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TESIS DOCTORAL:

Pilot-scale biorefinery of agri-food residual biomass oriented to valuable bioproducts

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Hemos modificado tan radicalmente nuestro entorno que ahora debemos modificarnos a nosotros mismos para poder existir dentro de él.

Norbert Wiener

CONTENTS

Abstract ..... I-VI
Introduction 1
1. Environmental problems 1
1.1. Dependence on fossil sources 1
1.2. Plastic 1
1.3. Solutions and alternative sources for the production of products 2
2. Biorefinery of lignocellulosics 5
2.1. Implementation of the biorefinery concept 5
2.2. Cellulose 6
2.3. Hemicelluloses 8
2.4. Pectins 11
2.5. Lignin 12
2.6. Extractives 13
3. Biorefinery processes 15
3.1. Separation of extractives 15
3.2. Fermentation of free sugars 15
3.3. Formulation of bioactive extractives 15
3.4. Extraction of biopolymers 16
3.5. Concentration, separation, and purification of biopolymers 18
3.6. Drying of biopolymers 22
3.7. Characterization of biopolymers 23
3.8. Biopolymer transformation for specific applications 23
4. Agricultural and agri-food waste 25
4.1. The problem of agricultural and agri-food waste 25
4.2. Agricultural and agri-food waste in Castilla y León region 27
4.3. Case studies: biomass used in this thesis 28
5. Spent coffee grounds 29
5.1. Spent coffee grounds as residue 29
5.2. Spent coffee oil extraction 30
5.3. Spent coffee oil valorization 32
5.4. Valorization of the solid 33

6. Discarded carrots 36
6.1. Discarded carrots as residue 36
6.2. Discarded carrots in Castilla y León region 38
6.3. Separation of juice and pulp 39
6.4. Valorization of the juice 40
6.5. Valorization of the pulp 44
7. References 58
Aims and contents 77
Chapter 1. Production of molecular weight fractionated hemicelluloses hydrolyzates from spent coffee grounds combining hydrothermal extraction and a multistep ultrafiltration/diafiltration 81
1. Introduction 81
2. Materials and methods 86
3. Results and discussion 92
4. Conclusions 107
5. Acknowledgments 108
6. References 108
7. Supplementary material 114
Chapter 2. Hydrothermal production of high-molecular weight hemicellulose-pectin, free sugars and residual cellulose pulp from discarded carrots 116
1. Introduction 116
2. Materials and methods 120
3. Results and discussion 123
4. Conclusions 139
5. Acknowledgments 139
6. References 139
7. Supplementary material 146
Chapter 3. Biorefinery of discarded carrot juice to produce carotenoids and fermentation products 147
1. Introduction 147
2. Materials and methods 151
3. Results and discussion 156
4. Conclusions 172
5. Acknowledgments 173
6. References 173
7. Supplementary material 178

Chapter 4. Production of purified hemicellulose-pectin fractions of different molecular weight from discarded carrots by hydrothermal treatment followed by multistep ultrafiltration/diafiltration 179

1. Introduction 179

2. Materials and methods 184

3. Results and discussion 189

4. Conclusions 212

5. Acknowledgments 213

6. References 213

7. Supplementary material 217

Chapter 5. Pilot-scale biorefinery for the production of purified biopolymers based on hydrothermal treatment in flow-through reactor cycles 219

1. Introduction 219

2. Materials and methods 224

3. Results and discussion 233

4. Conclusions 256

5. Acknowledgments 257

6. References 257

7. Supplementary material 261

Chapter 6. Biofilms from purified hemicellulose and pectin fractions and residual pulp from hydrothermally treated discarded carrots 263

1. Introduction 263

2. Materials and methods 271

3. Results and discussion 276

4. Conclusions 297

5. Acknowledgments 298

6. References 298

7. Supplementary material 303

Conclusions 305

Resumen R1-R32

Acknowledgments A1-A2

About the author B1-B4

CONTENTS

Resumen (inglés) ..... I-VI
Introducción 1
1. Problemas medioambientales 1
1.1. Dependencia de fuentes fósiles 1
1.2. Plástico 1
1.3. Soluciones y fuentes alternativas para la producción de productos 2
2. Biorrefinería de biomasa lignocelulósica 5
2.1. Implementación del concepto de biorrefinería 5
2.2. Celulosa 6
2.3. Hemicelulosas 8
2.4. Pectinas 11
2.5. Lignina 12
2.6. Extractivos 13
3. Procesos de biorrefinería 15
3.1. Separación de extractivos 15
3.2. Fermentación de azúcares libres 15
3.3. Formulación de extractivos bioactivos 15
3.4. Extracción de biopolímeros 16
3.5. Concentración, separación y purificación de biopolímeros 18
3.6. Secado de biopolímeros 22
3.7. Caracterización de biopolímeros 23
3.8. Transformación de biopolímeros para aplicaciones específicas 23
4. Residuo agrícola y agroalimentario 25
4.1. El problema del residuo agrícola y agroalimentario 25
4.2. Residuo agrícola y agroalimentario en Castilla y León 27
4.3. Casos de estudio: biomasas utilizadas en esta tesis 28
5. Marro de café 29
5.1. Marro de café como residuo 29
5.2. Extracción del aceite del marro de café 30
5.3. Valorización del aceite del marro de café 32
5.4. Valorización del sólido 33

