraction, ultrasonic-assisted extraction (UAE), microwave-assisted extraction, and the use of non-thermal technologies such as sub-critical fluids, high hydrostatic pressure, pulsed electric field and deep eutectic solvents (Bayar et al., 2017, 2018; M. Chen & Lahaye, 2021; Cui et al., 2021; de Farias et al., 2023; Zamboi et al., 2024).

In general, the functional properties of pectin are linked to the degree of esterification, that are classified as low methoxyl (LM) pectin (DE < 50%) and high methoxyl (HM) pectin (DE > 50%). The DE of the pectin is directly related to the source and has a fundamental influence on a proper future application (Sevgi et al., 2022). Pectin from OFI is generally classified as low methoxyl pectin (Aragona et al., 2018; del Socorro Santos-Díaz et al., 2017; Lefsih et al., 2016; Sevgi et al., 2022).

An example of pectin extraction from OFI cladodes was done by Chaouch et al. (2016). In this study three different extraction conditions were used: acidic (pH=2, 2h, 40°C), neutral (pH=6, 2h, 40°C) and basic (pH=10, 2h, 4°C) and samples were submitted to 10 min ultrasound prior to extraction. In conclusion, the highest yield of extraction was obtained with neutral pH, however, acidic conditions presented a higher galacturonic content (Chaouch et al., 2016). Moreover, in the work of Zamboi et al. (2024), pectin from OFI was extracted using UAE maintaining the power and frequency of the ultrasound bath constant and varying the solid-liquid ratio (SL), temperature, pH and time. The authors found the optimal operating conditions were obtained using a solid liquid ratio of 1:10 w/v, pH = 2.5, temperature of 65 °C and time

of extraction of 20 min. The extraction yield was around 11 % dw, and pectin was mainly composed of galacturonic acid, arabinose and galactose, with 42.82 % degree of esterification (DE), thus it was characterized as low-methylated pectin (Zamboi et al., 2024).

Hence, the present study is focused on the optimization of the extraction method of polysaccharides (pectin) from by-product cladodes of *Opuntia ficus indica*. To achieve this, cladodes were first characterized in terms of physical, chemical and bioactive properties. Focusing on the sustainability of the extraction process and thorough literature review, three different solvents were tested (water, citric acid aqueous solution and acetic acid aqueous solution), avoiding the use of strong acids. Additionally, the impact of pH on extraction was investigated by testing a range of pH values (1.5, 2, 2.5, 3, 4, and 7). Once the optimal solvent and pH were determined based on extraction yield, further parameters, including liquid-to-solid ratio, extraction time, and temperature, were optimized using Response Surface Methodology (RSM). RSM allows to minimize the number of experiments and gather more information in order to optimize various parameters and the effect of their interaction in the desired goal (Filho et al., 2022) The best extraction conditions resulting from the optimization were then used to obtain pectin that was further characterized in terms of degree of esterification, galacturonic acid content and identification of functional groups. Ultimately, this study seeks to identify sustainable and efficient methods for pectin extraction from an alternative source, envisaging the possibility to be used for food packaging applications.

4.2 Materials and Methods

4.2.1 Materials and reagents

O. ficus-indica cladodes were harvested in the period of September 2019 and kindly provided by *Pepe Aromas* company, located in Vale Pereiro, Évora, Portugal.

The water used was purified using Milli-Q system (Millipore, USA) and all chemicals had analytical reagent grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, catechin, aluminium chloride, sulphuric acid, 3,5-Dimethylphenol, D-(+)-Galacturonic acid monohydrate and pectin from citrus peel were purchased from Sigma Aldrich (Germany). Glacial acetic acid and gallic acid were acquired from Alfa Aesar (Germany). Folin–Ciocalteu reagent, citric acid, sodium chloride, copper sulfate pentahydrate, phenolphthalein, potassium hexacyanoferrate II trihydrate and, sodium carbonate anhydrous were obtained from PanReac (Spain). 2,6-Dichlorophenol-indophenol, hydrochloric acid, and ammonium molybdate

tetrahydrate were purchased from Fisher Chemical (United Kingdom). Potassium antimony tartrate, sodium nitrite, and zinc sulphate heptahydrate were purchased from Merck (Germany). Ethanol absolute, nitric acid, sulphuric acid and sodium sulphate were purchased from Riedel-de-Haen (Germany). Potassium sodium tartrate tetrahydrate was purchased from Scharlau (Spain). Potassium dihydrogen phosphate was purchased from Chem-Lab (Belgium).

4.2.2 Cladode characterization

Cladodes were separated into inner (pulp) and outer (peel) layers and characterized in terms of the following methods: pH, titratable acidity, total protein content, moisture, and ash according to the Association of Official Analytical Chemists (AOAC) methods (AOAC, 1990). The moisture content was determined by drying the samples at 105 °C for 1 hour in an oven WTB Binder type E28 (Binder, Germany) and the ash content by using a muffle furnace Heraeus Eletronic KR 170 E (Heraeus, Germany) at 550 °C and the results expressed in percentage of dry basis. In order to assess the pH and titratable acidity, an extract of 5 g of cladode in 10 mL of water was made. The pH values were measured using a potentiometer HI 2210 (Hanna Instruments, USA) and titratable acidity was evaluated by titrating 10 mL of extract with 0.1 N NaOH. The results of titratable acidity were expressed as a percentage of citric acid. The ascorbic acid content was determined by the 2,6 – dichlorophenol indophenol titrimetric method according to AOAC (AOAC, 1990). Fiber content was accessed through sequential extraction of the various fiber fractions (hemicellulose, cellulose and lignin) (Goering & Van Soest, 1970) and the results were expressed in mg per 100 g on a dry basis. The total sugar composition, reducing and non-reducing sugar was evaluated through the Munson and Walker method (NP 1419, 1988). Protein content was measured by nitrogen content using the Kjeldahl method and a conversion factor of 6.25 was used to obtain the total protein content (Watts & Halliwell, 1996). The phosphorus content was determined by digestion with H₂SO₄ and the determination of the phosphates in the digestates by molecular absorption spectrophotometry, through the formation of a colored complex with an ammonium molybdate solution, in the presence of ascorbic acid and potassium and antimony tartrate (Watanabe & Olsen, 1965) and the samples were read at 880 nm in the UV-Vis spectrophotometer E-1000UV (Peak Instruments, USA). The P content was obtained by using a calibration curve with known concentrations of P (0-0.25 mg/L) and read in UV-Vis spectrophotometer at 880 nm. Results were expressed in mg of P per 100 g. Total phenolic content (TPC), antioxidant activity and

total flavonoid content of the cladodes were quantified by Folin–Ciocalteu method (Singleton et al., 1999) , DPPH assays (Brand-Williams et al., 1995), and aluminum complexation in the presence of nitrite method (Barros et al., 2010), respectively. Ethanolic extracts in the proportion of cladodes/ethanol of 1/10 g /mL were used in the analysis. The TPC was obtained by using a calibration curve with known concentrations (0-120 mg/L) of gallic acid solution and the sample's absorbance at 760 nm read in the UV-Vis spectrophotometer. Results were expressed in mg of gallic acid equivalent (GAE) per 100 g of cladodes. To evaluate the antioxidant activity, DPPH assay was carried out and the absorbance was read at 517 nm in a UV-Vis spectrophotometer and the results were expressed in mg of Trolox equivalent per kg. For total flavonoid content, the samples were read at 510 nm in a UV-Vis spectrophotometer and the results were expressed in mg of catechin (CE) per 100 g of cladodes. The results were obtained by using a calibration curve of catechin. All the analysis was performed in triplicate.

4.2.3 Pectin extraction and characterization

To optimize the conditions for pectin extraction based on extraction yield, a series of experiments were conducted. The general procedure for pectin extraction is detailed in 4.2.3.1, while specific experiments were designed to evaluate and optimize the key extraction parameters. In order to define the solvent to be used three different solvents were tested, namely: deionized water, aqueous solutions of acetic acid (10% w/v) and citric acid (1% w/v) at a pH 2 adjusted with HCl 0.1 N – experimental set 1(J. Luo et al., 2019; Yuliarti et al., 2015). Then, experiments were conducted to study the impact of pH by testing different pH values (1.5, 2, 2.5, 3, 4 and 7) – experimental set 2. The remaining parameters for these experiments were defined as shown in Table 4.1. After these two parameters (pH and Solvent) were defined, liquid-solid ratio, time and temperature were optimized using RSM as described in 4.2.3.2. The extracted pectin was subsequently characterized following the procedures outlined in section 4.2.3.3.

4.2.3.1 Extraction

The extraction general procedure is described as follows. Cladodes were previously washed and separated into peel and pulp and then cut into small pieces. The extraction was performed according to Espino-Díaz and others (2010) with modifications (Espino-Díaz et al., 2010). The

extraction step was performed in a thermostatic water bath WB01(Labbox Labware, Spain) with the parameters described in Table 4.1.

Table 4.1 - Experimental range and levels of each factor used in the response surface design.

Experimental set	Solvent	pH	L/S ratio	Time (min)	Temperature (°C)
1	Deionized water, citric acid (1% w/v), acetic acid (10% v/v)	2	10	50	80
2	Deionized water	1.5, 2, 2.5, 3, 4, 7	10	50	80

Samples were centrifuged at 10000 rpm/ 15 min/ 0°C. The supernatant was left overnight at 4 °C in ethanol (ratio of 1:2 (v/v)) to precipitate. After precipitation, the samples were again centrifuged and dried in an oven at 45 °C for 2h and then placed in a desiccator containing silica. The final samples were reduced into powder for further analysis. All the extractions were performed in triplicate.

4.2.3.2 Experimental design

A Central Composite Design (CCD) with a 2³ factorial design with the addition of six replicates at the central point and six Alfa face centered essays were performed to study and optimize the three extraction parameters (L/S, time and temperature), chosen based on preliminary experiments and literature (Espitia, Soares, et al., 2013; Zamboi et al., 2024). The experiment was carried out according to a CCD based on the Response Surface Methodology with three variables: the L/S (X₁) ranged between 5 -15 (w/v), time (X₂) ranged between 40-60 min and temperature (X₃) between 70-90°C as it is demonstrated in Table 4.2.

Table 4.2 - Experimental range and levels of each factor used in the response surface design.

Variables	Factor	Coded levels		
		-1	0	+1
L/S (% w/v)	X ₁	5	10	15
Time (min)	X ₂	40	50	60
Temperature (°C)	X ₃	70	80	90

The dependent variable was pectin extraction yield, and the data were analyzed by regression using Minitab statistical software, version 19.2. The complete second-order model was initially tested (Equation 5). Only the significant coefficients ($p < 0.05$) were taken into account for the regression analysis.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (5)$$

where Y represents the yield of extraction, X₁ is the real variable L/S ratio, X₂ is the real variable for time, X₃ is the real variable for temperature, and $\beta_0, \beta_1, \beta_2, \beta_3, \beta_{11}, \beta_{22}, \beta_{33}, \beta_{12}, \beta_{13}, \beta_{23}$ represent the equation coefficients.

The statistical significance of the terms in the regression equation was determined with 95% confidence level.

4.2.3.3 Characterization

The yield of extraction was performed through gravimetric method and the results were expressed in percentage (%) of dry weight.

Fourier-transform infrared (FTIR) spectra of the resulting materials were acquired in the range 400 – 4000 cm⁻¹ using a FTIR spectrometer PerkinElmer Spectrum Two (Perkin Elmer, USA). The powder was directly analysed, with 4 scans and a resolution of 4 cm⁻¹. The FTIR spectra analysed were used to calculate the degree of esterification (DE), using the peak areas corresponding to COO⁻ asymmetric stretching and at C=O using the Equation 6 (Sato et al., 2011):

$$DE (\%) = \frac{Abs (C = O_{esterified})}{(Abs (C = O_{esterified}) + Abs (COO^{-} \text{ asymmetric stretching}))} \times 100 \quad (6)$$

The determination of galacturonic acid content followed the methodology of Van Hung et al. (2021) and expressed as (%) (Van Hung et al., 2021).

A commercial pectin from citrus was used to compare with the experimental results.

4.2.3.4 Statistical analysis

A statistical analysis of data was performed through a one-way analysis of variance using Software Minitab, version 19.2, and differences among mean values were processed by the Tukey test. Significance was defined at $p < 0.05$.

4.3 Results and Discussion

4.3.1 Cladode characterization

The composition of cladodes varies depending on several factors such as species, cultivar, maturity stage, climatic, soil, water conditions, geographical location and, harvest/post-harvest conditions. These variables contribute to the differences observed in the literature regarding cladodes composition (Astello-García et al., 2015; Dubeux et al., 2021). The chemical composition of peel and pulp from *O. ficus-indica* cladodes is shown in Table 4.3.

Peel and pulp from OFI cladodes showed no differences in terms of P content, Total reducing sugars, and total fiber content. On the other hand, other parameters demonstrated statistically significant differences ($p < 0.05$). This was specially more evident in terms of bioactive compounds, showing higher values in the external part, specifically the total phenolic content, antioxidant activity and total flavonoids. A higher amount of bioactive compounds is generally observed in outer layer of fruits and vegetables when compared to other parts of the plants such as pulp and seeds (Rifna et al., 2023).

Table 4.3 - Chemical composition of peel and pulp of OFI cladodes.

	Peel	Pulp	Literature
	Average $\pm$ SD	Average $\pm$ SD	
Moisture % (wb)	89.65 $\pm$ 0.37 ^b	94.73 $\pm$ 0.22 ^a	OFI Cladodes powder – 94.12% db (Cheikh Rouhou et al., 2018) OFI 89.90 % (Razzak et al., 2024)
Ash %(db)	19.75 $\pm$ 0.42 ^a	16.47 $\pm$ 0.23 ^b	OFI Cladodes powder – 23.31% db (Cheikh Rouhou et al., 2018) OFI 24.89 % (Razzak et al., 2024) OFI f. amyloceae – 25.65% db (Ayadi et al., 2009)
Crude Protein (% db)	4.03 $\pm$ 0.16 ^a	3.40 $\pm$ 0.25 ^b	OFI 0.30 % FW(L. P. Méndez et al., 2015) OFI 8.44% (Razzak et al., 2024) OFI 7.07 % db (Hernández-Urbiola et al., 2011)
P content (mg P/100g)	27.55 $\pm$ 2.15 ^a	20.43 $\pm$ 4.22 ^a	OFI – 16.38 mg/100g FW (L. P. Méndez et al., 2015)
pH	4.96 $\pm$ 0.01 ^a	4.88 $\pm$ 0.03 ^b	4.4 (Astello-García et al., 2015) 4.64 (L. P. Méndez et al., 2015)
Total titratable acidity (% w/w of citric acid)	0.44 $\pm$ 0.02 ^a	0.17 $\pm$ 0.01 ^b	286.0 mg/100 g FW (L. P. Méndez et al., 2015)
Total sugars (%w/w) db	14.54 $\pm$ 2.50 ^b	30.34 $\pm$ 0.76 ^a	41.20 % (Mounir et al., 2020)
Total reducing sugars (%w/w db)	7.85 $\pm$ 2.56 ^a	15.26 $\pm$ 10.55 ^a	2.87 % (Mounir et al., 2020)

	Peel	Pulp	Literature
	Average $\pm$ SD	Average $\pm$ SD	
Total fiber content (g/100g db)	26.43 $\pm$ 2.79 ^a	29.53 $\pm$ 1.96 ^a	56.68% (Cheikh Rouhou et al., 2018)
Cellulose (g/100g db)	10.86 $\pm$ 0.36 ^a	11.07 $\pm$ 1.58 ^a	21.6 % dry weight (Malainine et al., 2003) 26.7% db (Cheikh Rouhou et al., 2018)
Hemicellulose (g/100g db)	8.79 $\pm$ 1.68 ^b	13.11 $\pm$ 0.82 ^a	15.3% db (Cheikh Rouhou et al., 2018)
Lignin (g/100g db)	6.78 $\pm$ 0.84 ^a	5.35 $\pm$ 0.93 ^a	3.6 % dry weight (Malainine et al., 2003) 2.51 % (Cheikh Rouhou et al., 2018)
Ascorbic acid content (mg/100g)	5.67 $\pm$ 1.03 ^a	4.78 $\pm$ 1.03 ^a	26.78 – 77.40 mg/100g (Du Toit et al., 2018) 10-15 mg/100g (Martins, Ribeiro, & Almeida, 2023)
Total Phenolic content (mg GAE/100g)	5.30 $\pm$ 0.11 ^a	1.62 $\pm$ 0.13 ^b	2600 mg GAE/kg fresh weight (Rocchetti et al., 2018) 16.11 mg/100g (De Santiago et al., 2018)
Antioxidant activity (mg Eq Trolox/kg)	7.7 $\pm$ 2.97 ^a	2.3 $\pm$ 0.37 ^b	45.05 mg Eq Trolox/kg (De Santiago et al., 2018)
Total Flavonoids (mg Eq catechin/100g)	4.72 $\pm$ 0.44 ^a	1.23 $\pm$ 0.66 ^b	12.55 mg/100 g (De Santiago et al., 2018)

Superscript letters (a, b): different superscripts within the same row indicate significant differences among samples ($p < 0.05$). wb – wet basis; db – dry basis; SD – Standard Deviation.

Results showed a high moisture content in both peel (89.65%) and pulp (94.73%) and similar to those reported by other authors in OFI of 89.90% in cladodes (Razzak et al., 2024) and 94.12% for cladodes powder (Cheikh Rouhou et al., 2018). The ash content is slightly lower than the ones presented in literature for OFI of 23.31% (Cheikh Rouhou et al., 2018), 24.89% (Razzak et al., 2024) and 25.65% (Ayadi et al., 2009). The protein content obtained (4.03% for peel and 3.40% for pulp) is slightly lower than values reported in the literature such as 8.44% (Razzak et al., 2024) and 7.07% (Hernández-Urbiola et al., 2011). The phosphorus content results were slightly higher than the 16.38 mg/100g reported by Pérez Méndez et al. (2015) in *O. ficus indica* cladodes (L. P. Méndez et al., 2015). The pH values and titratable acidity obtained are considered as low (acidic), most likely because of the presence of organic acid such as malic, oxalic and citric acids due to the Crassulacean Acid Metabolism (CAM) metabolism performed by cactus and these values align with previous studies (L. P. Méndez et al., 2015; Stintzing & Carle, 2005). Sugars are one of the main components of cladodes, as demonstrated by the total sugar values obtained of 14.54 % w/w in the peel and 30.34 % w/w, in the pulp. The reducing sugar content was 7.85% w/w in the peel and 15.26% in the pulp. These values are consistent with those reported Mounir et al. (2020), who found total sugar and reducing sugar values of 41.20% and 2.97%, respectively (Mounir et al., 2020). Fibers represent a major fraction in both the peel (26.43 g/100g) and the pulp (29.53 g/100g), with no significant differences observed ($p \geq 0.05$). The lignocellulosic composition of cladodes revealed considerable amounts of lignin and hemicellulose, along with cellulose, further highlighting their potential for lignocellulosic compound extraction (Cheikh Rouhou et al., 2018). The ascorbic acid content in the peel and pulp was lower than the values reported for fresh OFI cladodes from four different cultivars (26.78-77.40 mg/100g) (Du Toit et al., 2018) but similar to the 10-15 mg/100g reported for young OFI cladodes (Martins, Ribeiro, & Almeida, 2023). OFI cladodes are also a source of phenolic compounds and flavonoids (Barba et al., 2020; Msaddak et al., 2015). However, the total phenolic content obtained in this study was lower than the values reported for OFI cladodes in Spain (De Santiago et al., 2018) but similar to the 2.6 mg GAE/ 100g reported by other authors (Rocchetti et al., 2018). The antioxidant activity of the peel (7.7 mg Eq Trolox/kg) and pulp (2.3 mg Eq Trolox/kg) was notably lower than values found in the literature (De Santiago et al., 2018). Similarly, total flavonoid content was lower than the results obtained in a study with OFI cladodes (12.55 mg/100g) (De Santiago et al., 2018). Thus, the chemical composition confirms that cladodes are a rich source of dietary fibre, sugars, and lignocellulosic compounds, supporting their potential use in various applications. In addition,

slight compositional differences were observed between peel and pulp of the cladodes, with the pulp showing lower ash and protein but higher sugar contents and peel showing higher bioactive activity. These variations likely reflect their distinct physiological functions, with the peel accumulating more structural components and minerals, while the pulp mainly stores water and soluble sugars (Astello-García et al., 2015; Barba et al., 2020; Hernández-Urbiola et al., 2011). Furthermore, *Opuntia ficus-indica* cladodes are recognized as a valuable source of polysaccharides, particularly mucilage rich in pectic substances. Reported yields of pectin range from about 5 to 12% of the dry cladode weight, with galacturonic acid contents between 30 and 80%, while mucilage represents approximately 6 to 15% of the dry mass (Aragona et al., 2018; del Socorro Santos-Díaz et al., 2017; Lefsih et al., 2016, 2017). These components, rich in uronic acids and neutral sugars, impart the characteristic hydration and gelling behavior of cladodes, reinforcing their potential as natural hydrocolloids. Although this work focused on the extraction of pectin, the close association and overlapping composition of pectin and mucilage suggest that both contribute to the functional properties that are going to be investigated in the following section.

4.3.2 Pectin extraction

4.3.2.1 Experimental set 1 – Yield of extraction with different solvents

Opuntia ficus indica cladodes have an appreciable content in macromolecules such as pectin and mucilage, which exhibit high water retention capacity (De Santiago et al., 2021). The results obtained for the extraction with different solvents are summarized in Figure 4.1. According to these results, the yield of extraction obtained ranged from 3.69 to 14.76 % all performed under the same conditions (time of extraction- 50 min, liquid/solid ratio - 10, pH - 2 and temperature - 80°C). Overall, the extraction yield from the pulp was consistently higher than from the peel, regardless of the solvent used. In the peel, statistically significant differences ($p < 0.05$) were observed between citric acid extractions and those performed with the other solvents. The highest extraction yield obtained in pulp (14.76% $\pm$ 1.02) was obtained using deionized water acidified to a pH of 2 when compared to citric acid 1% (10.39% $\pm$ 1.23) and acetic acid 10% (9.54%). In the pulp, significant differences were found using deionized water acidified to a pH of 2 and the other solvents. The highest extraction yield (7.57% $\pm$ 0.77) was achieved by using 10% (v/v) acetic acid as the solvent, whilst only 6.02% $\pm$ 0.72 was obtained for deionized water acidified to a pH of 2, and 3.69% $\pm$ 0.47 was obtained with 1% citric acid.

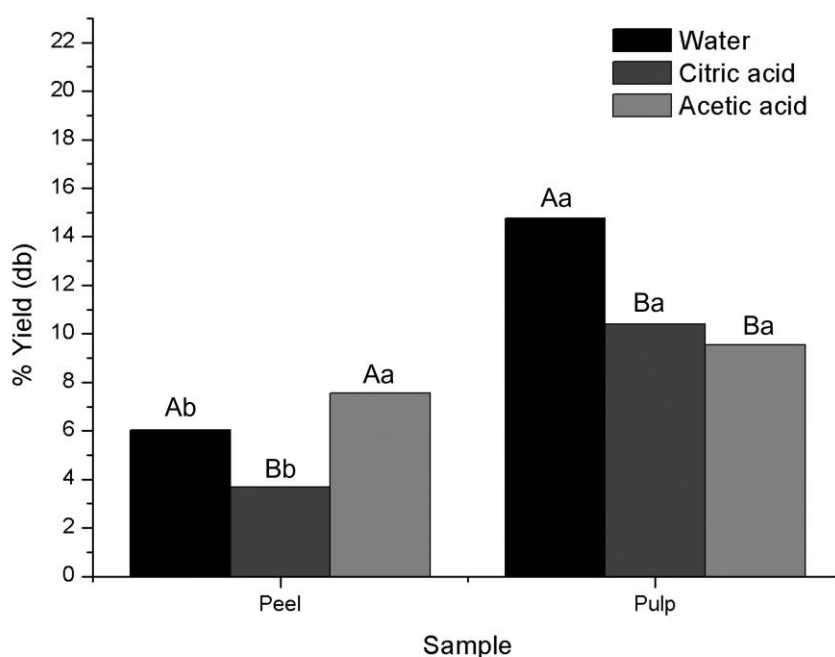


Figure 4.1 - Yield of extraction (% db) obtained with different solvents.

Superscript letters (a, b): different superscripts indicate significant differences between peel and pulp ($p < 0.05$). Superscript letters (A, B): different superscripts indicate significant differences between solvents ($p < 0.05$).

These results are aligned with those reported by Quintero-Garcia et al. (2021) for fresh cladodes (Quintero-García et al., 2021) and are slightly lower than the yields reported by Contreras-Padilla et al. (2016) (Contreras-Padilla et al., 2016). Specifically, the extraction yield using deionized water acidified to a pH of 2 (14.76% DW in the pulp) is comparable to the 12.01% DW reported by Dick et al. (2019) for *O. monacantha* (Dick et al., 2019) and higher than the 6.2% DW obtained by Kalegowda et al. (2017) in *O. dilenii* cladodes (Kalegowda et al., 2017). Similarly, other authors reported a value of 6.13% DW for the pectic fraction from cladodes that were extracted with water under a pH of 2.8 (Bayar et al., 2016).

The variation in extraction yield reported in the literature may be attributed to several factors, including differences in extraction conditions, maturity stage of the cladodes, species, and experimental methods (Quintero-García et al., 2021). Moreover, the choice of solvent plays a crucial role in optimizing extraction yield. The use of acids plays crucial role in the extraction since by facilitating cell wall degradation, thereby increasing access to cellular contents and

enhancing the efficiency of the extraction process (Van Hung et al., 2021). Organic acids, such as acetic and citric acids were explored in this study due to their cost-effectiveness and environmental friendliness compared to inorganic acids (e.g. hydrochloric acid and sulphuric acid) (Freitas et al., 2020; Marić et al., 2018). Focusing exclusively on the performance of the two organic acids tested, citric acid achieved a higher yield on the pulp. Due to the fact that the difference between the two solvents in pulp yield was not statistically significant ($p \geq 0.05$), citric acid was ultimately chosen as the solvent to proceed with the pectin extraction from OFI. This decision highlights the importance of optimizing solvent selection to maximize yield while maintaining the process sustainability.

4.3.2.2 Experimental set 2 - Yield of extraction under different pH values

pH is one of the most important features of the extraction process and is stated that acid conditions favor pectin extraction (Freitas et al., 2020). The results of extraction with different pH values are presented in Figure 4.2.

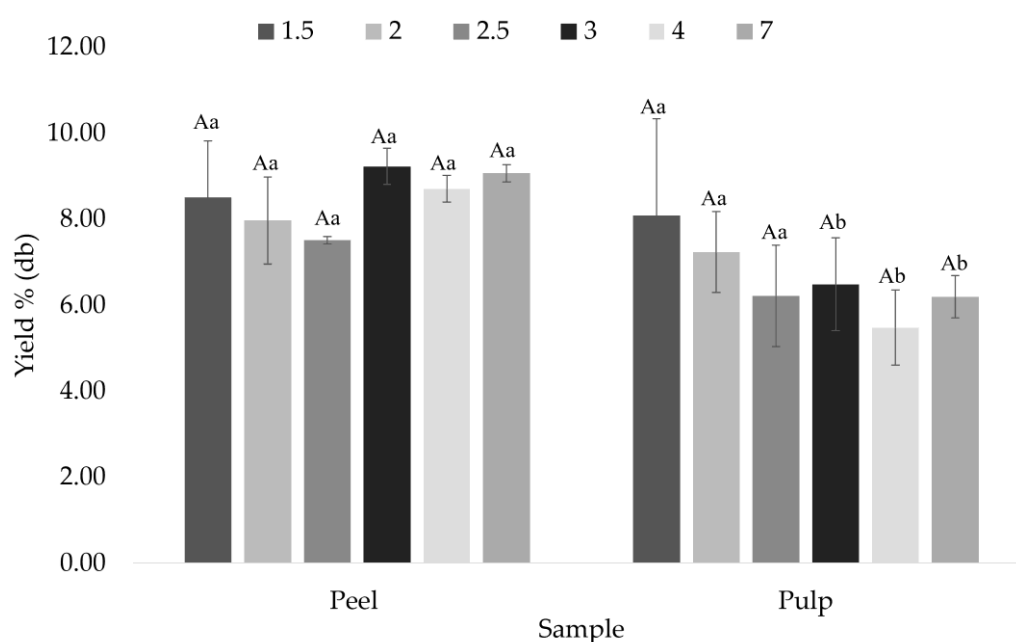


Figure 4.2 - Yield of extraction (% db) obtained at different pH (1.5,2,2.5,3,4 and 7) using water as solvent.

Lowercase letters (a,b): different lowercase letters indicate significant differences between peel and pulp ($p < 0.05$). Uppercase letter (A): different uppercase indicates significant differences between pH values ($p < 0.05$).

Pectin yield in peel varied between 7.51% (pH 2.5) and 9.23% (pH 3) and in pulp the variations occurred between 5.48% (pH 4) and 8.09% (pH 1.5). No statistical differences were observed among different pH values in the pectin extracted from OFI cladode peel ($p > 0.05$) showing that pH did not have a significant effect on the pectin extraction from peel under the tested conditions. Similarly, the same behavior was observed in the pectin extracted from OFI cladodes pulp, within the test pH range. Comparing the yield of extraction between peel and pulp at each pH level, at pH 1.5, 2, and 2.5, no significant differences ($p > 0.05$) were found between peel and pulp. On the other hand, at pH 3, 4, and 7, significant differences ($p < 0.05$) were observed. The values of the extraction at a pH of 2 ($7.97\% \pm 1.01$ w/w db - peel; $7.24\% \pm 0.94$ w/w db - pulp) are similar to the ones reported by Chaouch *et al.*, 2016 ($8.8\% \pm 0.7$ w/w db) at the same pH. At a pH of 6, the same authors reported a yield of extraction of $24.5\% \pm 0.5$ w/w db and, at a pH of 10 a value of $4.6\% \pm 0.4$ w/w db which reflects the fact that under basic conditions the yield of extraction starts to decrease (Chaouch *et al.*, 2016). This behavior is in agreement with the behavior observed in the extractions from pulp, where the results suggested that lower acid conditions (pH of 1.5, 2, 2.5,3) allowed the hydrolysis of the insoluble pectin into soluble pectin, increasing the yield of extraction which is in agreement with (Lefsih *et al.*, 2017). Based on these previous studies, the solvent and pH were fixed for subsequent experiments. Citric acid 1% (w/v) was selected as the extraction solvent without pH adjustment, as its natural pH was approximately 2.2–2.3. This pH value aligns with the findings presented in Figure 4.2, indicating that, within the tested range, pH had no significant impact on the extraction process.

4.3.2.3 Experimental design

A total of 20 experiments, following the methodology described in 4.2.3.2 were conducted in a randomized form, including six replicates at the central point to validate the model, in order to optimize the extraction conditions of pectin.

The experimental conditions tested and the results of yield of extractions for each experiment are described in Table 4.4, expressed as percentage on a dry basis.

Table 4.4 - Yield of pectin extraction (% on a dry basis) from OFI peel and pulp cladodes at pH around 2, using 1% citric acid solution, as affected by L/S ratio, time and temperature.

L/S	Time (min)	Temperature (°C)	Yield (%)	Yield (%)
			Peel	Pulp
10	50	80	13.74	23.38
15	40	90	16.65	24.08
15	50	80	10.78	18.93
15	40	70	8.52	19.34
10	40	80	17.88	24.56
10	50	80	12.75	22.94
5	50	80	16.27	17.26
10	50	90	15.80	26.32
10	50	80	14.62	23.73
10	60	80	13.79	18.86
10	50	70	13.84	19.70
5	40	90	19.28	21.79
5	60	90	7.70	13.98
15	60	90	11.27	17.29
10	50	80	14.45	21.21
5	40	70	16.75	14.00
5	60	70	13.81	14.16
10	50	80	14.00	23.34
10	50	80	12.76	21.11
15	60	70	6.18	12.09

The statistically non-significant terms were removed from the initial model, and the experimental data were re-fitted using only the significant parameters ($p < 0.05$). The model adjustments representing the variations in yield of pectin extraction from OFI peel and pulp cladodes in response to the different extraction conditions are presented in Table 4.5.

Table 4.5 - Model adjustment with the respective determination coefficients for yield of pectin extraction from OFI peel and pulp cladodes at pH ~2, using 1% citric acid solution, as affected by L/S ratio, time and temperature.

Sample	Adjusted model	R ²
	-9.0 - 2.455 L/S (% w/v) + 0.905 Time (min) + 0.426 Temperature (°C)	
Yield (%)	- 0.0657 L/S (% w/v)*L/S (% w/v)	88.79%
Peel	+ 0.04200 L/S (% w/v)*Temperature (°C)	
	- 0.01460 Time (min)*Temperature (°C)	
Yield (%)	-6.13 + 4.389 L/S (% w/v) - 0.2739 Time (min)	89.20%
Pulp	+ 0.2417 Temperature (°C)	
	- 0.2089 L/S (% w/v)*L/S (% w/v)	

Pectin yields ranged from 6.18 to 19.28 % in peel and 12.09 to 26.32 %, on a dry basis in pulp (Table 4.4), suggesting that pulp contains more extractable pectin than the peel. The fitted models were able to explain 88.79% in the extraction from peel and 89.20% in the extraction from pulp. Figure 4.3 shows the 2D representation of RSM of yield variation as a function of the experimental conditions of pectin extraction from OFI cladodes peel. The representation of the effects on yield of extraction of pectin from OFI cladodes pulp is represented in Figure 4.4.

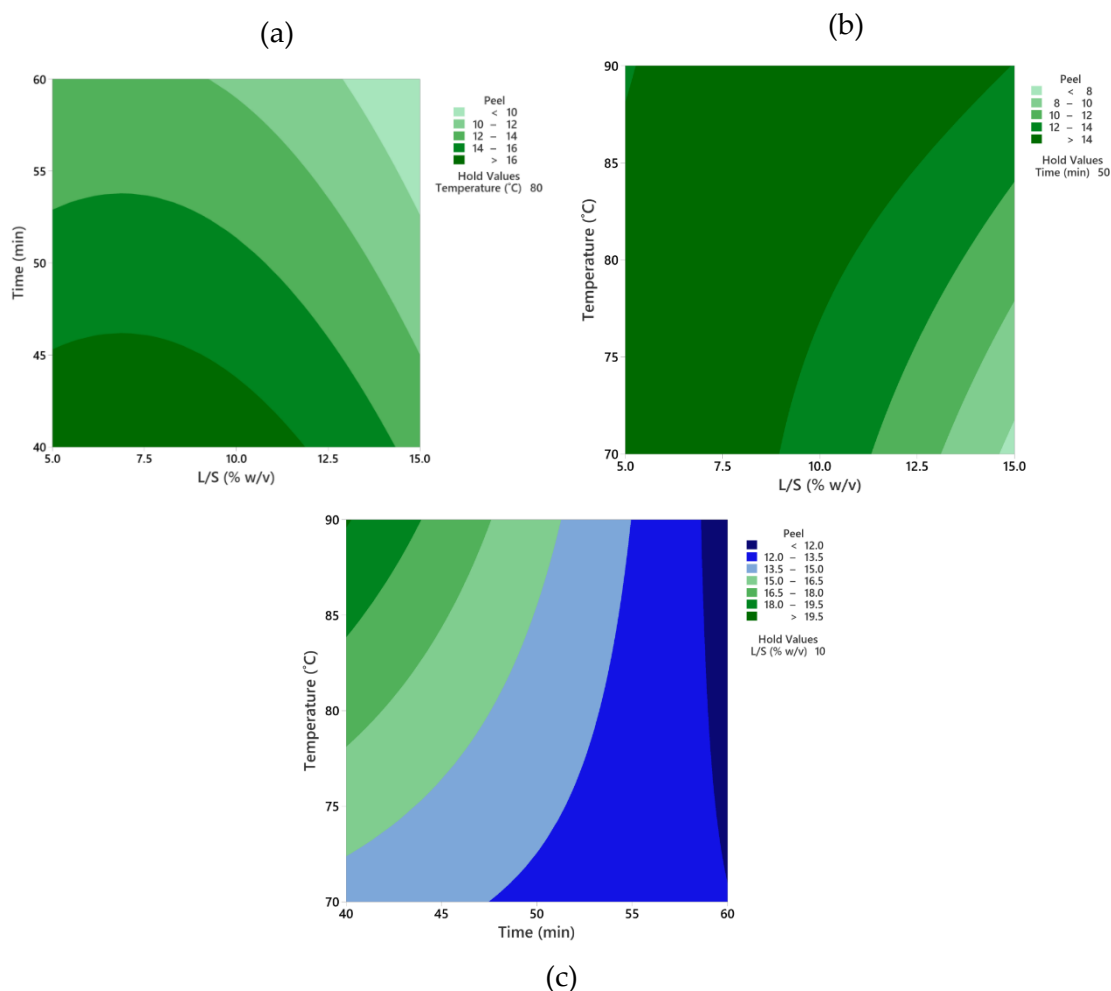


Figure 4.3 - Response Surfaces (a) shows the correlation between time and L/S, fixing the Temperature at their middle value, (b) shows the correlation between Temperature and L/S, fixing the time at their middle value and (c) shows the correlation between Temperature and time, fixing time at their middle value in 2D, on yield of extraction of pectin extracted from OFI cladodes peel.

Yield of pectin extraction from peel was influenced by the linear effect of L/S, time, and Temperature as well as the interaction of L/S and temperature and Time and Temperature and the quadratic effect of L/S ($p < 0.05$) according to the analysis of regression coefficients of the response function. The linear regression coefficient of L/S was negative, indicating that a lower L/S led to a higher yield of pectin extraction in peel, while the positive coefficient of time and temperature indicated that extraction yield could increase with longer extraction time and high temperatures. The positive coefficient value of the interaction between L/S and temperature, indicated that increasing both variables simultaneously enhanced the extraction efficiency. On the other side, the negative coefficient of the interaction between Time and Temperature suggested that longer extraction times combined with high temperatures reduced the pectin extraction yield. For pectin extraction from OFI cladodes peel, the best extraction

conditions should involve a moderate L/S ratio and extraction time, combined with a sufficiently high temperature.

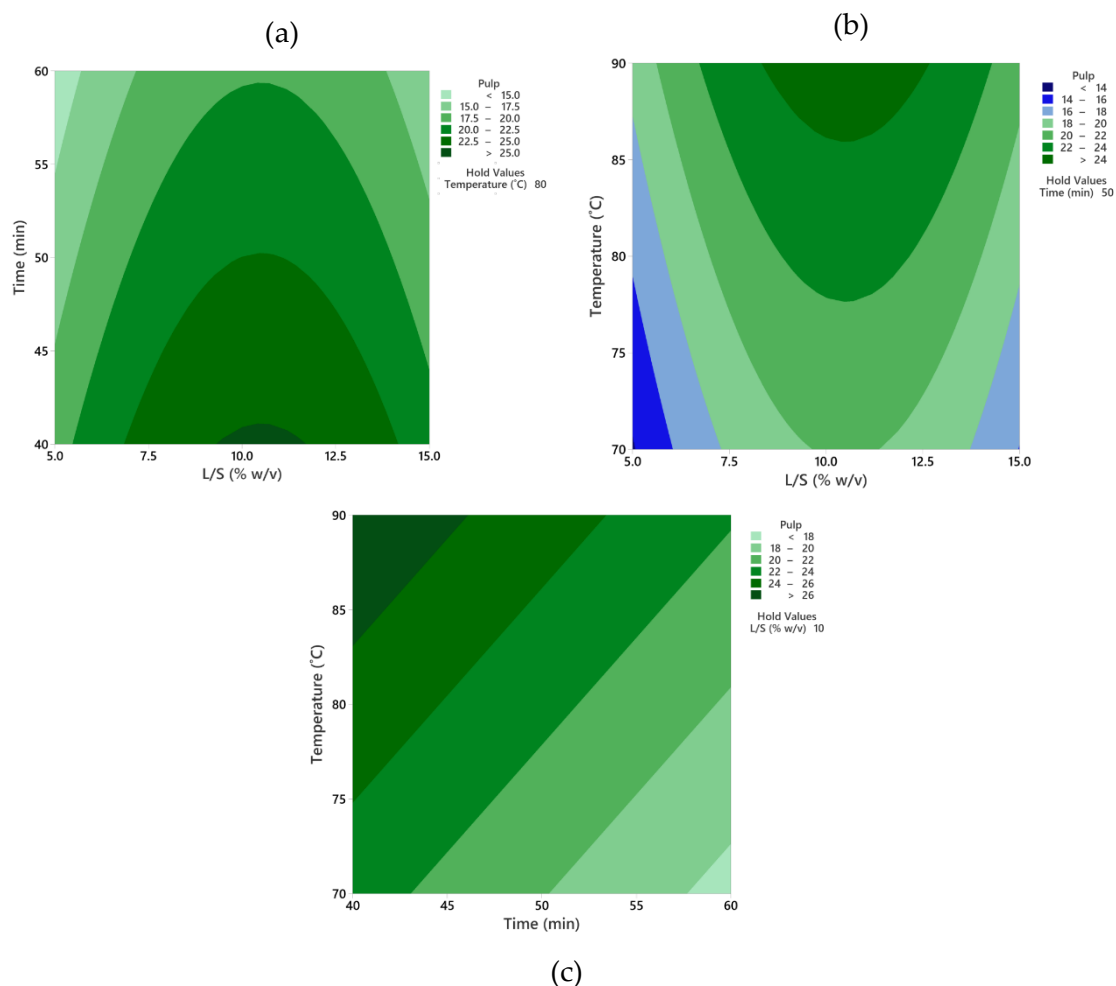


Figure 4.4 - Response Surfaces (a) shows the correlation between time and L/S, fixing the Temperature at their middle value, (b) shows the correlation between Temperature and L/S, fixing the time at their middle value and (c) shows the correlation between Temperature and time, fixing time at their middle value in 2D, on yield of extraction of pectin extracted from OFI cladodes pulp.

In pectin extracted from pulp, the yield of extraction was only affected by the linear effects of time, temperature and L/S, as well as by quadratic effect of L/S ($p < 0.05$). The negative coefficient of time, indicated that increasing the extraction time, leads to a slight decrease in pectin yield, while the positive coefficients of L/S and temperature showed that higher values of these parameters favor the extraction, up to an optimum point, and beyond that the yield decreases due to the negative quadratic effect of L/S. This suggests that prolonged extraction time may cause thermal degradation, reducing the pectin extraction or changing the

pectin characteristics (Chandel et al., 2022). Even though both models showed good fit, they indicated that longer extraction times led to a decrease in yield of extraction.

Based on both models, the optimized extraction conditions should balance the most significant parameters affecting yield in both pulp and peel from cladodes OFI. Thus, the L/S ratio should be kept moderate (10 w/v), since very low ratios may limit solubilization while high ratios reduce yield, particularly in peel and due to the quadratic effect observed in both models. The extraction time should be short to moderate (40-45 min), as prolonged extraction periods negatively affect yield, especially in pulp. Concerning to temperature, it should be moderate to high (75-85°C), as it enhances pectin extraction when combined with a relatively low L/S ratio. Therefore, in this work and based on the resulting models, the selected optimized pectin extraction conditions were L/S of 10 w/v, time of 40 min and Temperature of 80°C using citric acid 1% w/v at a pH around 2.

Bayar et al. (2016), reported a value of 6.13% of dry weight for pectin extraction from OFI cladodes, using HCl as solvent, and a pH of 2.8 using a L/S of 15, 90 °C for 2h. Also, Sevgi et al. (2022), conducted acidified hot water extraction using HCl under conditions of L/S 15, pH 2.8, temperature of 90°C, and extraction time of 4 hours, achieving an extraction yield of 0.18–0.06% fw (Sevgi et al., 2022). The results of the present study are relatively higher than those reported by these authors. Despite the similarity in extraction conditions and the use of a different solvent, no optimization of the extraction parameters was performed. In the extraction of pectin from banana peels using a 1:20 w/v solid-to-liquid ratio with citric acid, the optimal conditions were found to be 87°C, 160 minutes, and pH 2, resulting in a pectin yield of 14.23 g/100 g showing similarities with the optimal conditions obtained in the present study (T. Í. S. Oliveira et al., 2016). In this case, only the time of extraction was not in accordance with the results obtained. A prolonged time of extraction can result in a degradation of methyl ester groups into carboxyl acid, leading to pectin with a lower degree of esterification (Chandel et al., 2022). Zamboi et al. (2024) used ultrasound-assisted extraction to extract pectin from *Opuntia* cladodes obtaining optimal conditions of L/S 10 w/v, pH 2.5, time of 20 min and temperature of 65°C with the model validated with a 11.32% extraction yield (Zamboi et al., 2024). Even though the use of ultrasound was not contemplated in this study and for that it is more difficult to compare the results, the optimal conditions for L/S ratio identified closely aligns with the results obtained in the present work.

4.3.3 Characterization

The pectin extracted under the optimized conditions was subsequently characterized based on its functional groups, degree of esterification and galacturonic acid content.

4.3.3.1 FTIR

To obtain a more comprehensive understanding of the extracted material from OFI cladode peel and pulp, FTIR spectra were acquired and compared with a commercial pectin from citrus peel (CP). The peaks and the respective assignments are presented in Figure 4.5 and Table 4.6.

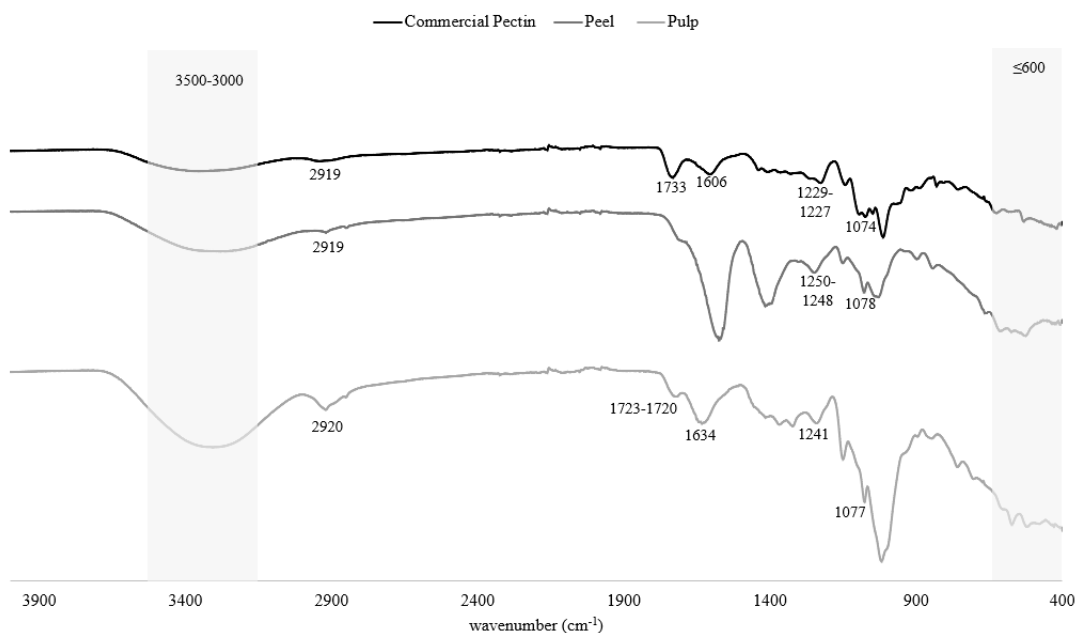


Figure 4.5 - FTIR assignments wavenumber (cm⁻¹) of CP and pectin extracted from peel and pulp of OFI.