6. Zanahorias descartadas 36
6.1. Zanahorias descartadas como residuo 36
6.2. Zanahorias descartadas en Castilla y León 38
6.3. Separación del zumo y la pulpa 39
6.4. Valorización del zumo 40
6.5. Valorización de la pulpa 44
7. Referencias 58
Objetivos y contenidos 77
Capítulo 1. Producción de hidrolizados de hemicelulosas fraccionados en función del peso molecular a partir de marro de café combinando extracción hidrotermal y ultrafiltración/diafiltración multietapa 81
1. Introducción 81
2. Materiales y métodos 86
3. Resultados y discusión 92
4. Conclusiones 107
5. Agradecimientos 108
6. Referencias 108
7. Material suplementario 114
Capítulo 2. Producción hidrotermal de hemicelulosa-pectina de alto peso molecular, azúcares libres y pulpa residual celulósica a partir de zanahorias descartadas 116
1. Introducción 116
2. Materiales y métodos 120
3. Resultados y discusión 123
4. Conclusiones 139
5. Agradecimientos 139
6. Referencias 139
7. Material suplementario 146
Capítulo 3. Biorrefinería de zumo de zanahorias descartadas para producir carotenoides y productos de fermentación 147
1. Introducción 147
2. Materiales y métodos 151
3. Resultados y discusión 156
4. Conclusiones 172
5. Agradecimientos 173
6. Referencias 173
7. Material suplementario 178

Capítulo 4. Producción de fracciones purificadas de hemicelulosa-pectina de distinto peso molecular a partir de zanahorias descartadas mediante tratamiento hidrotermal seguido por ultrafiltración/diafiltración multietapa 179

1. Introducción 179
2. Materiales y métodos 184
3. Resultados y discusión 189
4. Conclusiones 212
5. Agradecimientos 213
6. Referencias 213
7. Material suplementario 217

Capítulo 5. Biorrefinería a escala piloto para la producción de biopolímeros purificados mediante tratamiento hidrotermal en ciclos de reactores flow-through 219

1. Introducción 219
2. Materiales y métodos 224
3. Resultados y discusión 233
4. Conclusiones 256
5. Agradecimientos 257
6. Referencias 257
7. Material suplementario 261

Capítulo 6. Biofilms a partir de fracciones purificadas de hemicelulosa y pectina y de pulpa residual provenientes de zanahorias descartadas sometidas a tratamiento hidrotermal 263

1. Introducción 263
2. Materiales y métodos 271
3. Resultados y discusión 276
4. Conclusiones 297
5. Agradecimientos 298
6. Referencias 298
7. Material suplementario 303

Conclusiones 305

Resumen R1-R32

Agradecimientos A1-A2

Sobre la autora B1-B4

CONTENIDOS

Abstract

Our society is based on a series of products dependent on crude oil. This model is problematic and unsustainable for three main reasons: (1) the clear depletion of fossil resources, (2) the environmental problems arising from their use as raw material and fuel, and (3) the increasing population that demands more and more products and energy.

Among the collateral problems are the dumping of plastics and the global warming related to the emission of greenhouse gases. Plastic is one of the most widely produced materials worldwide due to its application in all industrial sectors. It is a material that stands out for its excellent barrier, mechanical and technical properties, making it very versatile. The main problem with plastic is that it is produced from non-renewable fossil fuels and is not biodegradable, which leads to its accumulation in the environment causing problems for living beings. As an alternative to the use of fossil fuels, the use of renewable sources such as lignocellulosic biomass stands out. Lignocellulosic biomass from vegetable waste is an abundant and inexpensive material containing interesting compounds. The biorefinery concept is equivalent to that of a traditional refinery, but instead of starting from fossil sources, lignocellulosic biomass is used. High value-added products are produced applying fractionation and several purification and transformation processes. The processes applied in biorefineries aim to be environmentally friendly, giving rise to a production model that fits in with the concept of circular economy and sustainability.