Table 4.6 - FTIR assignments wavenumber (cm^{-1}) of commercial pectin and pectin extracted from peel and pulp of OFI.

Wavenumber (cm^{-1})			Assignments
Peel	Pulp	CP	
3232-3353	3250-3349	3073-3613	O-H (Zamboi et al., 2024)
2919	2920	2971	C-H (Gopi et al., 2015)
-	1720-1723	1733	C=O (vibration of the esterified carboxyl group) (Lefsih et al., 2016; D. A. Méndez et al., 2021)
-	1634	1606	COO ⁻ (asymmetric stretching)(Lefsih et al., 2016; D. A. Méndez et al., 2021)
1250-1248	1241	1229-1227	O-acetyl ester (Nejatzadeh-Barandozi & Enferadi, 2012; Zamboi et al., 2024)
1200-800	1200-800	1200-800	Carbohydrates fingerprint (Bayar et al., 2016)
1078	1077	1074	Glycosidic linkage (typical from backbone vibrations of pectin) (Bayar et al., 2016)
≤ 600	≤ 600	≤ 600	Vibration due to monosaccharides and oligosaccharides molecules (Sevgi et al., 2022)

In both figures a band between 3500-3000 cm^{-1} corresponding to the stretching of hydroxyl groups (-OH) is observed (Zamboi et al., 2024). Bands in the region of 3000-2840 cm^{-1} are related with C-H (methyl) stretching bands (Zamboi et al., 2024) and are observed in both figures. In peel, a peak at 2919 cm^{-1} revealed the symmetric stretching vibration of the C-H group of the methyl ester of galacturonic acid (Bayar et al., 2016). In pulp, a peak at 2920 cm^{-1} , showed the same behavior. A peak at 1733 cm^{-1} (Commercial Pectin) and at 1720-1723 cm^{-1} (Pulp) correspond to vibration of the esterified carboxyl group (C=O) (Lefsih et al., 2016; D. A. Méndez et al., 2021). Bands around 1600-1650 cm^{-1} and 1400-1450 cm^{-1} corresponds to free carboxylic groups and are characteristic of pectin, that belongs to the class of carboxypolysaccharides (Lefsih et al., 2016). A band around 1600 cm^{-1} is related to the stretching vibration of the free carboxylic groups, present in commercial pectin and Pulp (Bayar et al., 2016). In the region of 1400 – 1450 cm^{-1} , which also corresponds to free carboxylic groups, a band is observed for peel but not for commercial pectin. The same behaviour is not observed for samples from pulp, which revealed a band around 1370 cm^{-1} which may correspond to the same functional groups.

Bands between 1200-800 cm^{-1} correspond to carbohydrates fingerprint region (Bayar et al., 2016). A peak around 1200 cm^{-1} is detectable in commercial pectin, peel and pulp, and it is assigned to O-acetyl-ester (Zamboi et al., 2024). Around 1150 cm^{-1} peaks are linked to C-O-H bonds of primary alcoholic, showed in Peel and Pulp. All samples showed a band around 1070 cm^{-1} , which corresponds to glycosidic linkage (C-O and C-C stretching bond) that is typical from backbone vibrations of pectin (Lefsih et al., 2016). Possibly linked with this connection, another band around 1010-1040 cm^{-1} was detected. More specifically, C-O-H bonds of primary D-arabinose were visible at 1013 cm^{-1} (commercial pectin) and 1019 cm^{-1} in Pulp (Sevgi et al., 2022). A peak at 1030 in Peel is assigned as glucan units (Zamboi et al., 2024). Peaks and bands around 850-900 cm^{-1} represent the α -glycosidic linkage (Bayar et al., 2016) and are detected in commercial pectin (831 cm^{-1}), and in Peel (843; 896-898 cm^{-1}). In pulp sample there were no observations around those wavenumbers, but peaks are detected around 760 cm^{-1} . Bands and peaks below 600 cm^{-1} are related with the vibration of monosaccharides and oligosaccharides molecules that are present in all samples (Sevgi et al., 2022).

4.3.3.2 Degree of esterification and galacturonic acid content

The values obtained for the degree of esterification and galacturonic acid content of Commercial Pectin and pectin from OFI peel and pulp are presented in Table 4.7.

The degree of esterification of pectin extracted from OFI cladodes pulp was 35.6% and 52.5% for commercial pectin (Table 4.7). It was not possible to calculate the degree of esterification in OFI cladodes peel due to the absence of peaks in the region of carboxyl groups (Table 4.6 and Figure 4.5). Thus, the pectin from OFI pulp can be classified into Low methylated-pectin whereas the commercial pectin can be categorized as High methoxyl-pectin (Bayar et al., 2016; Lefsih et al., 2016; Zamboi et al., 2024). Those values are comparable with others reported, that presented a value of 38.5% for OFI cladodes pectin (Sevgi et al., 2022). Through the analysis of degree of esterification and considering that the pectin obtained in this study had a Low degree of esterification, it is also possible to compare with a commercial citrus pectin with low methoxyl content with a value of 45.20% (Sevgi et al., 2022). The values obtained in the present study are in line with other research on pectin extracted from OFI cladodes that refer that pectin from OFI is mostly LM (Bayar et al., 2017; Yilmaz, 2020; Zamboi et al., 2024).

Table 4.7 - Degree of esterification and Galacturonic acid content of CP and pectin from OFI peel and pulp.

Sample	DE (%)	Galacturonic acid content (%)
CP	52.5	88.7
Peel	n.d.	41.4
Pulp	35.6	76.9

CP – Commercial pectin; DE – degree of esterification; n.d – not determined.

Galacturonic acid is the major component present in pectin and the presence of high percentages of galacturonic acid suggests the presence of pectin polymers (Sevgi et al., 2022). The results obtained revealed a considerable amount of galacturonic acid content with values 76.9% from pectin extracted from OFI pulp. The CP galacturonic acid content was 88.7%, which is consistent with products specification sheet, that indicates a content of over 74%. The results align with findings from other studies: (Bayar et al., 2017) reported 68.87% using ultrasonic extraction; (Sevgi et al., 2022) reported 56.16% using acidified hot water extraction; (Yilmaz, 2020) reported 68.8% using ultrasonic extraction and 60.66% using microwave extraction; (Zamboi et al., 2024) found 25.55% of galacturonic acid content by using ultrasound assisted extraction.

4.3.4 Film preparation

To explore the potential of the extracted *Opuntia ficus-indica* pectin as a film-forming material for food packaging applications, a filmogenic solution with 2% *Opuntia* pectin from cladodes pulp and 50% glycerol (in relation to *Opuntia* pectin dry matter) was prepared (Espino-Díaz et al., 2010). Although a continuous film was obtained, it presented a fragile structure, limiting its applicability as it is demonstrated in Figure 4.6.

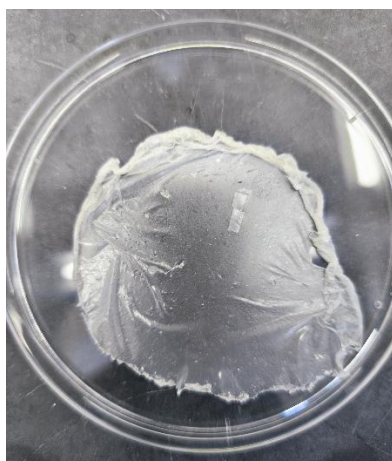


Figure 4.6 - Film prepared with 2% *Opuntia* pectin from cladodes pulp and 50% glycerol (*Opuntia* pectin dry matter).

These preliminary results indicate that while film formation is feasible, further optimization is required, such as vary the percentage of OFI pectin/mucilage, blend it with commercial pectin or other biopolymers, study the effect of cross-linking (time and concentration), aiming the improvement of its flexibility and mechanical performance. This study is presented in the chapter 5.

4.4 Conclusions

This study aimed to optimize pectin extraction from *Opuntia ficus-indica* cladodes, evaluating the effects of different solvents, pH, L/S ratio, time and temperature. Citric acid (1% w/v) was selected as the extraction solvent due to its effectiveness in maximizing yield without requiring pH adjustment (≈ 2.2 – 2.3). Moreover, it is a solvent with several advantages, namely cost-effectiveness as well as a more sustainable alternative to the conventional solvents usually used in this process. Optimization through Response Surface Methodology (RSM) of the remaining parameters (L/S ratio, time and temperature) was performed separately for peel and pulp. The regression models for both peel and pulp demonstrated good fit, with coefficients of determination of 88.79% and 89.20%, respectively, indicating that the selected variables were adequate to explain the extraction behavior. In the peel model, yield was mainly affected by the linear effects of L/S, time, and temperature, together with their interactions and the quadratic effect of L/S, while in the pulp model, yield was influenced by the linear effects of L/S, time, and temperature, as well as the quadratic term of L/S. Despite slight differences in parameter significance, both models revealed similar optimal regions. The optimized pectin extraction conditions for both pulp and peel from cladodes OFI are an L/S ratio of 10 w/v,

extraction time of 40 minutes, and a temperature of 80°C, using 1% w/v citric acid at a pH of around 2. The FTIR characterization confirmed the structural integrity of the extracted pectin, with galacturonic acid content aligning with values reported in the literature.

The degree of esterification (DE) and galacturonic acid content were also determined, showing values consistent with literature reports and further validating the quality of the extracted pectin. The DE obtained for pectin from pulp can be categorized as Low methoxyl pectin, which is also reported in the literature as a characteristic of pectin extracted from OFI cladodes. The findings of this study demonstrate the potential of *Opuntia ficus-indica* cladodes as an alternative source of pectin. To further enhance extraction efficiency, future research could explore advanced techniques such as ultrasound and pulsed electric fields, which may be valuable to improve yield of extraction. The potential use of pectin from OFI in food packaging applications should be strongly addressed, by studying the development of films and edible coatings.

DEVELOPMENT AND CHARACTERIZATION OF *OPUNTIA*-BASED FILMS FOR INTELLIGENT FOOD PACKAGING

This chapter presents the development and characterization of *Opuntia ficus-indica*-based films designed for intelligent food packaging. Based on the pectin extracted in the previous chapter, composite films were produced by blending OFI and commercial pectin with montmorillonite (MMT) and betalain-rich extracts from prickly pear peels as well as reticulation effect with CaCl_2 . The effects of these components on the structural, mechanical, barrier, and optical properties of the films were investigated. In addition, their performance as colorimetric indicators was tested in a food matrix, demonstrating their ability to detect spoilage.

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5.1 Introduction

Food waste is considered a global issue as one-third of all the food produced globally does not reach the consumers table, this represents an estimate monetary loss around US\$1 trillion/year (FAO, 2014; UNEP, 2024). According to FAO 2023 (FAO et al., 2023), 29.6 per cent of global population was moderately or severely food insecure, meaning that 783 million people were in hunger condition. Thus, reducing food waste can increase food availability for those populations affected by hunger. In this context, the development of sustainable and functional food packaging (active and intelligent packaging) using bio-based biodegradable

polymeric materials capable of extending shelf life while providing real-time quality monitoring can be highlighted as a strategy to fight such problems (Bhargava et al., 2020; Müller & Schmid, 2019; C. Rodrigues et al., 2021a).

It is known that petroleum-based polymeric materials, traditional plastics, have interesting and desired features, such as high durability and barrier properties. However, due to their non-biodegradability, they contribute significantly to environmental pollution, which aroused the interest to explore renewable and biodegradable alternatives derived from agro-industrial residues (Madhusudan et al., 2018; Mujtaba et al., 2022; Pires et al., 2022). Among these biopolymers, pectin, a complex polysaccharide polymer, is highlighted as a promising candidate due to its film-forming ability, biodegradability, non-toxicity, compatibility with bioactive compounds and good oxygen barrier property (Freitas et al., 2021; Y. Zhang et al., 2024).

Intelligent packaging is capable of monitoring the conditions of food and the environment, providing information (communicating) to the consumer throughout the shelf-life of the packaged food (Prasad & Kochhar, 2014; Silvestre et al., 2011). Natural pigments, such as anthocyanins and betalains, found in a variety of plants in nature, are considered environmentally friendly pH indicators, and are highlighted as colorimetric sensor to monitor the quality of food (C. Rodrigues et al., 2021c). This is possible once the food deterioration process occurs with microorganism growth and volatile compounds release, resulting in pH changes (Q. Guo et al., 2023). Recent studies have demonstrated the successful incorporation of betalain extracts from pitaya (*Hylocereus* spp.) into pectin, yielding multifunctional packaging with antioxidant, antimicrobial, and colorimetric freshness-sensing properties (X. Zhang et al., 2024).

Opuntia ficus-indica, a cactus species, is a crop adapted to arid and semi-arid climates, widely distributed on the Globe, and represents a promising yet underexplored source of biopolymers and natural pigments for intelligent packaging production (C. Rodrigues et al., 2023). Its mucilage, found in the cladodes, and betalain, found in the fruit (prickly pear), offer both functional and sensory benefits, as the former can be used to produce a bio-based composite films with reinforced properties, while the latter add antioxidant activity and pH-responsive color change to the composite (Debeaufort et al., 2025; C. Rodrigues et al., 2023).

Despite these advances, challenges remain in optimizing the physicochemical, mechanical, and barrier properties of biobased intelligent films to meet industrial requirements (J. Pires et al., 2021). Strategies such as nanocomposite reinforcement, blending polymers, bilayer structures, and cationic crosslinking have shown promise in enhancing film performance while maintaining responsiveness to environmental stimuli (Imran et al., 2010; J. Pires et al.,

2021; V. G. L. Souza et al., 2022). Integrating these approaches with *O. ficus-indica*-derived bioactive compounds can potentially produce multifunctional, sustainable packaging capable of improving food safety and reducing food waste and petroleum-fossil dependency.

Thus, this study aimed to develop a biobased intelligent film using *Opuntia ficus-indica* and fully characterize it. The mucilage/pectin extracted from the cladodes (by-product of the agriculture) blended with commercial pectin, and the betalain-rich extract from the peel of red prickly pears (by-product of juice industry) were used to prepare the intelligent packaging. For reinforcing purpose, natural montmorillonite was incorporated and cross linking with Ca^{2+} was tested. This work investigated their structural, mechanical, and functional properties, as well as their efficacy as pH-responsive indicators for food freshness, aiming to expand the application of cactus-derived biomaterials in sustainable intelligent food packaging. Ultimately, as proof of concept, the developed films were used to monitor the spoilage of fresh salmon.

5.2 Materials and Methods

5.2.1 Materials and Reagents

All chemical reagents were of analytical reagent grade and were used as purchased. Gallic acid (99% purity), sulphuric acid (H_2SO_4) and glycerol were purchased from Alfa Aesar (Karlsruhe, Germany). Folin-Ciocalteu reagent, sodium carbonate anhydrous (99.5% purity), sodium hydroxide ($\geq 99\%$ purity), trichloroacetic acid, and magnesium nitrate hexahydrate, were obtained from Panreac (Barcelona, Spain). Calcium chloride ($\geq 97\%$ purity) and ammonia solution ca. 25% NH_3 was purchased from Honeywell (Charlotte, NC, USA). Pectin from citrus peel (galacturonic acid of $\geq 74\%$ of dried basis), ethanol absolute ($\geq 99.8\%$ purity), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and potassium peroxodisulfate were supplied by Sigma-Aldrich (USA). Commercial montmorillonite (Cloisite[®]Na⁺) was kindly supplied by BYK Additives & Instruments (Wesel, Germany). All the water used was purified using a Milli-Q system (Millipore, St. Louis, MO, USA).

5.2.2 Betalain extraction and characterization

Red Prickly pear peel was freeze-dried in a SCANVAC CoolSystem (LABOGENE, Lillerød, Denmark) and reduced into powder using a coffee grinder Mill Pro (Create, Madrid, Spain). The extraction was performed according to Gómez-López et al. (2024). with some modifications Firstly, red prickly pear peel powder was mixed in a 50 % ethanolic solution with a ratio 1:5 (m/v) (Gómez-López et al., 2024). Two different methodologies were tested: agitation for 15 minutes at room temperature using a mixing plate (ARGO LAB, Carpi, Italy) or through ultrasound bath (JP Selecta, Barcelona, Spain) for 15 minutes. After this process, the mixture was placed under refrigeration for 1h. The mixture was then centrifuged at 10 956 g, 4 °C for 10 minutes.

The supernatant was recovered and kept under refrigeration (4°C). This process was repeated 2 more times with the pellet resulting from the centrifugation. The resulting supernatants were mixed, filtered, and evaporated under controlled ventilation to allow solvent removal. The remaining extract was placed at -80 °C, then freeze-dried until a powder was obtained. The betalain-rich powder was then characterized in terms of color and bioactive properties: phenolic compounds through Folin-Ciocalteu test, antioxidant activity through DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and ABTS method (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method, and Betalain determination (C. Rodrigues et al., 2025; V. G. L. Souza, Rodrigues, et al., 2018b).

The sensitivity to pH was assessed by preparing solutions of 10 mg/L with betalain-rich extract powder in ultrapure water. The pH of the solutions was adjusted from 1-12 and measured using a potentiometer CRISON micropH 2001 (CRISON, Barcelona, Spain) and CIE Lab color coordinates using a CR 410 colorimeter (Minolta Co., Tokyo, Japan).

5.2.3 Film forming solution and film preparation

As it was mentioned in Chapter 4, it was possible to obtain films using pectin extracted from *Opuntia ficus-indica* cladodes (2% w/v, plasticized with 50% w/w glycerol relative to pectin). However, the films were brittle and mechanically fragile, indicating the need for further optimization. To improve their properties, different formulations were tested (data not shown), varying the proportion of extracted and commercial pectin, glycerol content, and reticulation time with CaCl₂. The optimized composition was established as 3 g of total polymer (50% extracted *Opuntia* pectin and 50% commercial pectin), plasticized with 50% w/w glycerol,

and reticulated for 5 min in CaCl_2 solution. This formulation was therefore selected for the preparation of the OFIPC films used in this study.

Both pectins were dissolved at 1.5% (w/v) (to obtain a 3% (w/v) of polymer) in ultrapure water under magnetic stirring at 40 °C for 30 min and kept under continuous agitation overnight. Homogenization was performed using a T18 Ultra-Turrax (IKA, Staufen, Germany) at 10 956 g rpm for three cycles of 1 min each. Glycerol (50% w/w relative to pectin dry matter) was then incorporated as plasticizer. MMT (2.5% w/w relative to pectin dry matter) was dispersed in the solution by three additional cycles of 5 min homogenization at 10,000 rpm in an Ultra-Turrax followed by sonication in an ultrasonic bath (Selecta, Barcelona, Spain) for 15 min (V. G. L. Souza et al., 2022). Betalain-rich extract powder was incorporated during the last homogenization cycle at two concentrations, corresponding to 50% and 100% w/w of pectin dry matter.

Films were prepared through casting method by pouring 18 mL of filmogenic solution into a polystyrene petri dish with 85 mm diameter. The films were left to dry at room temperature in ventilated environment until complete solvent evaporation.

The dried films were immersed in 50 mL of a 2% (w/v) CaCl_2 solution containing 5 % (w/v) glycerol, and 50% (v/v) ethanol for 5 min to induce ionic crosslinking. After treatment, films were gently dried with tissue paper and left to dry completely at room temperature. Films were stored in a desiccator at 52% RH (with supersaturated solution of magnesium nitrate hexahydrate $[\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}]$) until further use.

Based on the optimized formulation, a set of composite films was developed to evaluate the effect of montmorillonite (MMT), betalain-rich extract powder concentration, and ionic crosslinking on film structure and properties. Formulations differed by the incorporation of MMT (2.5% w/w, relative to total pectin dry matter), betalain-rich extract (50% or 100% w/w, relative to pectin dry matter), and the presence or absence of reticulation with of CaCl_2 . The composition and respective designations of the prepared films are summarized in Table 5.1.

Table 5.1 -Composition and designation of the prepared films.

Film code	Composition
PC	Film containing 3% (w/v) commercial pectin
PC_R	PC film crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC	Film containing 3% (w/v) total polymer (1:1 blend of <i>Opuntia ficus-indica</i> cladode pectin and commercial pectin)
OFIPC_R	OFIPC film crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_MMT	OFIPC film incorporating 2.5% (w/w, relative to pectin) montmorillonite (MMT)
OFIPC_MMT_R	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_50EXT	OFIPC film incorporating 50% (w/w, relative to pectin) betalain-rich extract powder
OFIPC_50EXT_R	OFIPC_50EXT film crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_100EXT	OFIPC film incorporating 100% (w/w, relative to pectin) betalain-rich extract powder
OFIPC_100EXT_R	OFIPC_100EXT film crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_MMT_50EXT	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 50% (w/w, relative to pectin) betalain-rich extract powder
OFIPC_MMT_50EXT_R	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 50% (w/w, relative to pectin) betalain-rich extract powder, crosslinked with 2% (w/v) CaCl ₂ solution
OFIPC_MMT_100EXT	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 100% (w/w, relative to pectin) betalain-rich extract powder
OFIPC_MMT_100EXT_R	OFIPC film incorporating 2.5% (w/w, relative to pectin) MMT and 100% (w/w, relative to pectin) betalain-rich extract powder, crosslinked with 2% (w/v) CaCl ₂ solution

5.2.4 Films characterization

5.2.4.1 Optical properties

The color of the films was measured through the determination of the CIELab coordinates (L^* , a^* and b^*) using a CR 410 colorimeter (Minolta Co., Tokyo, Japan). L^* represents

lightness, where L=0 (black) to L= 100 (white), a^* represents redness from -(green) to + (red), and b^* from -(blue) to +(yellow). Five measurements were taken on each film.

The $L^*a^*b^*$ coordinates allowed calculation of the chroma (C^*) according to the following equation (Equation (7)) (Mohammadi et al., 2008; V. G. L. Souza et al., 2022):

$$C^* = (a^2 + b^2)^{0.5} \quad (7)$$

The opacity was evaluated by measuring the absorbance at 600 nm using a UV/Vis spectrophotometer (SPEKOL 1500, Analytikjena, Germany). The determination was made in triplicate and obtained through Equation 8 (V. G. L. Souza, Pires, Rodrigues, et al., 2019):

$$Opacity = \frac{Abs_{600nm}}{\delta} \quad (8)$$

where δ expresses the film thickness (mm).

The transparency of the films was made through a spectrum scan in a range of 200-900 nm in a UV/Vis spectrophotometer, expressed as transmittance (%).

5.2.4.2 Thickness and Mechanical Properties

Thickness was measured by using an electronic micrometer (Mitutoyo, Tokyo, Japan) at five random different points of each film. The results were expressed in micrometers (μm).

The mechanical properties of the films were evaluated through tensile tests using a texturometer TA.XTplusC (Stable Micro Systems, Surrey, UK) according to ASTM D822 (ASTM, 2012). Films strips of (70 mm x 20 mm) were placed in the tensile grips with an initial distance of 50mm of the film between the grip and stretched until rupture at a constant speed of 12.5 mm/s. The tests were performed with five replicates for each film. The stress-strain curves were used to determine the Tensile Strength (expressed in MPa), Young's Modulus (expressed in MPa) and Elongation-at-Break (expressed in %) were obtained from the stress-strain curve.

5.2.4.3 Contact Angle

The static contact angle was measured by the sessile drop method using a Drop Shape Analyses DSA25 (KRÜSS, Hamburg, Germany) equipped with Advance Software. Measurements were conducted at room temperature within 10 seconds after placing a water droplet on the surface of the film. The angle was obtained as an average on both side of the drop. For

each film, at least two measurements were taken at different locations for each film type (Salvada et al., 2022).

5.2.4.4 Moisture and Solubility in Water

Films with a dimension of 2 cm x 2 cm were used for the determination of humidity and solubility in water. Moisture content of the films was obtained by initially weighing the films and then dry them at 70 °C for 24h. The moisture was obtained through the percentage of weight loss, that relates to the initial weight (M_i) and the weight after drying (M_f), as expressed in Equation 9.

$$\text{Moisture (\%)} = \frac{M_i - M_f}{M_i} \times 100 \quad (9)$$

The solubility of the films in water was determined by immersion of the previously weighed dried films (M_i) in a petri dish containing 30 mL of ultrapure water at room temperature, for 24 h. After this time, the remaining fragments of the films were taken out of the water and dried in a natural convection oven till a constant weight was obtained at 70°C (M_f). The water solubility (%) of the films was obtained by Equation 10 using the weight of the M_i and M_f (V. G. L. Souza et al., 2022).

$$\text{Water solubility (\%)} = \frac{M_i - M_f}{M_i} \times 100 \quad (10)$$

5.2.4.5 Water Vapor Permeability (WVP)

Water Vapor Permeability (WVP) was determined through gravimetry following the ASTM E96/E96M-10 (ASTM, 2013). Permeability cups (Elcometer 5100, Nieuwegein, The Netherlands) were filled with anhydrous calcium chloride (Honeywell, Charlotte, NC, USA), creating an internal environment with ~ 0% relative humidity (RH). The films were attached to the top of the cups to seal and subsequently placed inside of a desiccator containing a supersaturated solution of magnesium nitrate hexahydrate [$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] to keep the RH at ~ 52%. The dessicator was equipped with a fan to promote air circulation and a thermohygrometer to obtain the real temperature and relative humidity. The permeability cups were weighed every hour for 8 h and after 24 h at room temperature. The rate of water vapor transmission (WVTR) was determined by calculating the slope of the linear relationship between mass gain (g) and time (s), divided by the surface area of the film exposed (m^2). WVP

(g·m⁻¹·Pa⁻¹·s⁻¹) was then calculated using Equation 11, where e is the film thickness (m), and ΔP represents the difference of water vapor partial pressure across the film. Each film formulation was tested in triplicate.

$$WVP = WVTR \times \frac{e}{\Delta P} \quad (11)$$

5.2.4.6 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared (FTIR) spectra of the resultant materials were acquired in the range 400 – 4000 cm⁻¹ using a FTIR spectrometer PerkinElmer Spectrum Two (Perkin Elmer, USA) with 1 cm⁻¹ resolution and 4 scans.

5.2.4.7 Scanning electron microscopy (SEM)

The surface morphology of the films was analysed by scanning electron microscopy (Quanta FEG 650, FEI, Hillsboro, OR, USA) operated at an accelerating voltage of 5 kV at different magnifications (Moura-Alves et al., 2023). Prior to the analysis, the samples were mounted on aluminum stubs with carbon adhesive tape and sputter-coated with gold (approximately 10 nm of thickness) using a vacuum coater (Leica EM ACE200, Wetzlar, Germany).

5.2.4.8 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) of the films was performed in a Setaram Labsys instrument, EVO model, with a 20 cm³·min⁻¹ Argon flow and a heating rate of 10 °C·min⁻¹ up to 700 °C.

5.2.4.9 Differential Scanning Calorimetry (DSC)

The thermal behavior of the films was evaluated through differential scanning calorimetry (DSC) using a DSC Q2000 from TA instruments Inc. (Tzero DSC technology) coupled to RCS 90 cooling system operating in the Heat Flow T4P option.

For each experiment, approximately 9-10 mg of film were weighed and encapsulated in a non-recyclable aluminum hermetic pan and lid and the capsules were sealed before analysis. The lids were perforated to allow water evaporation and avoid pressure increase inside the system. The experimental procedure started at 20°C, followed by cooling run until -90 °C and subsequently a heating run up 110°C. This procedure was repeated twice, with all measurements carried out at a rate of 10°C min⁻¹ under anhydrous conditions, while purging with

high-purity nitrogen at a flow rate of 50 mL min⁻¹. To remove the adsorbed water, in the first heating run, an isotherm at 110°C for 5 minutes was performed. Data treatment was carried out by using the Universal Analysis 2000 Software (TA Instruments).

5.2.5 Film's sensitivity to ammonia

The sensitivity to ammonia was evaluated on films of OFIPC_100EXT; OFIPC_100EXT_R; OFIPC_MMT_100EXT; OFIPC_MMT_100EXT_R. The films' response to ammonia (NH₃) vapor were evaluated by exposing them to a basic environment for 30 min. Films with a dimension of 20 x 20 mm were placed on the top of a petri dish and 1 mL of aqueous NH₄OH solution of 5% v/v on the bottom of the petri dish. The petri dish was sealed, and the color of the films were recorded every 2 min. The images of the films were obtained with a digital camera and the values of L^* , a^* and b^* were obtained by Image J software (Koop et al., 2024). With CIE Lab coordinates it was possible to calculate the Hue Angle (h°) (Equations 12, 13, and 14).

$$h^\circ = \arctan^{-1}\left(\frac{b^*}{a^*}\right), \text{ if } a^* > 0; \quad (12)$$

$$h^\circ = \arctan^{-1}\left(\frac{b^*}{a^*}\right) + 180, \text{ if } a^* < 0; \quad (13)$$

$$h^\circ = \arctan^{-1}\left(\frac{b^*}{a^*}\right) + 360, \text{ if } a^* > 0 \text{ and } b^* < 0 \quad (14)$$

5.2.6 Food packaging application

The ability of the films to monitor the freshness of salmon through color changes was evaluated. The films tested were OFIPC, OFIPC_R, OFIPC_MTT, OFIPC_MMT_R, OFIPC_100EXT, OFIPC_100EXT_R, OFIPC_MMT_100EXT, and OFIPC_MMT_100EXT_R.

In order to make a proof of concept, 20 x 20 mm of film was placed with tape on top of a petri dish (85 mm diameter). At first, 18 to 23 g of salmon were placed inside the petri dish and the cover with the film was placed on top. The system was sealed and stored at room temperature (23 °C, ~50% RH), for 72h. To assess the color changes, images of the films were taken at 0, 12, 24, 36, 48, 60 and 72h and converted into L^* , a^* and b^* through ImageJ software. The hue angle (h°) and ΔE using Equations 12,13, and 14 and Equation 15, respectively.

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (15)$$

The pH of the salmon at every time of experiment was assessed by using a CRISON micropH 2001 (CRISON, Barcelona, Spain). 5 g of salmon were placed under agitation in 50 mL of ultrapure water at 40°C for 20 minutes. The samples were filtered, and the pH was measured (AOAC, 1990). The analysis was performed in triplicate.

The total basic volatile nitrogen (TVB-N) of the salmon was also evaluated at every time-point. Firstly, 5 g of salmon were homogenized with 10 mL 7.5% TCA solution (w/v) for 30 minutes at room temperature. The TVB-N analysis was then performed according to AOAC method (AOAC, 1990). The TVB-N content was then obtained through Equation 16, and expressed as mg of N per 100 g of salmon:

$$TVBN (mg\ N/100g) = \frac{V \times N \times 14}{m} \times \frac{10}{V_{extract}} \times 100 \quad (16)$$

where V is the volume (mL) of H_2SO_4 0.02N used in the titration, N the normality of H_2SO_4 0.02N, m the mass (g) of salmon used, and $V_{extract}$ the volume (mL) of extracted obtained after filtration.

5.2.7 Statistical Analysis

The statistical analysis of the data was carried out through multivariate analysis of variance (MANOVA) to detect overall effects, followed by a one-way analysis of variance (ANOVA) using Software Minitab, version 22, and differences among mean values were processed by the Tukey test. Results were regarded as statistically significant at $p < 0.05$.

5.3 Results and Discussion

5.3.1 Betalain-rich extract powder characterization

The betalains were extracted according two methods and their bioactive properties were evaluated (Table 5.2). No statistically significant differences ($p > 0.05$) were found between the two extractions for all the parameters analysed. The betalain-rich extract obtained from *Opuntia ficus-indica* peel presented a total phenolic content of approximately 90–100 mg GAE/100 g, reflecting the presence of bioactive compounds typical of prickly pear by-products. The antioxidant activity reached around 60 μ mol Trolox/L in DPPH assay and 510 μ mol Trolox/L in ABTS assay. The extract showed betacyanin and betaxanthin contents of about 1.3–

1.9 mg/L, with hue angle values close to 5°, confirming the predominance of red–violet pigments. These results demonstrate that the extraction conditions efficiently recovered phenolic and betalain compounds with relevant antioxidant potential. Similar findings have been reported for *O. ficus-indica* extracts obtained with hydroethanolic solvents (Yao et al., 2020).

Table 5.2 - Bioactive properties and hue angle of betalain-rich extracts.

Extraction	TPC (mg GAE/ L)	DPPH assay	ABTS assay	Betacyanin (mg/100g)	Betaxanthin (mg/100g)	Hue Angle (h°)
		(μmol Trolox/L)	(μmol Trolox/L)			
Agitation	89.4±3.8	56.8±7.3	500.2±23.6	1.35±0.38	1.46±0.38	5.4±4.3
Ultrasound	98.2±12.1	64.6±3.8	519.5±6.49	1.62±0.21	1.93±0.60	5.3±4.3

*TPC: Total Phenolic Content; GAE: Gallic acid equivalent.

Since no statistically significant differences were observed in the parameters characterized between the two methods of extraction, the following experiments were made with a mixture of both betalain-rich extract powder.

The ability of the betalain-rich powder extract to change color was evaluated at different pH values (Figure 5.1 and Table 5.3). It was possible to visualize the reaction through the color change, and it was evident that the extract remained visually stable between pH 2 and 7, while above this range, the betalains exhibited noticeable color alterations. The hue angle values supported these observations, remaining nearly constant in the range of around 0–6°/360° from pH 2 to 7, corresponding to red to purplish-red tones characteristic of the protonated forms of betacyanins (C. Rodrigues et al., 2021a). It is worth to mention that the apparent numerical difference between hue values near 0° and 360° results from the circular nature of hue angle and does not indicate an actual chromatic shift. In fact, the hue angle values, reflect approximately the same color.

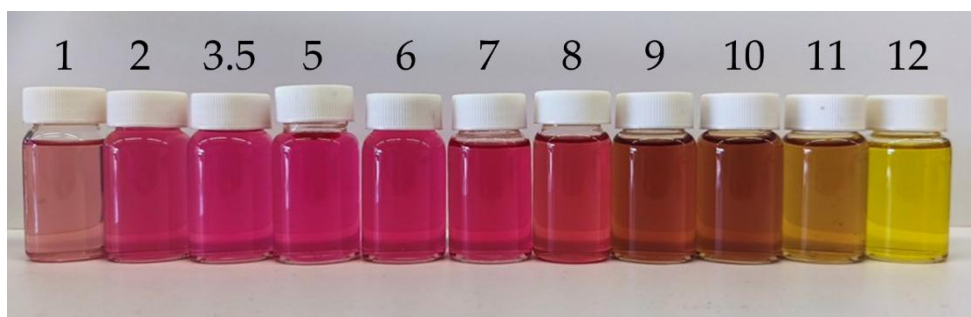


Figure 5.1 - Betalain-rich extract aspect under different pH values.

Table 5.3 - Hue angle values of betalain-rich extract under different pH.

pH	1	2	3.5	5	6	7	8	9	10	11	12
h°	22.1	2.54	358.6	359.74	1.78	5.50	15.57	36.01	44.35	76.43	107.4

In contrast, from pH 8 to 12, hue angle values increased markedly, corresponding to orange to yellow hues. These changes are associated with the deprotonation and degradation of betacyanins and the greater relative contribution of betaxanthins under alkaline conditions (Abedi-Firoozjah et al., 2023; Gengatharan et al., 2015; Melgar et al., 2017). Similar pH-dependent color changes of betalain-rich extracts have been reported in previous studies (Halloub et al., 2023; Kanatt, 2020; Qin et al., 2020; Yao et al., 2020). Overall, the extract demonstrated good colour stability in the acidic to neutral range (pH 2–7), with progressive hue shifts under basic conditions, confirming its potential application as a natural pH-sensitive pigment for intelligent or active packaging systems.

5.3.2 Optical properties

In the development of novel active/intelligent food packaging system, where active compounds are incorporated into the polymeric matrix, to study the material's optical properties is fundamental. Consumers value more those products packaged in transparent material, where it is possible to see the content packaged, thus the packaging should not interfere in the appearance of the food (C. Rodrigues et al., 2021a). The optical parameters (L, Chroma and Opacity) are shown in Table 5.4.

Table 5.4 - Optical properties of PC and OFIPC films with MMT, extract addition, and Reticulation effect.

	Ext	PC	PC_R	OFIPC	OFIPC_R	OFIPC_ MMT	OFIPC_ MMT_R
L	0	82.9±0.4 ^d	84.2±0.2 ^c	85.8±0.4 ^{Aa}	85.6±0.2 ^{Aab}	84.7±0.2 ^{Abc}	83.6±0.7 ^{Ac}
	50	-	-	66.0±1.1 ^{Bb}	69.2±2.9 ^{Bab}	70.6±0.6 ^{Ba}	72.0±0.7 ^{Ba}
	100	-	-	58.5±2.0 ^{Cb}	60.1±0.5 ^{Cb}	59.1±0.5 ^{Cb}	66.1±1.5 ^{Ca}
Chroma	0	7.0±0.3 ^a	5.3±0.2 ^{cd}	4.8±0.3 ^{Cd}	4.6±0.3 ^{Cd}	5.7±0.2 ^{Cbc}	6.0±0.1 ^{Cb}
	50	-	-	20.0±0.8 ^{Ba}	15.1±2.6 ^{Bb}	14.7±0.4 ^{Bb}	12.0±0.6 ^{Bb}
	100	-	-	29.0±1.9 ^{Aa}	24.7±0.4 ^{Ab}	27.4±0.5 ^{Aab}	18.0±1.3 ^{Ac}
Opacity (mm ⁻¹)	0	1.04±0.0 ^d	1.27±0.0 ^{cd}	1.56±0.1 ^{Ac}	2.06±0.3 ^{Ab}	2.26±0.0 ^{Ab}	3.64±0.1 ^{Aa}
	50	-	-	1.09±0.0 ^{Bb}	1.13±0.1 ^{Bb}	1.31±0.0 ^{Bb}	2.12±0.1 ^{Ba}
	100	-	-	1.07±0.0 ^{Bc}	1.69±0.0 ^{Ab}	1.08±0.0 ^{Cc}	2.04±0.1 ^{Ba}

*A-C: values in the same column not sharing upper case superscript letters indicate significant differences among films ($p < 0.05$); a-d: values in the same row not sharing lower case superscript letters indicate significant differences among films ($p < 0.05$);

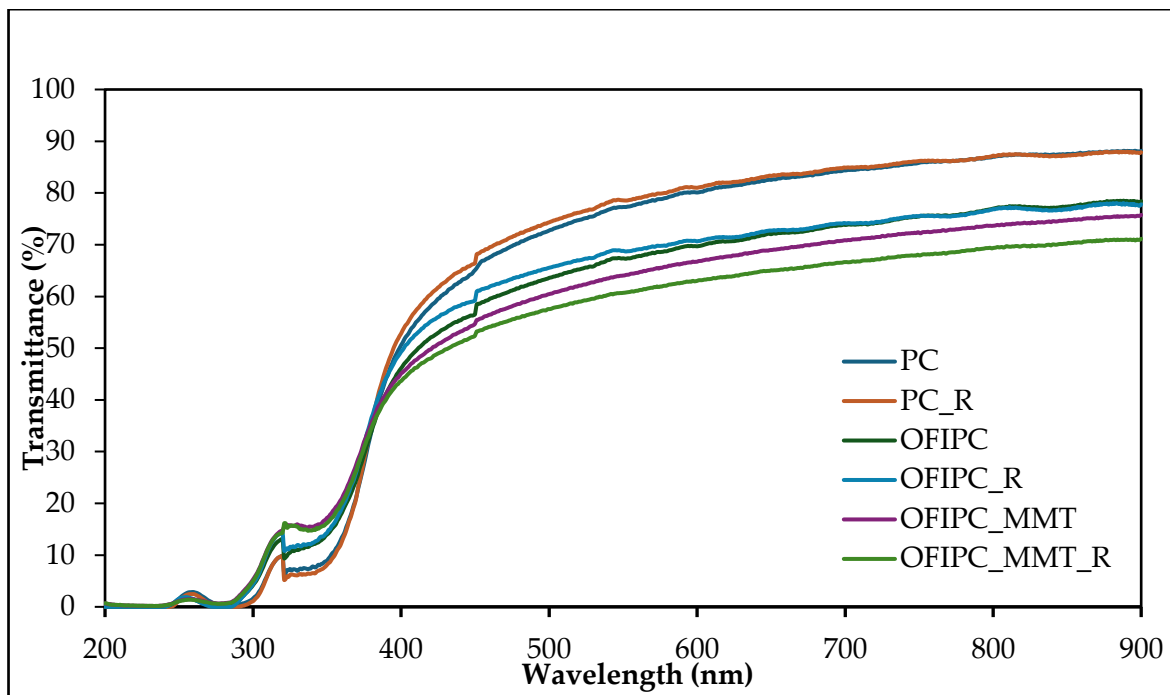
The lightness of the films decreased with the increase of the extract concentration in film formulation ($p < 0.05$). Films without extract (0% EXT) were the lightest, with values of L around 83 to 86. The increase of extract in the film formulation, decreased the Lightness of the films. This is directly related to the increase in betalain pigments present in betalain-rich extract. Yao et al. (2020) documented similar behavior with the incorporation of cactus pear extract in quaternary ammonium chitosan and polyvinyl alcohol films (Yao et al., 2020). Within each extract level, some variation among formulations was observed, namely when MMT was incorporated or the films were reticulated, often showing slightly higher L values when compared to their counterparts. The use of MMT contributed to changes in lightness which is also reported by other authors in white-fleshed pitaya peel pectin films with MMT (H. Jiang et al., 2022, 2023). Furthermore, the same authors added betacyanins to the film, which altogether contributed to L^* parameter decrease. Even though some statistically significant differences ($p < 0.05$) were observed between reticulated and non-reticulated films, the lightness remained high, which behavior is similar to mango-peel pectin films crosslinked with Ca^{2+} ions (S. Zhang et al., 2023).

In Chroma results, films without addition of extract presented low levels of color saturation. Although some statistically significant differences ($p < 0.05$) were observed between films reticulated and non-reticulated without extract addition, Chroma values remained similar. No statistically significant differences ($p > 0.05$) were observed between the same films without extract addition that were reticulated and non-reticulated, showing that the crosslinking agent did not affect chroma values. Same behaviour was described in mango-peel pectin films crosslinked with Ca^{2+} ions (S. Zhang et al., 2023). The addition of 50% of betalain-rich extract to the film formulation increased Chroma levels (12 – 20) and with 100% of extract the values raised even more (18-29) suggesting that strong intensification of color saturation with the extract addition. Differences among formulations were evident, with reticulated films generally presenting lower Chroma values compared to non-reticulated films with the same percentage of extract added.

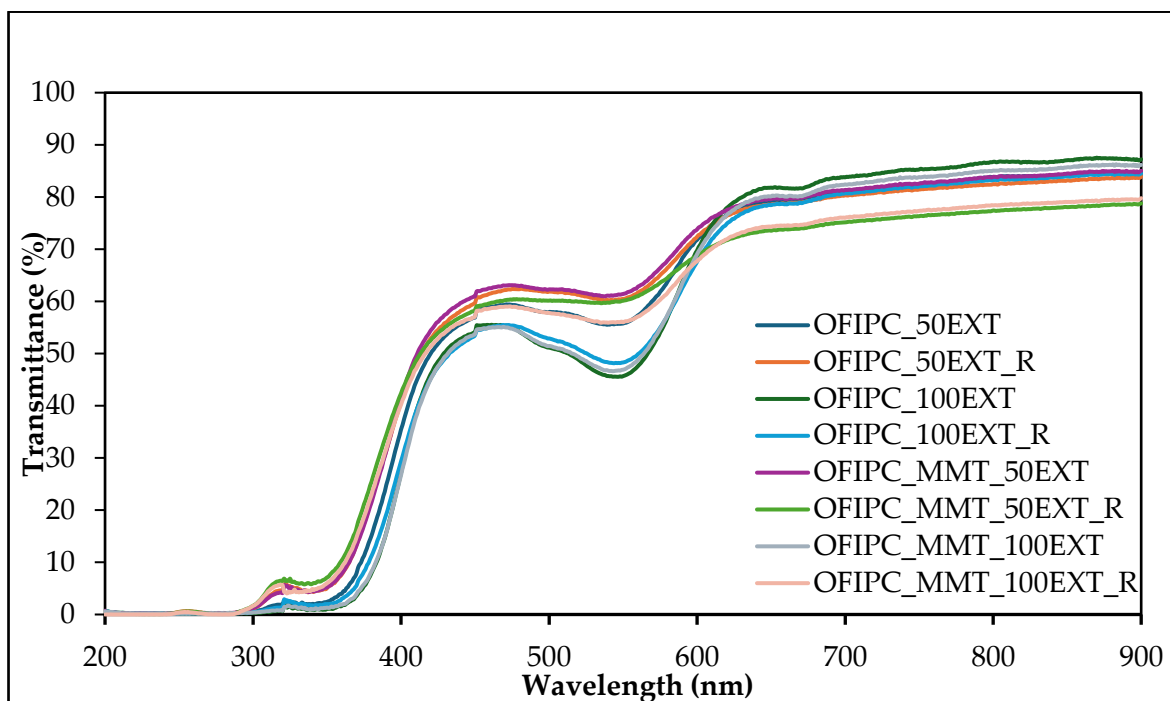
Although the differences in L and Chroma between reticulated and non-reticulated films with extract were not statistically significant ($p > 0.05$), a tendency toward lower values was observed, which may be related to lixiviation of betalains during the crosslinking step, since these water-soluble pigments can diffuse into the CaCl_2 solution (Zhai et al., 2025). In contrast, in control films without extract, reticulation led to a slight increase in both L and Chroma.

Regarding opacity, the addition of extract had a less direct influence on the films when compared to L and Chroma parameters. The PC film showed less opacity ($1.04 \pm 0.0 \text{ mm}^{-1}$) amongst all the films studied. The OFIPC films were more opaque than the PC films and when 2.5% of MMT was added to the films the opacity increased even more. The addition of extracts did not contribute to the increase of opacity, but the combination with MMT and Reticulation enhanced light scattering. The addition of MMT by itself enhanced light scattering, increasing the opacity of the films which is also reported by other authors (H. Jiang et al., 2022; V. G. L. Souza et al., 2022). The interaction of the betalain-rich extract in higher concentration and MMT (OFIPC_MMT_100EXT film) might have contributed to the decrease in opacity when compared to OFIPC_MMT that may have caused a decrease in reflective index of MMT (Jiang 2023).

The light transmittance analysis of the films is fundamental to better understand the UV-Vis barrier properties. The study of the transparency of the films is shown in Figure 5.2 A and B. In both Figure 5.2 A and B, it is possible to verify an abrupt change at 320 nm which corresponds to the lamp change from Visible to Ultraviolet.



A



B

Figure 5.2 - UV-Vis transmittance spectra of PC and OFIPC films with MMT, and Reticulation effect (A) and OFIPC films with MMT, extract addition, and Reticulation effect (B).