The most abundant components of lignocellulosic biomass are the extractives (non-structural components) and the structural biopolymers, namely cellulose, hemicellulose, pectin, and lignin. All biomass components can be valorized and transformed through biorefinery processes. Extractives are first extracted and can be formulated if they are bioactive compounds of interest, or they can be transformed into other compounds by e.g., biotechnology. Structural biopolymers can be extracted in their polymeric form from biomass and used as substitutes for traditional polymers from fossil sources. Once biopolymers are extracted, separated, and purified, if their properties are suitable, they can lead to high value-added products such as bioplastics. Bioplastics are biodegradable materials from biomass that have similar properties to

plastics and are therefore potential substitutes with enormous environmental and economic advantages.

Agricultural and agri-food residues are among the most abundant lignocellulosic biomass residues worldwide. They represent a double problem as they imply an economic loss for producers and are also an environmental issue due to their difficult management. These wastes are often characterized by a high moisture content, which makes them more expensive to transport and accelerates their degradation. Incineration of this waste is discarded because the moisture content makes it inefficient. It is usual for a small proportion of the waste to be used as animal feed and the majority ends up in landfill sites, causing environmental problems in the area. The use as raw material in biorefinery is attractive due to the compounds of interest that this type of waste contains and because they are easily harvested as they are located in specific places (cultivation land, processing plant, etc.). The biomass used in this thesis was the agri-food residue (spent coffee grounds) and the agriculture/agri-food residue (discarded carrots).

This thesis demonstrates that a pilot-scale biorefinery can be applied to agricultural and agri-food residues to produce valuable products. We have applied hydrothermal treatment in a multi-reactor system with downstream processes such as ultrafiltration/diafiltration, spray drying, encapsulation, and fermentation. As a result, several intermediate products have been obtained, e.g., carotenoids, sugars, hemicellulose, pectin and lignin-containing cellulose nanofibers. These intermediates have been successfully used to produce biofilms, encapsulated pigments, and fermentation products (lactic acid and ethanol).

In Chapter 1, the valorization of spent coffee grounds on a laboratory scale was studied by applying environmentally friendly processes. The first step was to extract the oil, as it is the most abundant extractive. The extraction was performed with supercritical CO_2, which is a cheap and non-toxic compound compared to the usual organic solvents. The extracted oil was characterized for potential applications in the food, cosmetic and pharmaceutical industries. The defatted solid was subjected to hydrothermal treatment in a flow-through reactor system for the extraction of the hemicellulose biopolymer, leaving the cellulose and lignin biopolymers in the solid. The extraction of hemicelluloses

was studied over time operating at 140 and 160 °C. The hydrothermal extracts were characterized by hemicelluloses with a wide molecular weight distribution and some by-products such as free sugars, organic acids, and degradation compounds. Therefore, the extracts were subjected to a concentration, separation, and purification process through multistage ultrafiltration and diafiltration using membranes of different molecular weight cut-off (30, 10 and 5 kDa). This process allowed the fractionation of each extract into three liquid products. Certain groups of hemicelluloses were concentrated by a factor of up to 5 with respect to the concentration of the extract. The diafiltration system allowed to purify the hemicelluloses reducing the retention of by-products from 45.6 % to 8.7 %. In total, six hemicellulose fractions were obtained with purities ranging from 83.7-97.8 % and molecular weights from 1641 to 49,733 Da.

In Chapter 2, the lignocellulosic biomass used was discarded carrots, which accounted for 30 % of the total carrots harvested. Due to their high moisture content of around 95 %, the first step was the separation of juice and pulp. Most of the extractives from the carrot went into the juice, and this Chapter focused on the valorization of the pulp. The pulp was subjected to hydrothermal treatment for the extraction of the biopolymers hemicellulose and pectin, leaving the cellulose and lignin in the solid. The extraction was performed in a flow-through reactor system operating at 140, 160 and 180 °C. The extracted components reached 211 g/kg dry pulp of free sugars, 29.13 g/kg dry pulp of homogalacturonan pectin, and 70.45 g/kg dry pulp of arabinogalactan hemicellulose. The residual pulp had a majority cellulose content (57.5 %) followed by a lignin content of 15.7 %. It is therefore an interesting material for applications where cellulose is used while presenting the advantages of lignin, such as hydrophobicity. The liquid extracts were characterized by a low biopolymer purity (high content of free sugars) and a wide molecular weight distribution. Consequently, it is interesting to subject these extracts to conditioning for the utilization of the extracted biopolymers, which was developed in Chapter 4.