In Figure 5.2 B, it was noticeable that the higher the content in betalain-rich extract powder the more the decrease in light transmittance was observed thus there was a reduction

in transparency. The use of betalain-rich extract powder in the films led to a higher UV-Vis light barrier and this behaviour is linked to the betalains chromophore properties, such as C=C, C=N and C=O (H. Jiang et al., 2023; Qin et al., 2020). The reduction on transparency with betalain-rich extract is visible by the decrease in light transmittance in the region 500-600 nm, the same region of betacyanins (535/538 nm) (H. Jiang et al., 2023). Yao et al. (2020) also observed a decrease in UV-Vis light transmittance when cactus pear extract was added to quaternary ammonium chitosan and polyvinyl alcohol films (Yao et al., 2020). The incorporation of betalains from red pitaya peel also influenced the UV-Vis light transmittance in starch and polyvinyl alcohol films (Qin et al., 2020). Moreover, the presence in the films of extract and MMT simultaneously (OFIPC_MMT_100EXT), decreased the transparency of the films when compared to the other films with less percentage of extract. Nevertheless, and despite this reduction, the films remained sufficiently transparent to fit as a packaging material, allowing to visualize a food product (Figure 5.3).

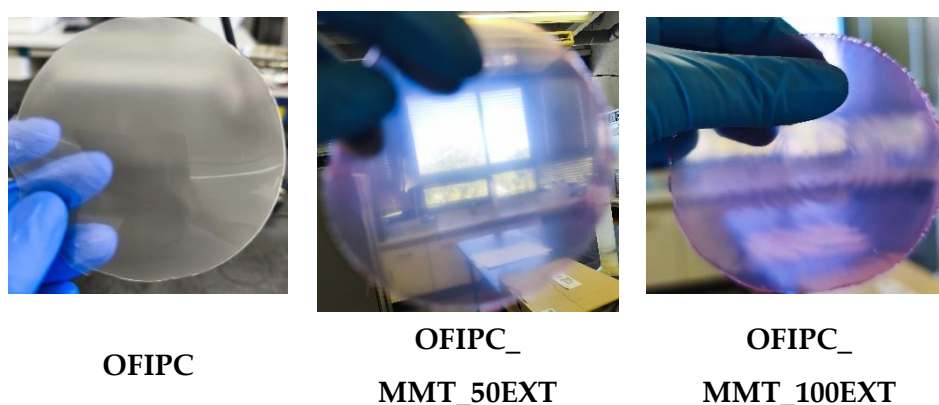


Figure 5.3 - Example of the films' aspect.

In most films, the use of a cross-linking agent to reticulate the films, lead to an increase in transparency when compared to the same films without reticulation and films became more opaque (Table 5.4). These results demonstrated that the use of betalain-rich extract powder, especially the films OFIPC_100EXT, and the films with extract and MMT (OFIPC_MMT_100EXT) could be more effective in the protection from UV-Vis light when compared with the PC and OFIPC films. The enhanced light-barrier properties are particularly valuable for packaging applications, as the decreased UV-Vis transmittance provides effective protection against photo-oxidative degradation pathways and reduces the risk of lipid autooxidation in food products (Said & Lee, 2025; V. G. L. Souza, Pires, Vieira, et al., 2019).

5.3.3 Mechanical Properties and Thickness

The mechanical performance of packaging films is a critical attribute, as they must be sufficiently resistant to ensure polymer integrity during handling and application to food products (Kurek et al., 2021). The evaluation of mechanical properties provides insights into how films behave under external forces and are strongly influenced by their internal structure and molecular interactions (Huang et al., 2022; H. Jiang et al., 2022). In addition, films thickness also plays an important role, as it is directly related to some properties such as mechanical, optical and barrier properties. The concentration of soluble substances in the film-forming dispersion solution has a high impact on this parameter (Ingale et al., 2025). To achieve that, the Young's Modulus (YM), Tensile Strength (TS) and Elongation at Break (EAB) of the films were studied through tensile tests. The results for mechanical properties and thickness of the films were studied and are demonstrated in Table 5.5.

Table 5.5 - Mechanical properties and Thickness of PC and OFIPC films with MMT, extract addition, and Reticulation effect.

	Ext	PC	PC_R	OFIPC	OFIPC_R	OFIPC_ MMT	OFIPC_ MMT_R
YM (MPa)	0	2.2±0.8 ^{cd}	18.3±3.5 ^a	0.94±0.14 ^{Ad}	9.4±0.4 ^{Ab}	0.93±0.19 ^{Ad}	9.3±5.2 ^{Abc}
	50	-	-	0.12±0.00 ^{Bb}	10.1±0.3 ^{Aa}	0.30±0.0 ^{Bb}	9.9±7.2 ^{Aa}
	100	-	-	0.04±0.00 ^{Bc}	1.50±0.25 ^{Bb}	0.10±0.01 ^{Bbc}	6.5±1.0 ^{Aa}
TS (MPa)	0	22.9±2.9 ^b	54.9±27.2 ^a	8.3±1.2 ^{Ab}	11.1±0.6 ^{Ab}	9.3±2.8 ^{Ab}	6.3±2.4 ^{Ab}
	50	-	-	3.6±0.1 ^{Ba}	5.8±3.9 ^{Aa}	4.2±0.5 ^{Ba}	8.1±7.2 ^{Aa}
	100	-	-	2.5±0.2 ^{Bb}	7.2±1.5 ^{Aa}	3.8±0.1 ^{Bb}	7.8±1.7 ^{Aa}
EAB (%)	0	20.8±6.1 ^a	13.3±11.5 ^{ab}	12.5±3.7 ^{Cab}	2.3±0.7 ^{Bb}	15.0±7.2 ^{Bab}	1.2±0.2 ^{Ab}
	50	-	-	30.0±3.9 ^{Ba}	0.9±0.2 ^{Cc}	17.5±1.4 ^{Bb}	4.2±3.0 ^{Ac}
	100	-	-	60.8±1.5 ^{Aa}	6.8±0.6 ^{Ac}	39.0±1.6 ^{Ab}	1.7±0.1 ^{Ad}
Thick ness (µm)	0	116±12 ^a	70±19 ^{bc}	101±9 ^{Bab}	76±14 ^{Abc}	87±7 ^{Babc}	55±5 ^{Bc}
	50	-	-	133±19 ^{ABa}	95±14 ^{Ab}	104±11 ^{Bab}	73±9 ^{ABb}
	100	-	-	146±13 ^{Aa}	101±1 ^{Ab}	139±13 ^{Aa}	86±16 ^{Ab}

*A-B: within each parameter, values in the same column not sharing upper case superscript letters indicate significant differences among films ($p < 0.05$); a-d: values in the same row not sharing lower case superscript letters indicate significant differences among films ($p < 0.05$);

The YM of the films was affected by the extract addition and reticulation ($p < 0.05$), but not by MMT addition or its interactions ($p > 0.05$). Reticulation consistently increased YM in OFIPC and OFIPC_MMT films, particularly in films without extract and with 50% extract, demonstrating an increase in films stiffness. In contrast, the increase of extract reduced the YM in non-reticulated films OFIPC and OFIPC_MMT with 100% extract addition. This reduction may be attributed to a plasticizing effect of betalains and other extract components, which interfere with molecular interactions, thereby decreasing rigidity (Huang et al., 2022; Qin et al., 2020).

The tensile strength (TS) was also highly influenced by the extract addition and reticulation ($p < 0.05$), but not by MMT ($p > 0.05$). Amongst all films, pristine commercial pectin films (PC and PC_R) showed the highest TS values, with 54.9 ± 27.2 MPa in PC_R. In general, all OFIPC films displayed lower TS values, when compared to PC films, which can be attributed to the composition of the film-forming dispersion (Van Rooyen et al., 2023a). Extract addition led to a decrease in TS values which can be associated to pigments aggregation, that formed an heterogenous film structure and the formation of discontinuous regions within the film matrix (Huang et al., 2022). It was observed a trend that reticulation improved strength relative to the corresponding non-reticulated films, although there were non-statistically significant differences ($p > 0.05$) between most formulations. This is in agreement with the findings of Zhang et al. (2022), that reported that the cross-linking of pectin-thymol films with CaCl_2 increased the TS.

The increasing of extract in non-reticulated films led to a significant reduction in TS ($p < 0.05$), while in reticulated films with extract addition no differences were observed ($p > 0.05$). Although the reinforcing effect of MMT was not statistically significant, its incorporation partially counterbalanced the weakening effect of the betalain-rich extract, resulting in intermediate TS values when both components were combined. A similar compensatory effect of MMT has been described in other polysaccharide-based films, where nanoclay addition improved stress transfer and mechanical resistance even in the presence of plasticizing or disruptive additives (V. G. L. Souza et al., 2017, 2018; V. G. L. Souza, Pires, Rodrigues, et al., 2019). Similar to this work, Jiang et al. (2023), reported that the incorporation of MMT reinforced white-fleshed pitaya peel pectin films, but the addition of betacyanin extract significantly reduced TS, particularly at higher concentrations, due to pigment aggregation and possible disruption of polymer-polymer interactions.

Elongation at break (EAB) was highly affected by all factors (MMT, extract, and reticulation) ($p < 0.05$). In non-reticulated films, it was verified that a marked increase in EAB with the increase in extract content with the highest levels observed for OFIPC_100EXT ($60.8 \% \pm 1.5$), showing more flexibility. This could be explained by the plasticizing effect caused by the extract addition that changed the molecular interaction in the film-forming dispersion (Qin et al., 2020). Moreover, this outcome was expected since in biopolymer films, an increase in flexibility is often linked to a drop in tensile strength (Abdullah et al., 2022; Figueroa Pires et al., 2024). The use of a cross-linking agent drastically reduced EAB in all formulations leading to a loss of flexibility in those films, thus becoming more fragile. Similarly to this present study, Zhang et al. (2022) reported a decrease in EAB in pectin-thymol films cross-linked with CaCl_2 when compared to the non-reticulated films. The use of MMT in non-reticulated films showed a decrease in EAB values ($p < 0.05$) and that reduction was even more marked in films with MMT and reticulation (with or without extract addition). In agreement with these results, Jiang et al. (2023) observed that the incorporation of MMT reduced white-fleshed pitaya peel pectin films' flexibility by decreasing EAB, and the addition of betacyanins further accentuated this effect. This could be caused by pigment-polymer interactions that caused a disruption in pectin network.

Regarding the thickness, it was possible to observe an increase with higher extract concentrations in films from $101 \pm 9 \mu\text{m}$ in OFIPC to $146 \pm 13 \mu\text{m}$ in OFIPC_100EXT. This effect can be possibly explained by the higher solid content from betalain-rich powder, leading to thicker films (Ingale et al., 2025; Le et al., 2024). Reticulation significantly ($p < 0.05$) affected the thickness of the films with a notorious loss between the non-reticulated and reticulated films. MMT addition also tended to reduce thickness compared to films without MMT, although the differences were not statistically significant ($p > 0.05$) (V. G. L. Souza et al., 2022).

5.3.4 Moisture, Solubility, Contact angle and Water Vapor Permeability

The moisture content, water solubility, contact angle, and water vapor permeability (WVP) are important parameters in the evaluation of biopolymer-based films, as they directly influence both functional performance and further potential applications. The moisture content and solubility reflect the affinity of the polymer network for water, which is particularly relevant for storage stability and potential interactions with high-moisture foods (Kurek et al., 2021). Solubility in water also provides insights into the film's resistance to external water and its effect on barrier and mechanical properties (D. Liu et al., 2023). The contact angle indicates

surface wettability, distinguishing hydrophilic from hydrophobic surfaces, and influencing the interaction of the film with aqueous environments (Zhai et al., 2025). WVP quantifies the rate of water vapor transmission through the film and is considered one of the most important parameters to ensure that packaging materials effectively regulate moisture transfer in order to maintain food quality (Qin et al., 2020; Zhai et al., 2025). Together, these parameters provide a comprehensive insight of the water–film interactions, with implications for barrier efficiency, mechanical performance, and potential food packaging applications. The results obtained for moisture content, solubility in water, contact angle, and Water Vapor Permeability are presented in Table 5.6 .

Table 5.6 - Moisture, solubility, contact angle and Water Vapor Permeability of PC and OFIPC films with MMT, extract addition, and Reticulation effect.

	Ext	PC	PC_R	OFIPC	OFIPC_R	OFIPC_ MMT	OFIPC_ MMT_R
Humidity (%)	0	17±1 ^b	13±1 ^{bc}	22±2 ^{Aa}	15±1 ^{Ab}	16±2 ^{Ab}	11±0 ^{Bc}
	50	-	-	26±7 ^{Aa}	12±1 ^{Bb}	14±0 ^{Ab}	11±1 ^{Bb}
	100	-	-	18±0 ^{Aa}	12±0 ^{Bc}	16±1 ^{Ab}	12±0 ^{Ac}
Solubility (%)	0	100±0 ^a	70±1 ^c	79±1 ^{Ab}	72±1 ^{Bc}	100±0 ^a	100±0 ^{Aa}
	50	-	-	76±4 ^{Ab}	72±3 ^{Bb}	100±0 ^a	72±1 ^{Cb}
	100	-	-	79±2 ^{Ab}	78±1 ^{Ab}	100±0 ^a	77±1 ^{Bb}
Contact Angle (°)	0	85±2 ^a	81±9 ^a	67±15 ^{Aab}	53±2 ^{Ab}	59±0 ^{Ab}	59±7 ^{Ab}
	50	-	-	68±5 ^{Aa}	67±6 ^{Aa}	58±2 ^{Aa}	56±6 ^{Aa}
	100	-	-	65±4 ^{Aa}	68±11 ^{Aa}	59±7 ^{Aa}	70±8 ^{Aa}
WVP (10 ⁻¹¹ g/s.m.Pa)	0	29.7±4.6 ^a	9.4±1.9 ^c	20.4±1.5 ^{Ab}	4.3±2.6 ^{Ac}	17.9±0.1 ^{Ab}	2.5±0.1 ^{Ad}
	50	-	-	14.4±1.8 ^{Ba}	2.8±1.0 ^{Ab}	12.5±1.2 ^{Ba}	1.8±0.2 ^{Ab}
	100	-	-	13.9±0.4 ^{Ba}	3.9±0.1 ^{Ac}	11.1±0.9 ^{Bb}	2.5±0.5 ^{Ac}

*A-C: within each parameter values in the same column not sharing upper case superscript letters indicate significant differences among films ($p < 0.05$); a-d: values in the same row not sharing lower case superscript letters indicate significant differences among films ($p < 0.05$);

Moisture content of the films was affected by MMT and Reticulation ($p < 0.05$) but the addition of extract did not seem to highly impact the moisture content ($p > 0.05$). The highest moisture content was obtained by OFIPC (22 %) and OFIPC_50EXT (26%) while the lowest

moisture contents were OFIPC_MMT_R and OFIPC_MMT_50EXT_R films, both with 11% of moisture. In most formulations, the addition of extract either maintained or slightly reduced film moisture, suggesting that the extract did not enhance water retention overall. This behaviour can be explained by the molecular interactions between betalains and phenolic compounds and pectin chains, which likely occupy hydrophilic sites on the polymer backbone that would otherwise bind water molecules. Similar interactions have been described in polysaccharide films containing natural pigments, where the formation of hydrogen bonds between pigment and polymer lead to denser structures and lower moisture levels (Halloub et al., 2023; Huang et al., 2022). This is also linked with mechanical properties, leading to higher elongation at break associated with the plasticizing effect of the extracts on films (Table 5.5) (Qin et al., 2020). The addition of MMT on films decreased significantly the moisture content in OFIPC films ($p < 0.05$). This could be caused by the formation of hydrogen bonds between hydroxyl groups of the polymer with MMT that reduce the water content in films (H. Jiang et al., 2022). The conjugation of MMT and reticulation in films decreased significantly ($p < 0.05$) the moisture content when compared to their counterparts, demonstrating a synergistic effect between ionic cross-linking and nanoclay reinforcement. These findings corroborate previous studies highlighting the important role of the interaction between polymer and filler and the network density in controlling water affinity of polysaccharide-based films (Kurek et al., 2021).

Solubility was highly affected by all the film-forming dispersion variables ($p < 0.05$). The use of pectin from *Opuntia cladodes* (OFIPC- 79%) highly reduced solubility of the films when compared to pristine commercial pectin films (100% soluble in water) that are usually referred of hydrophilic nature (Nastasi et al., 2022). The addition of betalain-rich extract powder may have impact on water solubility due to their hydrophilic nature, increasing the solubility in water (Chavoshizadeh et al., 2020; Huang et al., 2022). In the case of reticulation, there was significant differences between PC and PC_R films, showing that reticulation reduced films' solubility. The same behavior was observed in pectin films with Ca^{2+} crosslinking (Van Rooyen et al., 2024; W. Zhang et al., 2022). In OFIPC films, MMT addition did not help on the reduction of films solubility when compared to its counterparts. However, in films with MMT and reticulation a significant ($p < 0.05$) decrease in solubility was observed, which improved the film's resistance to dissolution in water.

Relative to contact angle, pristine commercial pectin films presented the higher values showing a more hydrophobic characteristic when compared to OFIPC films. Values of contact angle for OFIPC films were obtained between $53^\circ \pm 2$ for OFIPC_R and $70^\circ \pm 8$ for

OFIPC_MMT_100EXT_R, with no statistically significant differences among them ($p > 0.05$). MMT addition did not strongly change water contact angle which was also reported by other authors, likely because of the limited effect of the fillers in this parameter (H. Jiang et al., 2022). There seems to have a tendency for the increase in contact angle with extract addition and MMT, suggesting that this conjugation reduced the surface hydrophilicity which is desirable for films to be applied in food packaging (H. Jiang et al., 2023). Even though no major differences were observed with extract addition, the pigments present in the betalain-rich extract powder may affect negatively the hydrophobicity of the films, due to their hydrophilic nature (Huang et al., 2022).

After evaluating the moisture and solubility behavior, the barrier performance of the films was further investigated through water vapor permeability (WVP) measurements. PC films showed the higher WVP values of all films $29.7 \pm 4.6 \times 10^{-11}$ g/s.m.Pa in this study. The combination of pectin from *Opuntia cladodes* and pristine commercial pectin significantly improved the WVP ($p < 0.05$) when compared to pristine pectin films, resulting in films with a value of $20.4 \pm 1.5 \times 10^{-11}$ g/s.m.Pa in OFIPC.

The addition of MMT reduced WVP, even though not statistically significant, and that behaviour was also reported in other works (H. Jiang et al., 2022, 2023). Extract addition reduced WVP, with the strongest barrier achieved when combined with reticulation and MMT ($1.8 \pm 4.6 \times 10^{-11}$ g/s.m.Pa in OFIPC_MMT_50EXT_R and $2.5 \pm 0.1 \times 10^{-11}$ g/s.m.Pa in OFIPC_MMT_100EXT_R). The reduction of WVP with extract addition was also reported for other authors showing a less porous structure, enhancing the barrier properties (Huang et al., 2022; Ingale et al., 2025) significantly reduced WVP permeability, as it was also demonstrated by Zhang et al. (2022) in pectin films with Ca^{2+} crosslinking.

To contextualize these results, the best WVP values obtained for OFIPC films ($1.8\text{--}2.5 \times 10^{-11}$ g/m.s.Pa) are substantially lower than those typically reported for other polysaccharide-based films such as sodium alginate (22.1×10^{-11} g/m.s.Pa) (Moura-Alves et al., 2023)), chitosan (28.4×10^{-11} g/m.s.Pa) (Kurek et al., 2021), and pectin (29.6×10^{-11} g/m.s.Pa) (Kurek et al., 2021). They are also lower than carrageenan films (4.17×10^{-11} g/m.s.Pa) (Martiny et al., 2020) and PLA (87.7×10^{-11} g/m.s.Pa) (Moura-Alves et al., 2023). Although, the result in this work were still higher than the permeability of petroleum-derived PVC films (2.47×10^{-13} g m⁻¹ s⁻¹ Pa⁻¹) (Martiny et al., 2020), these results demonstrate a significant improvement in barrier performance among biodegradable polysaccharide-based matrices.

Moreover, this reduction is also correlated with mechanical properties demonstrating that films with low water vapour permeability exhibited higher TS and YM values (Table 5.5) which is a desirable feature for food packaging applications (Dirpan et al., 2024). Overall, the combined effects of MMT reinforcement, Ca^{2+} reticulation, and betalain-rich extract incorporation produced films with competitive barrier properties comparable to or better than other biopolymers currently investigated for active and intelligent packaging.

5.3.5 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra obtained of the different films are represented in Figure 5.4(a,b,c,d).

A broad band around $3300\text{--}3200\text{ cm}^{-1}$, assigned to O-H stretching vibrations of hydroxyl groups of pectin, glycerol, and bound water is observed in all films (S. Zhang et al., 2023). This band is less broad in films reticulated with Ca^{2+} , probably due to the reduction of bound water and the formation of a more compact network structure due to ionic crosslinking (S. Zhang et al., 2023). The peaks in the region of $2935\text{--}2921\text{ cm}^{-1}$ could be attributed to C-H stretching ($-\text{CH}$, $-\text{CH}_2$, $-\text{CH}_3$) and a small increase in intensity was observed when betalain-rich extract powder was added which may be related with additional aliphatic structures from the extract (H. Jiang et al., 2023; W. Zhang et al., 2022). All films presented the characteristic absorption peaks for pectin, around $1744\text{--}1733\text{ cm}^{-1}$ corresponding to $\text{C}=\text{O}$ of methyl-esterified carboxyl groups, and around $1611\text{--}1587\text{ cm}^{-1}$ related to asymmetric COO^- carboxyl groups (W. Zhang et al., 2022). In reticulated films, this later peak showed a decrease in intensity and a slight shift in reticulated films, which can be linked with the relation of carboxylate groups in ionic bridges with Ca^{2+} (S. Zhang et al., 2023). A peak around $1437\text{--}1412\text{ cm}^{-1}$, can be attributed to symmetric COO^- was observed in most films with different intensities. Around $1239\text{--}1225\text{ cm}^{-1}$, bands were observed that may correspond to C-O stretching of acetyl/ester groups; C-O-C glycosidic vibrations (Kurek et al., 2021), contributions that slightly smoothed with cross-linking. Peaks around $1146\text{--}1140\text{ cm}^{-1}$, $1101\text{--}1006\text{ cm}^{-1}$, and $971\text{--}913\text{ cm}^{-1}$ were observed in all films' spectra. They represent C-O-C (glycosidic) and ring vibrations (pectin backbone), C-O stretching of C-O-H/C-O-C (pectin and glycerol) and pyranose ring structure in pectin, respectively (Ezati & Rhim, 2020; H. Jiang et al., 2023; Qin et al., 2020)

The films with MMT and extract addition (Figure 5.4 c and d) did not show new absorption bands when compared to films without addition showing that the nanoclay addition and betalain-rich extract powder did not produce effects on chemical structure of OFIPC matrix films but mainly interacted through physical dispersion or hydrogen bonding. Same

behavior was reported by Jiang et al. (2023), in films prepared from white-fleshed pitaya peel pectin and betacyanins, and MMT. Moreover, Souza et al.(2022) showed no major effects in chemical structure when MMT was added to pectin films. This absence may be related to the low MMT content use, which could be insufficient to produce changes detectable in FTIR spectra. In all reticulated films, a general decrease in peak intensity was verified when compared to their non-reticulated counterparts (Figure 5.4 a and d). This can be attributed to the formation of a denser polymeric network with less water content, that is also confirmed by moisture values obtained (Table 5.6) for those films and reduced molecular mobility due to Ca^{2+} crosslinking.

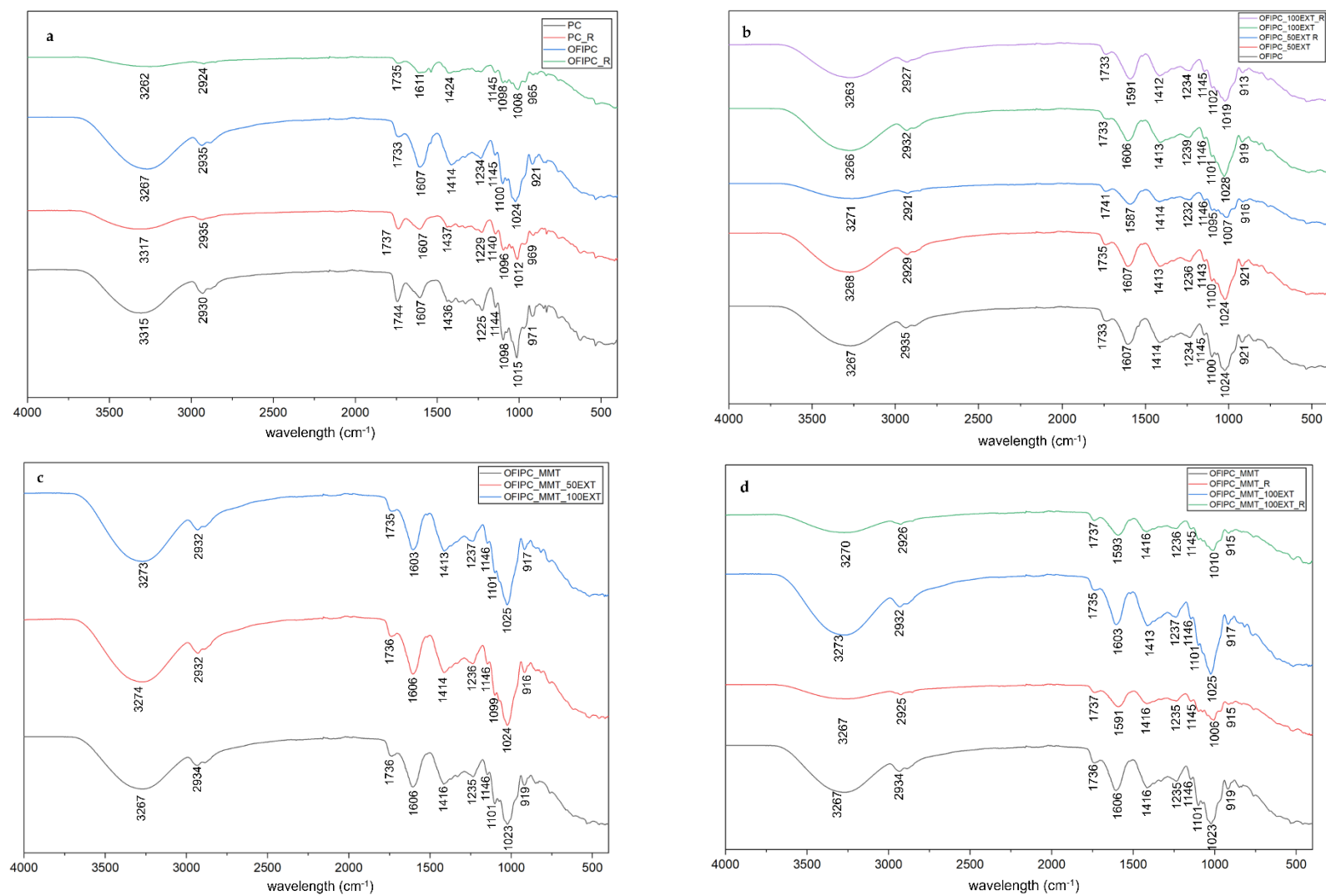


Figure 5.4 - FTIR spectra of PC and OFIPC films with MMT, extract addition, and Reticulation effect.

5.3.6 Morphological analysis

The morphological analysis of the composite films was performed by Scanning Electron Microscopy (SEM) to observe the microstructure of the films. The surface and the cross-section of the films are presented in Figure 5.5(A-G) and in Figure 5.6(A-G), respectively. Films of pristine commercial pectin (PC) show a homogenous, non-porous surface and cross-section as it is demonstrated in Figure 5.5-A and Figure 5.6-A, respectively. Similar uniform morphologies were reported by several authors for pectin-based films, evidencing that pectin matrices generally form continuous and compact structures (Luna-Sosa et al., 2020; Otálora González et al., 2021; V. G. L. Souza et al., 2022). This homogeneity can also be enhanced by the presence of glycerol, which acts as a plasticizer, reducing internal stress and promoting chain mobility during film formation (Luna-Sosa et al., 2020). On the other hand, the blend of commercial pectin with *Opuntia cladodes* pectin (OFIPC films) lead to a more heterogenous microstructure, with visible agglomerates (Figure 5.5-C), with some cracks and pores that can be visible in the cross-section of the film (Figure 5.6- C). Similar microstructural features were also reported by Van Rooyen et al. (2023), who reported that cactus pear mucilage films presented irregular and non-homogeneous surfaces with cracks and pores, in contrast to the smoother and more compact morphologies of pectin and alginate films. Such morphological heterogeneity is consistent with the lower tensile strength observed in the OFIPC films (Table 5.5), suggesting that structural irregularities directly affected their mechanical integrity. These results are also related to the opacity of the films (Table 5.4), where OFIPC films were more opaque than the PC films possibly due to the agglomerates observed in SEM images.

The reticulation with calcium ions led to noticeable modifications in the films' morphology, with more evident agglomerates on the surface of both PC_R and OFIPC_R films (Figure 5.5- C-D). This could be related to the formation of insoluble calcium pectate domains at the film surface, a phenomenon also reported in mango peel pectin-based films (S. Zhang et al., 2023). Moreover, the cross-section of OFIPC_R (Figure 5.6 -D) revealed a denser and more compact structure compared to the non-cross-linked OFIPC film, indicating the establishment of ionic bridges ($\text{Ca}^{2+}\text{--COO}^-$) between pectin chains. Similar structural densification has been described for calcium-cross-linked pectin matrices (S. Zhang et al., 2023; W. Zhang et al., 2022), where ionic crosslinking reduced porosity and increased internal cohesion. These microstructural modifications are consistent with the increased tensile strength and reduced elongation at break observed after reticulation (Table 5.5), as the formation of a more compact

and rigid network restricts polymer chain mobility, enhancing film stiffness but lowering flexibility.

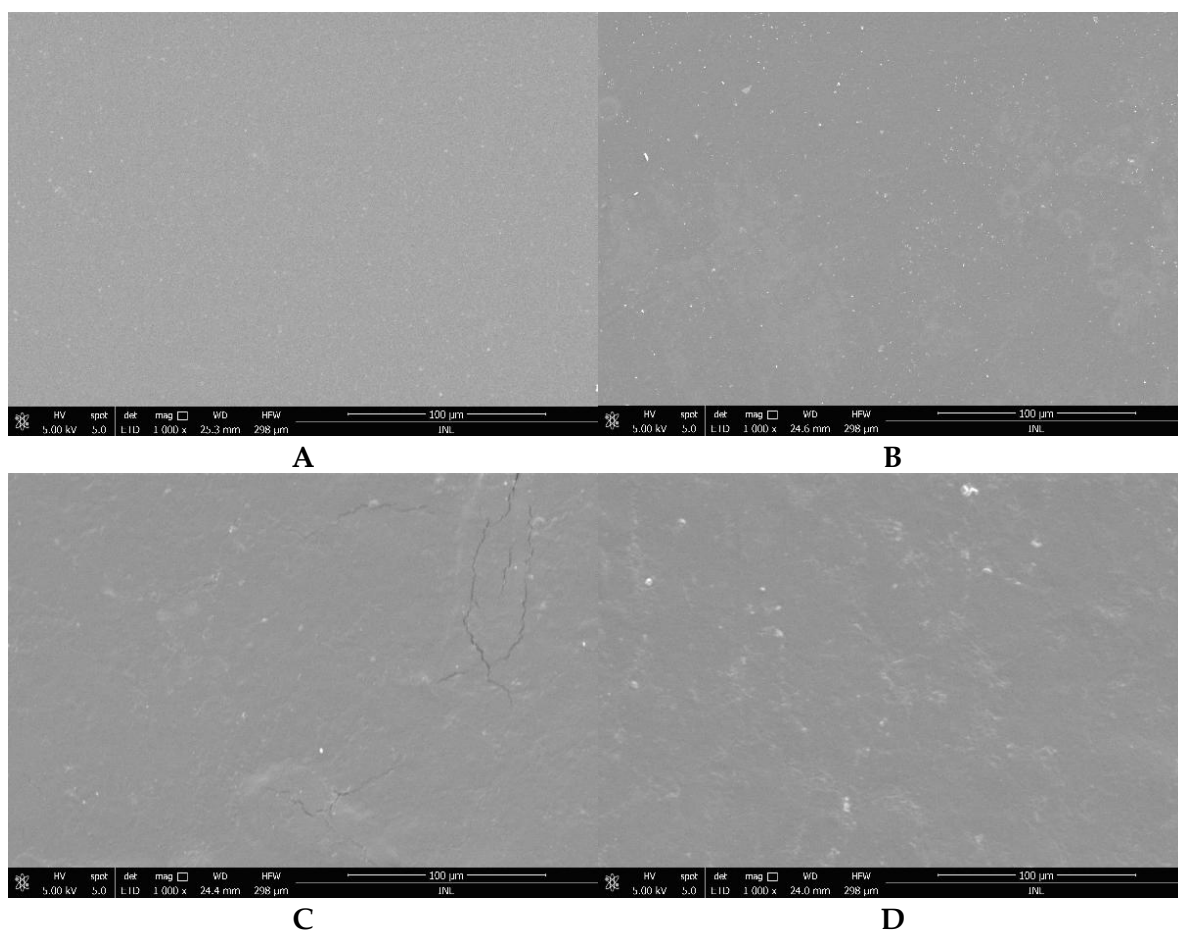


Figure 5.5 – SEM surface images of PC and OFIPC films with MMT, extract addition, and Reticulation effect: A - PC; B - PC_R; C - OFIPC; D - OFIPC_R; E - OFIPC_MMT; F - OFIPC_100EXT; G - OFIPC_MMT_100EXT.

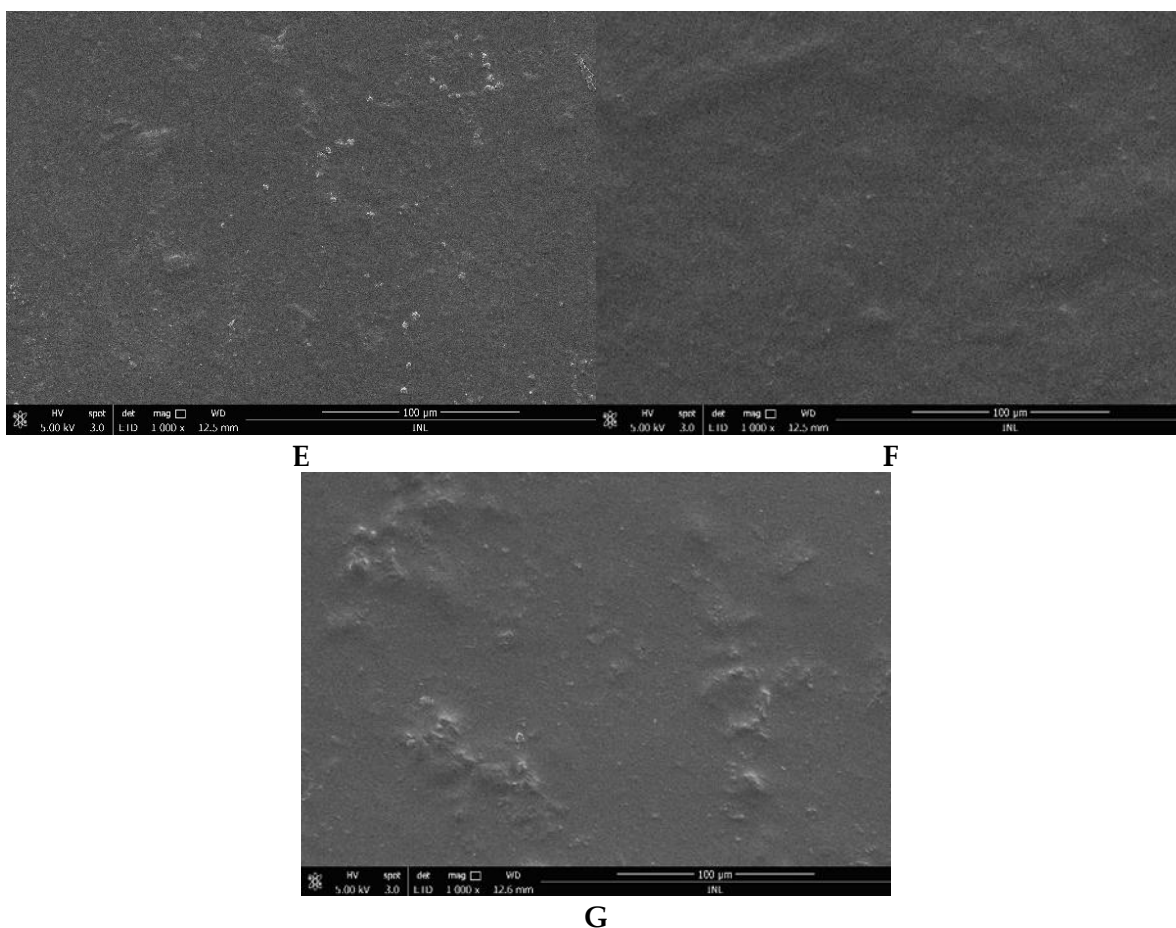


Figure 5.5 – SEM surface images of PC and OFIPC films with MMT, extract addition, and Reticulation effect: A - PC; B - PC_R; C - OFIPC; D - OFIPC_R; E – OFIPC_MMT; F – OFIPC_100EXT; G – OFIPC_MMT_100EXT (cont.).

The addition of MMT to films in OFIPC_MMT (Figure 5.5- E) led to a surface with a higher number of agglomerates and irregularities that increased in relation to OFIPC films, evidencing an incomplete dispersion of the nanoclay into the polymer matrix. This behavior is often attributed to the tendency of MMT to aggregate when not fully exfoliated during the casting process (Z. Guo et al., 2021; H. Jiang et al., 2022). Similar observations were reported for pectin/MMT systems, where the clay sheets distributed heterogeneously, producing surface bumps and a rougher texture (H. Jiang et al., 2022). The cross-section of this film (Figure 5.6 - E) demonstrates a more porous structure when compared to OFIPC films, showing that the addition of montmorillonite changed the microstructure of the film. Such microstructural heterogeneity is consistent with the slight increase in tensile strength observed for these films, even though not statistically significant (Table 5.5). The increased rigidity can be associated with the reinforcing effect of the dispersed silicate layers, whereas the decreased flexibility arises from restricted polymer mobility and poor interfacial adhesion between pectin chains and aggregated clay domains. Similar correlations were described for MMT-reinforced pectin

films, where incomplete clay exfoliation led to brittle and non-uniform matrices (H. Jiang et al., 2023).

In contrast, the addition of betalain-rich extract, in OFIPC_100EXT film (Figure 5.5- F), did not drastically alter the films surface homogeneity. It is possible to observe a smooth surface, with good dispersion of the extract within the polymer matrix. However, the cross-section micrograph (Figure 5.6– F), displayed a film structure with several pores, likely resulting from air entrapment during drying and the plasticizing effect of the betalain-rich extract and glycerol. The plasticizing action of the extract is consistent with the lower tensile strength and higher elongation at break recorded for these films (Table 5.5). This more porous structure is consistent with Jiang et al. (2023) reported a similar effect for pectin films containing pitaya peel betacyanins, where pigment addition increased the film's thickness and porosity due to the hydrophilic nature of the extract and its interference with polymer–polymer interactions.

The OFIPC_MMT_100EXT (Figure 5.5- G) revealed a surface morphology with small agglomerates and bumps, probably related to MMT insufficient dispersion on the film dispersion as was also observed for OFIPC_MMT films. Nevertheless, the cross-section of the film (Figure 5.6– G) can demonstrate that the nanoclay and extract addition led to a more dense and compact structure, suggesting a synergistic interaction between MMT and betalain-rich extract. This behavior is in accordance with the moderate increase in tensile strength and a decrease in elongation at break when compared with OFIPC_100EXT (Table 5.5), showing that the montmorillonite counteracted the softening effect of betalain-rich extract by enhancing interfacial reinforcement. Comparable findings were reported by Guo et al. (2021) and Jiang et al. (2023), who showed that combining nanoclay and natural pigments yielded more cohesive and less porous pectin-based films, leading into stronger but less extensible films.

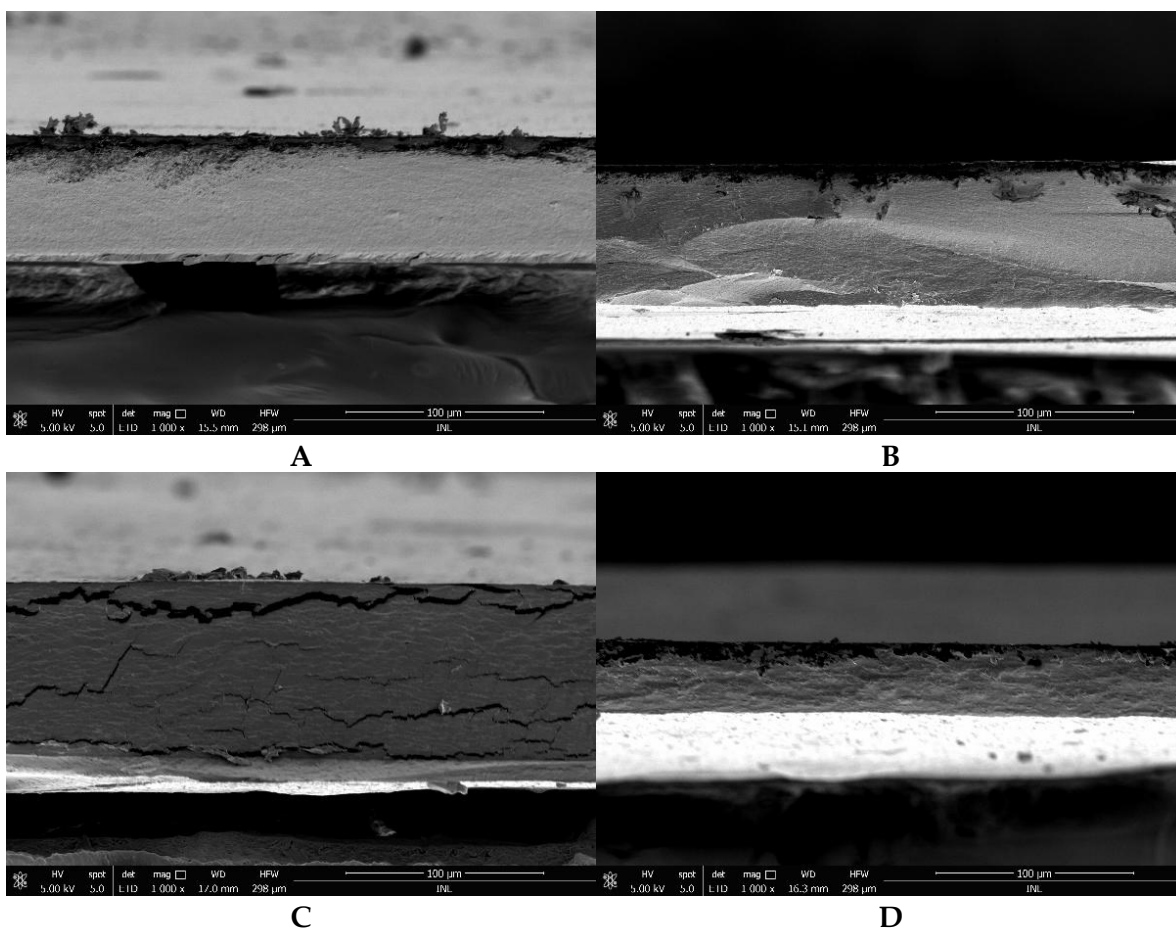


Figure 5.6 - Cross section images of PC and OFIPC films with MMT, extract addition, and Reticulation effect: A - PC; B - PC_R; C - OFIPC; D - OFIPC_R, E - OFIPC_MMT, F - OFIPC_100EXT, G - OFIPC_MMT_100EXT.

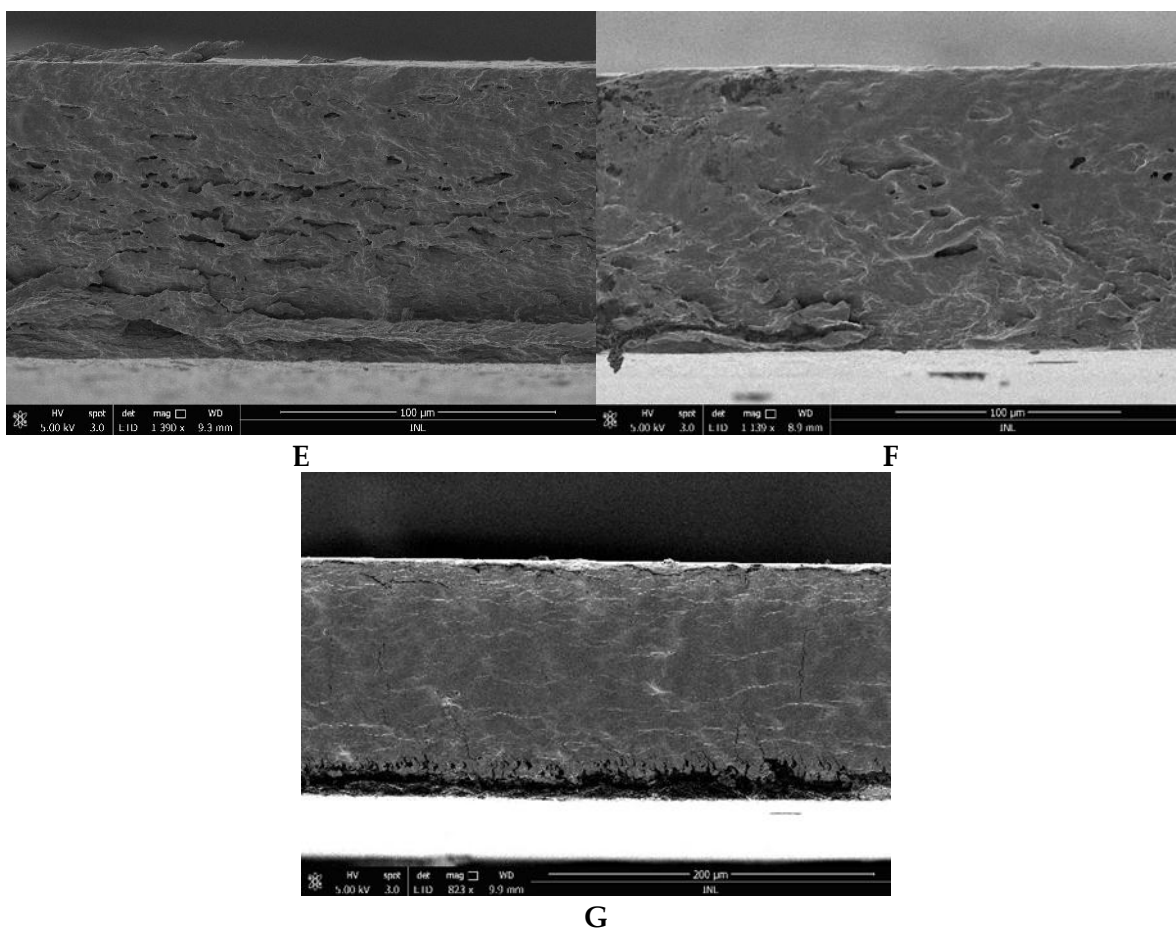


Figure 5.6 - Cross section images of PC and OFIPC films with MMT, extract addition, and Reticulation effect: A - PC; B - PC_R; C - OFIPC; D - OFIPC_R, E - OFIPC_MMT, F - OFIPC_100EXT, G - OFIPC_MMT_100EXT (cont).

5.3.7 Thermal Properties

Thermal properties of the films were evaluated through Differential Scanning Calorimetry (DSC) and thermogravimetric analysis (TGA) that allowed the determination of glass transition temperature (T_g) and the mass losses through thermal decomposition, respectively.

The DSC thermograms revealed that all films exhibited a single glass transition in negative temperature range (Table 5.7). The OFIPC film presented a T_g midpoint, similar to pristine commercial pectin, with values of $-65.45\text{ }^{\circ}\text{C}$ and $-69.39\text{ }^{\circ}\text{C}$, respectively. This could indicate that the blending process of commercial pectin with *Opuntia cladodes* pectin maintained molecular compatibility between the two polysaccharides. The addition of MMT to OFIPC film slightly increased T_g ($-59.71\text{ }^{\circ}\text{C}$ when compared to OFIPC: $-65.45\text{ }^{\circ}\text{C}$), suggesting restricted polymer chain mobility caused by hydrogen bonding and physical confinement at the clay-polymer interface. This finding is consistent with the behavior reported by Arora et al. (2025), where MMT addition increased the transition temperature of pectin-based composites due to improved dispersion and compatibility.

Table 5.7 - Glass transition temperature (T_g) obtained by Differential Scanning Calorimetry (DSC) of PC and OFIPC films with MMT, extract addition, and Reticulation effect

	T _g (°C)
PC	-69.4
OFIPC	-65.5
OFIPC_R	Not detected
OFIPC_MMT	-59.7
OFIPC_MMT_R	Not detected
OFIPC_100EXT	-45.7
OFIPC_100EXT_R	-10.3
OFIPC_MMT_100EXT	-42.2
OFIPC_MMT_100EXT_R	-16.9

The betalain-rich powder extract addition showed a marked increase in T_g values of films to -45.7 °C in OFIPC_100EXT. The conjugation of MMT and betalain-rich powder extract further raised the T_g to -42.2 °C, demonstrating the synergistic restriction of polymer mobility due to the possible simultaneous interaction of polymer with pigment and clay (Trigueiro et al., 2024).

The films reticulated with Ca²⁺ exhibited a marked different behavior. For both OFIPC_R and OFIPC_MMT_R, it was not possible to detect the glass transition in the scanned range. In reticulated films containing extract, T_g were observed at much higher temperatures, with -10.3 °C and -16.9 °C for OFIPC_100EXT_R and OFIPC_MMT_100EXT_R, respectively, indicating the formation of a more rigid and compact structure. Comparable T_g values (around -10 °C) were reported by Otálora González et al. (2021), for calcium-crosslinked pectin films plasticized with glycerol and incorporating 1.5% (w/w) beetroot or red cabbage powders as natural fillers. However, while their pigment-rich powders slightly decreased the T_g of the pectin-rich phase, suggesting partial interference with ionic crosslinking, the betalain-rich extract used in the present work promoted a significant T_g increase compared with the non-reticulated films, evidencing stronger polymer–calcium interactions and enhanced network rigidity.

The progressive increase in T_g observed with MMT, betalain-rich extract, and Ca²⁺ reticulation reflects the formation of a more rigid and cohesive polymer network. This trend aligns with the mechanical (Table 5.5) and barrier properties (Table 5.6), as films with higher T_g showed greater tensile strength, lower elongation at break, and reduced water vapor permeability. The restricted molecular mobility induced by clay dispersion, pigment and polymer interactions, and ionic crosslinking limited polymer chain mobility and water diffusion through the matrix. Overall, the increase in T_g correlates with enhanced stiffness and barrier

performance, confirming the synergistic reinforcing effects of MMT, betalain extract, and Ca²⁺ within the pectin-based films (A. Arora & Padua, 2010; Huang et al., 2022; Otálora González et al., 2021; Trigueiro et al., 2024).

The thermogravimetric analysis (TGA) of the individual materials (Commercial pectin, *Opuntia ficus-indica* pectin, montmorillonite and betalain-rich powder) and the films are presented in Figure 5.7-A and B, respectively. The different stages of thermal degradation are presented in Table 5.8 for materials and Table 5.9 for films.

Regarding the individual components, Commercial pectin and OFI pectin exhibited three and four steps of degradation, respectively. The first event occurred could be observed around 40-200 °C that corresponds to water evaporation (Ezati & Rhim, 2020) (Ezati 2020).

Table 5.8 – Thermal degradation parameters obtained by Thermogravimetric Analysis (TGA) parameters of the individual components used for film formulation.

	$\Delta 1$		$\Delta 2$		$\Delta 3$		$\Delta 4$		Residue (%)
	Td (°C)	Δm (%)	Td (°C)	Δm (%)	Td (°C)	Δm (%)	Td (°C)	Δm (%)	
PEC	113.0.	7.0.	226.7	40.1	283.4	20.5			32.4
OFI	101.7	12.3	294.0	33.2	442.3	18.4	698.3	3.7	32.4
MMT	81.7	2.1	646.6	4.4					93.4
EXT	185.9	40.6	250.8	25.8					33.6

n.d. – not determined

In commercial pectin, the second stage, occurred around 226.7 °C, with a mass loss of 40.1 %. This is attributed to depolymerization and decarboxylation of pectin backbone (W. Zhang et al., 2022). The third and final stage (275-625 °C) reflected the carbonization of the residual organic matrix that led to a final residue of 32.4%. The OFI pectin displayed a wider thermal degradation interval, with the main event occurring at 294 °C, with a mass loss of 40.1 %. This shift, compared to commercial pectin, indicates a more structural complexity and mineral content possibly due to its lower degree of esterification, which conferred increased thermal resistance (Coqueiro et al., 2025; Van Rooyen et al., 2024). A final residue of 32.4 % was obtained, similarly to commercial pectin. MMT exhibited an excellent thermal stability, showing a minor weight loss (2.1%) around 81°C due to the desorption of water molecules and another event occurred around 646.6 °C that is attributed to dehydroxylation of nanoclay layers (H. Jiang et al., 2023). The inorganic nature of MMT is reflected in the final residue of 93.4%, showing a higher resistance to heat. The betalain-rich extract exhibited two main degradation steps. The first stage occurred around 91.82-232.79 °C, and reflected the major mass loss (40.6%), directly related to water evaporation and volatile organic compounds such as

sugars, phenolics and betalain pigments (Huang et al., 2022). The second stage occurred around 233.89-612.81 °C, linked to pyrolytic decomposition of aromatic and pigment structures (Z. Guo et al., 2021), that reflected a mass loss of 25.8 %, a final residue of 33.6 % was obtained.

The TGA curves of the OFIPC films (Table 5.9 and Figure 5.7-B) showed a multistage degradation profile, characteristic of complex biopolymeric matrices.

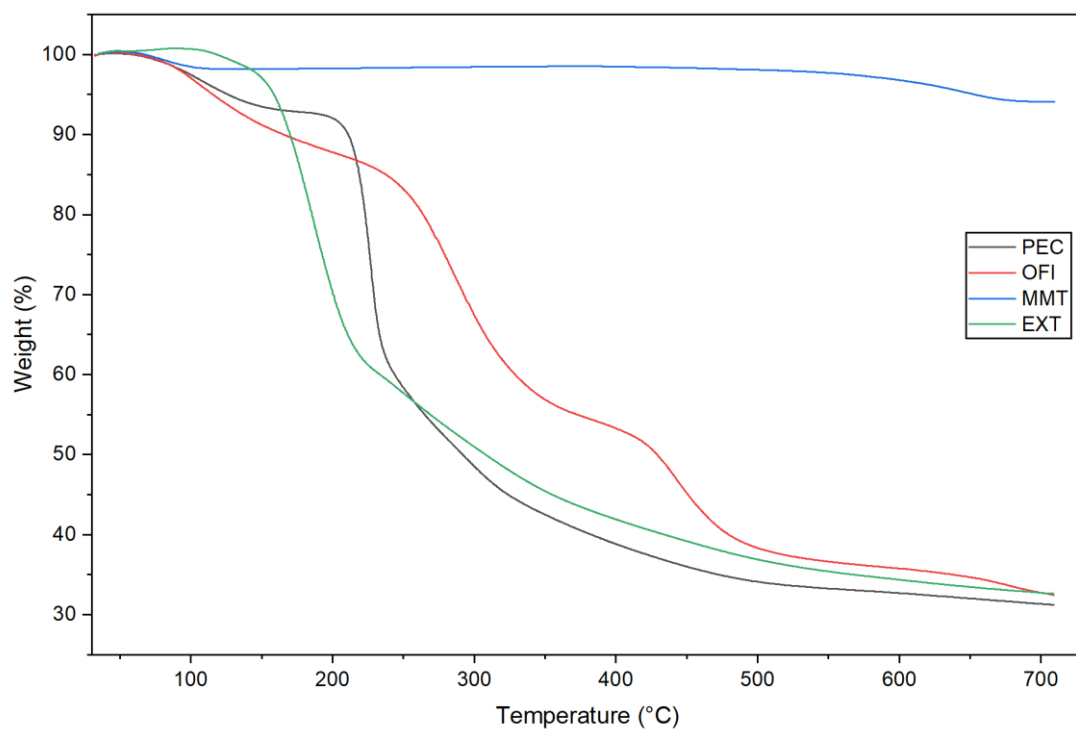
The 1st stage of weight loss in all films occurred around 86 to 146 °C, with weight losses ranging from around 10 to 16%. This phase corresponds to the evaporation of water, as well as the release of water retained within the polysaccharide matrix, and glycerol, according to typical thermal behavior in polysaccharide films (Halloub et al., 2023; S. Zhang et al., 2023).

The OFIPC film, showed its major degradation peaks at around 214–248 °C, corresponding to the depolymerization of the pectin backbone and scission of glycosidic linkages. The residual mass of 25 % reflected the formation of carbonaceous material characteristic of polysaccharide pyrolysis (Ezati & Rhim, 2020).

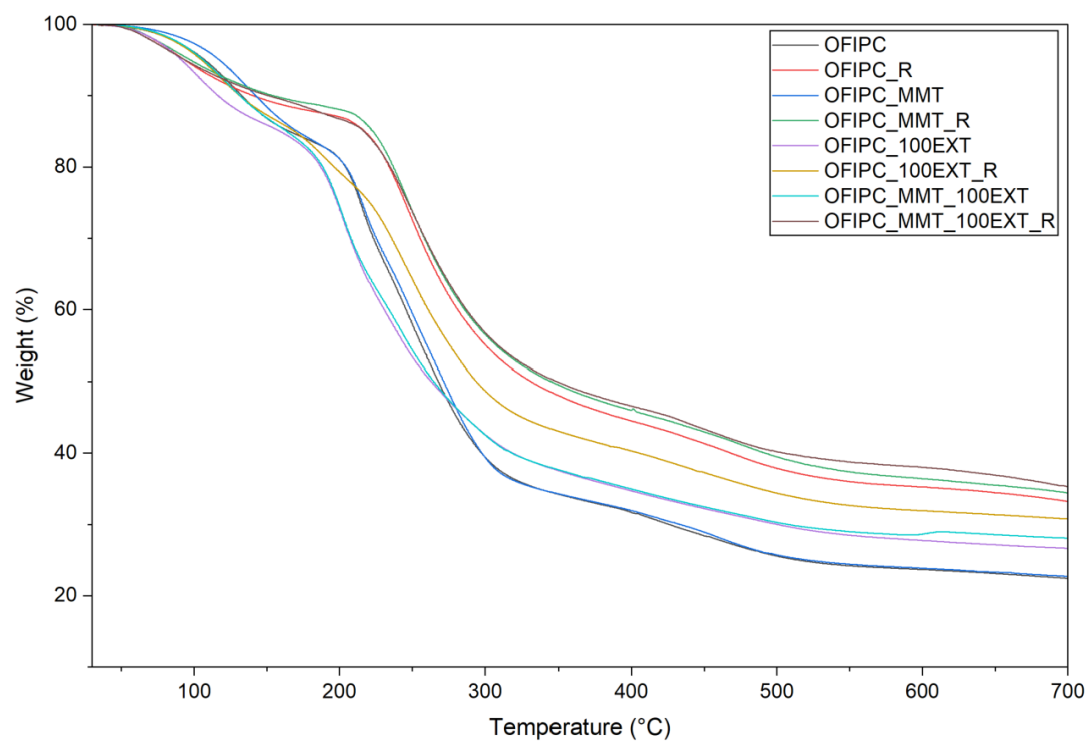
When calcium ions were introduced (OFIPC_R), the onset of degradation shifted toward higher temperatures, with the main decomposition occurring around 248.8 °C. This behavior demonstrates the stabilizing role of the Ca²⁺-induced “egg-box” crosslinks, which limit polymer chain mobility and promote a more compact structure (W. Zhang et al., 2022). As a result, the Ca-reticulated film yielded a larger amount of residue (35.5 %), indicating that crosslinking favours condensation and char formation during pyrolysis (H. Jiang et al., 2023; W. Zhang et al., 2022)

The addition of montmorillonite to the film’s matrix (OFIPC_MMT) produced a slight increase in the main decomposition temperature, to 216 °C, when compared to OFIPC film with a similar residue in the end (24.2%). This behavior can be attributed to the barrier and heat protective effects of the nanoclay, stabilizing the carbon matrix produced at elevated temperatures (H. Jiang et al., 2022; S. Zhang et al., 2023).

In OFIPC_MMT_R film, the stabilizing effect of nanoclay and reticulation became more evident. The main decomposition temperature was reached at 248.8 °C, with a mass loss of 42%, and the resulting residue in the end was 37%. The combined presence of the ionic crosslinking network and the inorganic reinforcement enhanced the cohesive character of the film and promoted the development of a dense, thermally robust carbonaceous structure (H. Jiang et al., 2023; S. Zhang et al., 2023).



A



B

Figure 5.7 - TGA curves of the individual components (A) and of OFIPC films with MMT, extract addition, and Reticulation effect (B).

Film with extract addition (OFIPC_100EXT) showed a decomposition at lower temperatures (148.21 – 233.20 °C) most likely due to the volatilization of low molecular-weight compounds, pigments, and sugars present in the extract (Huang et al., 2022). Despite this reduction in thermal stability, the final residue was of 37.2 %.

When the film containing extract was crosslinked (OFIPC_100EXT_R), a higher thermal stability was observed when compared to OFIPC_100EXT film. The main decomposition event shifted up to 205.18-354.47 °C with a mass loss of 35.6%, and a final residue around 32%. This improvement results from the effect of the ionic crosslinking, which enhanced the cohesion of the pectin chains by strengthening the film structure, thereby restricting chain mobility and promoting the formation of a more stable carbonaceous phase (W. Zhang et al., 2022).

The combination of MMT and betalain-rich extract powder into the film matrix resulted in intermediate behavior, with the main decomposition temperature around 241.1 °C (Δm - 35.6%), and a residue of 31.9%. The nanoclay partially counteracted the extract-induced destabilization by providing a tortuous diffusion path and acting as a framework for char formation (H. Jiang et al., 2022).

The OFIPC_MMT_100EXT_R presented the highest decomposition temperature (254.5 °C) and the largest final residue (38.6%), indicating a synergic interaction between all the components of the film (Arora & Saini, 2025; H. Jiang et al., 2023). The formed film structure seemed to restrict the release of components, leading to a delay in thermal degradation.

Table 5.9 -Thermal degradation parameters obtained by Thermogravimetric Analysis (TGA) OFIPC films with MMT, extract addition, and Reticulation effect.

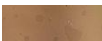
	$\Delta 1$		$\Delta 2$		$\Delta 3$		$\Delta 4$		Residue (%)
	Td (°C)	Δm (%)	Td (°C)	Δm (%)	Td (°C)	Δm (%)	Td (°C)	Δm (%)	
OFIPC	130.4	15.7	214.6	18.3	247.8	32.2	466.3	399.8	24.6
OFIPC_R	85.8	12.1	249.6	43.4	464.0	9.1	425.7		35.5
OFIPC_MMT	146.5	15.9	216.0	17.4	247.0	33.1	466.1	9.4	24.2
OFIPC_MMT_R	93.7	11.0	248.8	42.0	401.9	10.1	476.8		36.9
OFIPC_100EXT	103.4	13.8	204.6	26.9	233.3	22.0			37.2
OFIPC_100EXT_R	120.6	13.5	191.5	8.1	248.7	35.6	433.7	10.8	31.9
OFIPC_MMT_100EXT	122.9	13.6	202.0	24.1	241.1	32.9			29.5
OFIPC_MMT_100EXT_R	87.5	10.3	254.5	42.6	437.5	8.5	430.4		38.6

n.d. – not determined

5.3.8 Film's sensitivity to ammonia

The film's sensitivity to ammonia is particularly relevant to assessing the potential of the developed films to act as intelligent indicators, capable of changing color in response to volatile compounds released during food spoilage, such as ammonia. The colorimetric behavior of natural pigments under pH variation is a key property for intelligent/smart packaging (Alizadeh-Sani et al., 2020; Halloub et al., 2023). The sensitivity to ammonia was evaluated only in the films containing the highest concentration of extract (100% extract), as these samples exhibited the most intense initial coloration. The results of the test are presented in Table 5.10 showing the Hue Angle values and representative images of each film over 30 minutes of exposure.

Table 5.10 - Hue angle (h°) changes of films exposed to ammonia vapor for 30 min.

		Hue Angle (h°)									
		0	2	4	6	8	10	12	15	30	
OFIPC_	100EXT	15.1±0.9	28.9±0.2	44.3±2.1	46.0±4.	53.5±1.7	55.5±5.3	61.2±2.3 ^B	66.0±4.9 ^B	71.0±3.3 ^B	
		Cf	Ce	Bd	3Bd	Acđ	Bc	bc	ab	a	
											
OFIPC_	100EXT	16.2±0.8	25.6±0.1	39.5±3.4	41.5±1.	45.5±3.7	51.2±5.4	52.7±2.6 ^C	64.3±4.6 ^B	65.1±2.3 ^B	
		Ce	Dđ	Bc	9Bc	Bbc	Bb	b	a	Ca	
	_R										
OFIPC_	MMT_	24.8±0.7 ^B	40.6±1.2	46.4±2.6	48.3±3.	49.7±1.5	57.3±1.3	56.3±2.2 ^B	56.2±4.1 ^B	62.8±2.5	
		e	Bđ	Bcd	3Bc	Bbc	Bab	Cab	a	Ca	
	100EXT										
OFIPC_	MMT_	46.7±5.1	55.2±2.2	64.3±2.9	62.0±2.	n.d.		68.9±0.2	86.3±2.7 ^A	100.2±5.	103.7±0.
		8Ae	Ade	Acđ	0Ac			Ac	b	0Aa	5Aa
	100EXT					-					
	_R										

*A-C: values in the same column not sharing upper case superscript letters indicate significant differences among films ($p < 0.05$); a-f: values in the same row not sharing lower case superscript letters indicate significant differences among films ($p < 0.05$); n.d. – not determined.

The hue angle (h°) values of all films increased progressively upon exposure to ammonia vapor ($p < 0.05$), confirming their ability to change color due to betalain pigment reaction to alkaline conditions. At initial time (0 min), all films presented reddish-purple tones ($h^\circ \approx 15\text{--}46^\circ$), which gradually shifted towards yellowish hues with higher hue values, as exposure time increased, indicating color transition from red to orange/yellow associated with betalain degradation or conversion to betalamic derivatives at elevated pH. This change corresponds to the degradation of betacyanins into betalamic acid, a yellow derivative formed under basic conditions (H. Jiang et al., 2023; Qin et al., 2020; Yao et al., 2020b). The mechanism of NH_3 response involves the hydration and hydrolysis of ammonia molecules that generate hydroxyl ions and ammonium (NH_4^+), thus forming an alkaline environment on the film surface (Koop et al., 2024; Ma et al., 2017). Under these conditions, betalains undergo deprotonation and conversion of betacyanins to betalamic acid, resulting in the observed yellow hue (H. Jiang et al., 2023; Qin et al., 2020). In addition, studies have shown that relative humidity plays a significant role in accelerating the color transition, since higher water availability promotes the formation of OH^- ions and facilitates pigment transformation (J. Liu et al., 2019).

Among the films tested, OFIPC_MMT_100EXT_R exhibited the highest hue angle values throughout the exposure, reaching a h° value of 100° at 15 min, evidencing the strongest and fastest color response to ammonia. This suggests that the combination of MMT incorporation and crosslinking possibly enhanced pigment dispersion, facilitating the diffusion of ammonia molecules through the film. Jiang et al. (2023) similarly reported that pectin with MMT films and containing betacyanins showed enhanced ammonia sensitivity due to the improved permeability of volatile bases. The OFIPC_100EXT film, also showed a clear colour shift, reaching 71.0° after 30 minutes, indicating good responsiveness. However, even though a colour change was observed, the OFIPC_100EXT_R exhibited slightly lower final hue values (65.1°), which may be due to the denser crosslinked structure restricting ammonia penetration or limiting pigment mobility. The OFIPC_MMT_100EXT displayed higher initial hue angles compared with films without MMT, suggesting that MMT facilitated early pigment-environment interactions. Nevertheless, its final hue value (62.8°) was the lowest among all formulations, indicating that MMT alone may partially buffer or adsorb ammonia, slowing the overall colour transition, as also observed by other authors (Mang et al., 2025; Yao et al., 2020b).

The results obtained confirm that all tested films exhibit clear and rapid colour changes visible to the naked eye, validating their potential application as freshness indicators. These findings are consistent with the broader literature on ammonia-sensitive natural pigment

systems, reinforcing the suitability of betalain-based pectin films for real-time monitoring of food spoilage in intelligent packaging applications.

5.3.9 Food packaging application

The colorimetric response of the films was evaluated during salmon storage to assess their potential as visual indicators of fish freshness. Hue angle (h°) and total color difference (ΔE) were monitored over 72 h, and the results are presented in Table 5.11.

For the films without extract OFIPC, OFIPC_R, OFIPC_MMT, and OFIPC_MMT_R, only moderate fluctuations in hue angle were observed throughout the storage period. These films exhibited hue values in the yellow–green region, in a range from 63 to 100° , typical of pectin-based matrices without pigment addition (Yao et al., 2020b). Although the more relevant variations occurred particularly at 60–72 h, the overall ΔE values remained low up to 36 h, increasing afterwards, possibly due to minor color instability caused by matrix oxidation or moisture interaction rather than a response to volatile amines (Z. Guo et al., 2021).

Films containing extract (OFIPC_100EXT, OFIPC_100EXT_R, OFIPC_MMT_100EXT, and OFIPC_MMT_100EXT_R) exhibited pronounced and progressive color changes throughout the storage period. These films initially presented hue values in the red–purple range (between 31 and 46°), which gradually shifted towards yellowish tones (around 64 – 90°) with increasing storage time, corresponding to an evident visual color transition. The increase in ΔE values, especially after 48 h demonstrates the films' sensitivity to environmental changes induced by salmon spoilage. This behavior was consistent with previous reports where betalain-rich films displayed similar color transitions in response to fish and shrimp deterioration. Qin et al. (2020) observed that starch/PVA films containing red pitaya betalains changed from pink to yellow when the TVB-N of shrimp increased from 10 to 35 mg N/100 g, while Yao et al. (Yao et al., 2020b) reported a comparable purple-to-yellow transition in quaternary ammonium chitosan/PVA films with cactus pear extract as the TVB-N exceeded 20 mg N/100 g. Jiang et al. (2023) also confirmed this pH-driven transformation for pitaya peel pectin/MMT films, which turned from red to brown-yellow when the TVB-N of shrimp reached 38 mg N/100 g.

The reticulated films (OFIPC_100EXT_R and OFIPC_MMT_100EXT_R) showed the most marked response which can be observed through the final Hue angle values when compared to the other films with extract. Even though OFIPC_MMT_100EXT_R displayed a slightly slower initial response, possibly due to the more compact matrix structure that limits diffusion of ammonia and water molecules into the film at early stages, at 72h presented ΔE

values exceeding 30 when compared to initial values, indicating a very noticeable color change perceptible to the naked eye ($\Delta E > 3$) (Kurek et al., 2021). This strong variation can be attributed to the betalain and ammonia interaction resulting from the increase in total volatile basic nitrogen compounds and pH during spoilage (Abedi-Firoozjah et al., 2023). Betalains are known to undergo structural deprotonation and color transition from red–pink to yellow under alkaline conditions, serving as an efficient visual pH indicator (Abedi-Firoozjah et al., 2023). Similarly, Liu et al. (2023) demonstrated that diffusion of volatile ammonia into anthocyanin–betacyanin films induced an alkaline microenvironment, promoting structural transformations of the pigments responsible for the color shift. Moreover, Hu et al. (2020), reported that quaternary ammonium chitosan/fish gelatin films with higher pigment or crosslinking content exhibited delayed response to spoilage. Mang et al. (2025) also found that betalains encapsulated within microcrystalline cellulose diffused more slowly, leading to a gradual red-to-yellow transition in beef stored at 2–4 °C.

The OFIPC_100EXT and OFIPC_MMT_100EXT, showed significant differences ($p < 0.05$) in hue angle values at 36 h, but not that marked as the reticulated films. Also, the total difference color at the end was lower than the reticulated films. This could be explained by the less compact structure, allowing a faster volatile diffusion and betalain reaction. Similar trends have been reported for biopolymer indicator films, where higher permeability or more porous structures enhanced gas absorption and accelerated color response to ammonia (Halloub et al., 2023; H. Jiang et al., 2023; D. Liu et al., 2023).

Table 5.11 - Hue angle (h°) and total color difference (ΔE) of films during salmon storage over 72 h.

		0	12h	24h	36h	48h	60h	72h
OFIPC	h°	91.0±1.5 ^b	76.1±2.0 ^{de}	77.5±3.2 ^{cde}	83.9±1.4 ^c	81.4±2.3 ^{cd}	72.0±0.8 ^e	100.1±3.7 ^a
	ΔE	-	4.0±0.3 ^b	7.1±2.5 ^b	4.9±1.3 ^b	5.7±0.9 ^b	16.4±1.8 ^a	12.2±2.5 ^a
OFIPC_R	h°	78.1±0.0 ^c	82.8±1.0 ^b	82.4±0.9 ^b	77.7±1.2 ^{cd}	75.4±1.2 ^d	84.8±1.1 ^b	98.2±0.2 ^a
	ΔE	-	2.6±1.0 ^d	4.4±1.2 ^{cd}	2.5±1.3 ^d	6.6±1.6 ^c	18.7±0.4 ^a	12.2±0.9 ^b
OFIPC_MMT	h°	75.1±1.1 ^c	82.2±3.6 ^b	84.7±2.1 ^b	63.7±0.0 ^d	73.1±0.8 ^c	71.9±0.8 ^c	98.1±0.2 ^a
	ΔE	-	5.4±3.0 ^b	5.3±3.5 ^b	5.1±1.3 ^b	6.4±2.6 ^b	17.5±2.4 ^a	10.8±2.3 ^{ab}
OFIPC_MMT_R	h°	77.0±2.0 ^b	74.3±1.8 ^b	73.5±0.8 ^{bc}	90.9±1.7 ^a	67.6±1.9 ^c	78.4±4.9 ^b	93.5±0.2 ^a
	ΔE	-	5.1±0.9 ^b	5.2±1.0 ^b	4.0±0.4 ^b	3.5±0.7 ^b	11.6±0.3 ^a	5.3±0.1 ^b
OFIPC_100EXT	h°	31.0±1.0 ^e	34.9±0.7 ^d	36.2±0.8 ^d	40.2±1.1 ^c	39.7±2.0 ^c	47.4±0.3 ^b	64.1±1.2 ^a
	ΔE	-	6.5±3.4 ^b	4.2±1.9 ^b	6.6±0.4 ^b	6.8±0.6 ^b	18.7±1.1 ^a	17.3±0.8 ^a
OFIPC_100EXT_R	h°	34.4±4.4 ^d	37.8±1.2 ^d	38.6±0.8 ^d	40.9±1.5 ^d	54.2±4.0 ^c	69.3±1.3 ^b	90.8±0.3 ^a
	ΔE	-	7.0±3.1 ^c	7.7±4.6 ^c	7.9±4.1 ^c	11.5±2.5 ^c	23.0±3.7 ^b	32.5±1.5 ^a
OFIPC_MMT_100EXT	h°	33.9±3.9 ^d	36.5±2.3 ^d	38.6±2.5 ^d	39.5±2.2 ^d	49.5±1.6 ^c	61.7±2.3 ^b	72.4±1.5 ^a
	ΔE	-	4.1±1.7 ^b	5.4±1.6 ^b	5.2±3.5 ^b	9.1±1.3 ^b	19.8±0.9 ^a	19.3±2.0 ^a
OFIPC_MMT_100EXT_R	h°	45.6±3.4 ^e	45.4±0.4 ^e	45.9±1.6 ^e	61.6±1.2 ^d	70.2±1.4 ^c	77.8±0.5 ^b	90.2±0.2 ^a
	ΔE	-	7.6±1.4 ^b	6.3±2.5 ^b	9.2±1.5 ^b	10.9±2.4 ^b	19.8±1.4 ^a	17.3±0.8 ^a

*a-e: values in the same row not sharing lower case superscript letters indicate significant differences among films ($p < 0.05$);

The color changes of the films were also accompanied by the measurement of total volatile basic nitrogen (TVB-N) and pH of the salmon samples, as it is demonstrated in Figure 5.8.

A progressive increase in both parameters was observed, confirming the chemical and microbiological deterioration of the salmon. The initial pH of fresh salmon was around 6.2 and as storage time progressed, the pH increased significantly ($p < 0.05$), reaching a value of 8.20 ± 0.02 after 72 h. This alkalization is associated with the accumulation of basic nitrogenous compounds such as ammonia, and amines as degradation advances produced by microbial and enzymatic degradation of proteins and non-protein nitrogen compounds (Halloub et al., 2023; D. Liu et al., 2023; Qin et al., 2020). Similarly, TVB-N values remained low and stable during the first 24–36 h of storage, indicating minimal spoilage, but showed a sharp rise after 48 h, reaching a value of 270.28 ± 20.18 mg N/100 g at 72 h.

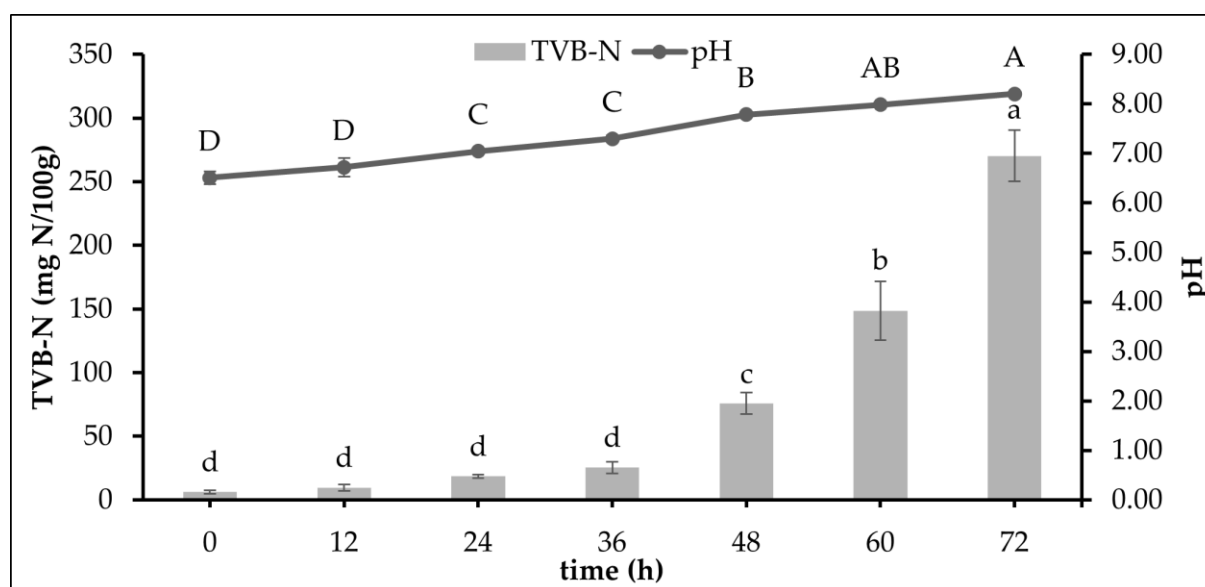


Figure 5.8 - Evolution of pH and total volatile basic nitrogen (TVB-N) values of salmon during 72h of storage at room temperature. Different uppercase letters (A–D) indicate significant differences in pH values, and different lowercase letters (a–d) indicate significant differences in TVB-N values between storage times ($p < 0.05$).

The pH and TVB-N evolution exhibited a clear correlation with the color transitions of the films containing betalain-rich extract powder. During the initial 24–36 h, when both parameters remained relatively low, the hue angle (h°) and ΔE values showed minimal variation, indicating that the surrounding environment was still within the acidic to near-neutral range where betalains are stable and retain their reddish coloration. After 48 h, as the pH of the salmon rose above 7.0 and volatile amines accumulated, a marked increase in ΔE and a shift

of h° toward higher values were recorded for the pigmented films, particularly OFIPC_100EXT_R and OFIPC_MMT_100EXT_R. According to European Commission legislation, fish products of the *Salmo salar* species are considered fresh and appropriate for consumption within a limit of TVB-N of 35 mg N/100g of muscular tissue (European Commission, 2005). In this work, the limit was surpassed between the 36 and 48 hours of experiment which was coincident with a more visible change of color in films with extract. A similar relationship between regulatory thresholds and visual response was reported by Yao et al. (2020) and Qin et al. (2020) for shrimp, as well as by Guo et al. (2021) for beef stored under refrigeration, where color transition occurred near the 20 mg N/100 g limit established by the Chinese standards.

This behavior confirms that the films' color change was triggered by the release of basic volatile compounds, primarily ammonia, in agreement with the observed physicochemical degradation of salmon. The betalain pigments, sensitive to pH changes, underwent structural deprotonation and conversion from betacyanins (red–purple) to betaxanthins (yellow–orange), providing a visual and quantifiable signal of freshness loss. Therefore, the good agreement between TVB-N, pH, and color parameters demonstrates that the developed films effectively translate the chemical spoilage of salmon into a clear visual indicator, reinforcing their suitability as intelligent freshness sensors for perishable food products.

Overall, the betalain-rich OFIPC films, particularly OFIPC_100EXT_R and OFIPC_MMT_100EXT_R, demonstrated strong potential as intelligent indicators for salmon freshness, capable of visually signaling deterioration through distinct and quantifiable color changes correlated with spoilage progression. These results are in line with other betalain-based films applied to fish and meat products (Z. Guo et al., 2021; Halloub et al., 2023; H. Jiang et al., 2023; Mang et al., 2025), confirming betalains' reliability and environmental safety as natural pigments for smart and active food packaging.

5.4 Conclusions

This chapter addressed the development and characterization of pectin-based composite films with incorporation of betalain-rich extract powder from prickly pear peel, montmorillonite reinforcement and calcium crosslinking, aiming their application as food packaging material. This study investigated how the combined effects of the film formulation influenced the films' optical, mechanical, barrier, thermal, and morphological properties. In addition, the films' responsiveness to ammonia and their performance as indicator for salmon freshness

were also evaluated. Overall, the results provided valuable insights into the relation between composition, structure, and functional behavior, while demonstrating the potential of *Opuntia*-derived by-products as sustainable raw materials for the production of environmentally friendly intelligent/smart packaging systems.

Betalain-rich extracts from *Opuntia ficus-indica* peels were efficiently recovered by both agitation and ultrasound methods, yielding comparable total phenolics, antioxidant capacity, and pigment composition. The extracts exhibited high chromatic stability between pH 2 and 7, with pronounced hue shifts under alkaline conditions attributable to betacyanin deprotonation/degradation and increasing relative contributions of betaxanthins. This well-defined pH responsiveness reinforces their suitability as colorimetric indicators for basic volatile nitrogenous compounds generated during fish spoilage.

When incorporated into pectin matrices, the extracts influenced optical, mechanical, and barrier performance with effects varying according to formulation.. Increasing extract content intensified chroma and enhanced UV–Vis attenuation, particularly near the betacyanin absorption region, while preserving sufficient transparency for product visibility. Calcium-induced ionic crosslinking systematically increased stiffness and reduced extensibility, whereas extract addition acted plastically in non-reticulated films; MMT alone did not significantly reinforce at the studied loading, probably due to the low concentration used, but partly mitigated extract-induced mechanical weakening. Film thickness scaled with extract solids, as expected.

Water and film's interactions were strongly governed by network densification (Ca^{2+} reticulation) and interactions between polymer and MMT. These factors collectively decreased moisture content, solubility, and water vapour permeability, with the best results achieved with values around $1.8\text{--}2.5 \times 10^{-11}$ g/m.s.Pa. These values are competitive with or superior to several biopolymers found in the literature. Contact angles remained within a moderate hydrophilicity range and were only poorly affected by MMT. FTIR data supported predominantly physical interactions (hydrogen bonding/dispersion) for extract and MMT with spectral signatures consistent with Ca^{2+} crosslinking. SEM micrographs corroborated a transition from homogenous morphology of PC films to a more heterogeneous morphology in OFIPC films and, upon reticulation, denser, less porous networks, aligning with the observed mechanical and barrier results obtained.

The films with the highest percentage of extract added displayed changes in terms of color upon ammonia exposure, confirming sensitivity to changes from acid to alkaline

environments. In terms of food packaging application of the films in salmon storage, the films exhibited progressive and visually perceptible color transitions, that correlated with the increase in pH of TVB-N values beyond the regulatory freshness limits, effectively demonstrating the spoilage process into a visual response. Among the tested formulations, the OFIPC_MMT_100EXT_R film presented the most balanced performance, combining improved barrier efficiency, suitable optical visibility, and a rapid yet distinct color response to spoilage progression.

Altogether, these findings demonstrate that OFIPC films with betalain-rich powder derived from *Opuntia ficus-indica* by-products constitute a promising and sustainable alternative for intelligent food packaging applications. The clear relation observed between film composition, microstructural organization, and functional performance highlights the effectiveness of combining ionic crosslinking and montmorillonite reinforcement to modulate mechanical strength, barrier efficiency, and colorimetric sensitivity. The developed films exhibited a rapid and visually distinct response to volatile amines produced during salmon storage, confirming their potential to be used as freshness indicators.

Overall, this work establishes a strong foundation for the valorization of *Opuntia* residues in the development of biodegradable, functionally active packaging materials. Future research should focus on improvement of mechanical and barrier properties, and assess long-term stability, safety and performance of these biobased materials.

GENERAL CONCLUSIONS

This Ph.D. work explored the valorization of *Opuntia ficus-indica* residues, more specifically cladodes and fruit peels, as raw materials for the development of sustainable and functional biopolymer films for food packaging applications. Through a sequence of complementary studies, it advanced from literature review and fundamental characterization to experimental extraction, material development, and application testing. The overall goal was to demonstrate that *Opuntia*-derived polysaccharides and pigments can serve as renewable sources for producing environmentally friendly packaging systems capable of responding to food spoilage through visible color changes.

The literature review (Chapter 2) consolidated existing knowledge on *Opuntia ficus-indica*, a cactus widely adapted to arid environments and abundant in regions affected by water scarcity. The review highlighted the underexploited potential of this species as a source of bioactive compounds, pectins, and pigments such as betacyanins and betaxanthins. The chapter also established the conceptual and technological basis of active and intelligent packaging. It reviewed the mechanisms of colorimetric indicators and the application of natural pigments, such as anthocyanins and betalains, for freshness monitoring. This overview revealed important research gaps related to (i) the limited exploration of *Opuntia*-derived materials for packaging, (ii) the instability of betalains under environmental stresses, and (iii) the challenge of integrating natural pigments and biopolymers into functional materials without synthetic additives. These gaps guided the experimental design of the subsequent chapters, forming a logical pathway from raw material selection to intelligent/smart film development.

As an initial step toward understanding the behavior of natural polysaccharides in food contact applications, Chapter 3 evaluated polysaccharide-based edible coatings for the postharvest preservation of prickly pear fruits. Coatings prepared with alginate, starch,

chitosan, and pectin were applied to two prickly pear varieties and assessed during refrigerated storage. The results showed that the coatings effectively delayed physicochemical degradation, particularly by reducing weight loss and maintaining color, firmness, and visual quality. Microbiological analysis revealed reduced fungal and bacterial growth in coated fruits, confirming that such polysaccharide matrices can act as moisture and gas barriers. Among the formulations, pectin-based coatings displayed a balanced performance, combining flexibility and compatibility with the fruit surface. This preliminary study provided crucial insight into the interfacial behavior of biopolymer coatings and validated their role in food preservation. It also reinforced the potential of using pectin, especially when derived from renewable sources like *Opuntia*, as a primary matrix for further film development, setting the stage for the more advanced material studies.

Pectin was extracted from *Opuntia ficus-indica* cladodes through an optimized process (Chapter 4). Response Surface Methodology (RSM) was applied to evaluate the effects of pH, temperature, and liquid-to-solid ratio on extraction yield and quality parameters. The optimized conditions led to the recovery of a pectin rich in galacturonic acid and with a low degree of esterification, classifying it as low-methoxyl pectin (LM), suitable for gelation and film formation. The study also characterized the structural and compositional differences between pectins extracted from cladode peel and pulp, contributing to a deeper understanding of *Opuntia* polysaccharides. The results validated the use of cladode residues as an alternative, low-cost source of functional pectin, supporting circular bioeconomy strategies and waste valorization.

Based on the previous findings, Chapter 5 focused on the preparation and characterization of composite films composed of *Opuntia* pectin blend with Commercial Pectin, montmorillonite (MMT), and betalain-rich extracts obtained from prickly pear fruit peels. The research explored the synergistic effects of nanoclay addition, extract incorporation, and calcium crosslinking on the films' structural, optical, and barrier properties. The inclusion of MMT improved the compactness of the polymeric network, leading to increased tensile strength and reduced water vapor permeability, even though the changes were not always statistically significant. The addition of betalain-rich extracts provide color to the films and pH-sensitive behavior, while the reticulation process contributed to structural stability and decreased solubility. The films were evaluated as colorimetric indicators in contact with volatile compounds released during salmon spoilage. The betalain-containing films showed a rapid and irreversible color change from pink to yellow in the presence of ammonia and total volatile basic

nitrogen (TVB-N), confirming their suitability as freshness indicators. Among all formulations, OFIPC_MMT_100EXT_R appeared to exhibit the best overall performance, combining mechanical strength, low permeability, and fast chromatic response.

This study represents an important advance in the sustainable use of *Opuntia ficus-indica* residues for intelligent packaging, while highlighting key areas for further research. These include refining extraction and structural characterization methods, improving betalain stability, enhancing film durability and functionality, and evaluating biodegradability and scalability. These aspects, and other potential research prospects, will be further explored in the next chapter dedicated to future perspectives.

Overall, the results presented in this thesis highlight that *Opuntia ficus-indica* by-products can be transformed into value-added materials suitable for intelligent food packaging applications that support sustainability goals and promote waste valorization. From extraction to application, each experimental stage contributed to a deeper understanding of how composition and structure influence functionality in natural polymer systems. The outcomes highlight the potential of this underutilized crop to contribute to the transition from fossil-based plastics toward bio-based, intelligent packaging systems that enhance food quality, safety, and environmental responsibility. From a socio-economic perspective, valorizing *Opuntia* residues could also benefit agricultural regions where the cactus is abundant, promoting local bioeconomies and rural innovation. By turning agricultural waste into advanced materials, this approach contributes to multiple Sustainable Development Goals (SDGs), including responsible consumption and production (SDG 12), industry and innovation (SDG 9), and climate action (SDG 13).

FUTURE WORK

This work has demonstrated the potential of *Opuntia ficus-indica* by-products for the development of intelligent packaging. However, several research directions remain open for exploration. Future research should focus on deepening the understanding of the structure–function relationships of *Opuntia* polysaccharides and betalains, as well as on assessing long-term stability, scalability, and environmental behavior.

The extraction of pectin from *Opuntia ficus-indica* cladodes in this work was carried out through a conventional acid-assisted process optimized by response surface methodology. Although the method produced low-methoxyl pectin, further refinement of extraction strategies is needed to improve efficiency, reduce environmental impact, and tailor pectin properties to specific applications. Future studies should explore non-conventional and greener extraction techniques, such as ultrasound-assisted, microwave-assisted, enzyme-assisted, or pressurized water extraction. These methods can enhance yield, shorten processing time, and minimize solvent and energy consumption while preserving the polymer’s native structure. Comprehensive structural and physicochemical characterization of *Opuntia*-derived polysaccharides is also crucial for understanding how molecular structure affects material properties. Future research should employ advanced analytical techniques such as NMR spectroscopy, size-exclusion chromatography, or rheology to investigate molecular weight, branching degree, and interaction with other polymers or fillers. This deeper knowledge would support future design of formulations that balance mechanical resistance, flexibility, and barrier performance according to the intended packaging function.

The combination of *Opuntia* pectin with other biopolymers (starch, chitosan, alginate) or reinforcing agents (nanocellulose or lignin nanoparticles) represents a promising strategy to improve mechanical properties and reduce hydrophilicity. Moreover, hydrophobic surface

treatments or multilayer configurations could increase moisture resistance and extend film durability under real storage conditions. The formulation of bilayer or multilayer systems, in which the sensing and structural layers are separated, would also allow independent optimization of mechanical stability and colorimetric responsiveness.

Studies on the stability of betalains is another essential area for progress. Since these pigments are sensitive to light, pH, and temperature, encapsulation or co-pigmentation strategies should be explored to enhance their color retention and functionality. Embedding betalains into natural carriers could mitigate degradation and increase the films' shelf life. Systematic stability studies under variable environmental conditions will be essential to validate the performance of the colorimetric response over time.

Additionally, evaluating the reversibility and reusability of the bio-based films with betalain-rich extract could open new possibilities for their use in reusable or multi-cycle freshness indicators. Complementary studies on biodegradability, compostability, and environmental safety under realistic conditions should also be undertaken to confirm the ecological benefits of these films and ensure their alignment with sustainable packaging requirements.

Finally, future efforts should aim to integrate both active and intelligent functionalities within the same system, creating fully smart packaging materials capable of extending food shelf life while monitoring freshness in real time. Since this study assessed only the sensing behavior of the films, future research should aim to evaluate their protective effect in food preservation. To achieve industrial relevance, further work should also address scaling-up challenges, including the adaptation of film casting or extrusion techniques, and assess food safety and migration behavior in accordance with regulatory standards. Performing life-cycle assessments (LCA) will be crucial to quantify environmental advantages and guide sustainable production pathways.

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DEVELOPMENT OF BIOPOLYMER FILMS FROM *OPUNTIA* SPP.
MUCILAGE FOR FOOD PACKAGING APPLICATION