Chapter 3 focused on the valorization of the juice of discarded carrots, complementing Chapter 2, which focused on the valorization of the pulp. The juice was obtained in a similar weight proportion to the pulp and was characterized by a high content of free sugars (sucrose, glucose and fructose) and by the carotenoids content in the form of

pulp microparticles. To valorize these two components, the juice was subjected to a physical separation process by means of several cycles of diafiltration with a 30 kDa MWCO membrane. This separation yielded a suspension rich in carotenoids (4996.4 µg/g) and with a very low sugar content. The other fraction was a solution containing free sugars (84.83 ± 3.26 g/L) and nutrients such as minerals and vitamins. Carotenoids are very interesting compounds with potential applications in the food and pharmaceutical industry. Therefore, their encapsulation was studied using gum Arabic and spray drying and freeze drying as drying methods. Encapsulation using spray drying reduced the degradation of the carotenoids by 51.9 % compared to non-encapsulation, and increased the stability of the carotenoids in water resulting in a more stable suspension. The sugar-rich fraction was valorized through three types of fermentation: with autochthonous microorganisms, with lactic acid bacteria, and with yeasts. Fermentation with autochthonous microorganisms and lactic acid bacteria resulted in lactic acid production (up to 17.64 ± 1.54 g/L), while fermentation with yeasts resulted in ethanol production (up to 49.46 ± 0.28 g/L). The addition of 6 % (w/v) NaCl to the medium as an additive prevented contamination by external microorganisms and allowed pure lactic acid to be obtained with both autochthonous microorganisms and lactic acid bacteria. The yeast fermentation resulted in a total consumption of sugars, while the lactic fermentation did not reach total consumption, probably due to the marked decreased in pH, a variable that should be controlled if a higher lactic acid production is desired.

In Chapter 4, the raw material were the three hydrothermal extracts obtained in Chapter 2, resulting from the hydrothermal treatment at 140, 160 and 180 °C. Given the compounds present in the extracts and the molecular weight distribution, the biopolymers present in these extracts were concentrated, separated, and purified by means of ultrafiltration and multistage diafiltration processes with membranes. The membranes were of larger scale than those of Chapter 1, and with MWCO of 30, 10, 5 and 1 kDa. The 140 and 160 °C extracts were subjected to a cascade treatment (30-10-5-1 kDa) versus the 180 °C extract which was subjected to a mixed configuration (5-10-1 kDa). The highest molecular weight hemicelluloses and pectins increased in concentration by a factor of up to 5 in the cascade configuration and by a factor of up

to 16.67 in the mixed configuration. The application of at least five diafiltration cycles on each retentate, with extra cycles reusing previous diafiltration waters, resulted in high removal of free sugars from the fractions (98.9-99.5 %) as well as of by-products (94.2-99.2 %) through the diafiltration waters and through the 1 kDa permeate. The system allowed going form feed with molecular weight, polydispersity and purity in the ranges 9.02-18.83 kDa, 16.2-31.6, and 30.12-33.51 % (w/w) to fractions with values in the ranges 2.59-102.75 kDa, 1.2-4.0, and 73.1-100 % (w/w). The purified solid fractions were dried using freeze drying and stored.

Chapter 5 developed the processes investigated in Chapters 2 and 4 on a larger scale to obtain fractions in sufficient quantity for further processing into products. Approximately 11.32 kg of fresh carrots were valorized in each experiment, applying hydrothermal treatment at 140 and 180 °C by means of cycles of several flow-through reactors operating in series at the same time. Free sugars, hemicelluloses and pectins were extracted with maximum yields of 379.51 g/kg dry pulp, 81.01 g/kg dry pulp and 5.35 g/kg dry pulp, respectively. The extraction yield of hemicelluloses reached 96.1 % (w/w). The two target extracts were conditioned applying ultrafiltration and several diafiltration cycles with membranes of 10 and 30 kDa (140 °C) and 10, 30 and 1 kDa (180 °C). By-products were removed at 99.8-100 % and free sugars at 98.7-100 %. Biopolymers from the 140 °C extract were recovered in two fractions (10-30 kDa and > 30 kDa) which yielded 31.4 % of the extracted hemicelluloses and 32.4 % of the extracted pectins. Biopolymers from the 180 °C extract were recovered in three fractions (1-10, 10-30 and > 30 kDa) with percentages of 36.8 % of the extracted hemicelluloses and 97.9 % of the extracted pectins. Fouling during membrane operation was evaluated. The membrane system allowed to go from feeds with molecular weight, polydispersity and purity values of 8.08-14.77 kDa, 18.2-19.2 and 14.9-22.2 % (w/w) to five fractions with the following values: 1) 14.77 kDa, 19.2 and 22.2 % (140 °C) and 2) 8.08 kDa, 18.2 and 14.9 % (180 °C), to fractions with values of 1) 80.36 kDa, 2.4 and 100 % (140 °C), 2) 9.85 kDa, 2.1 and 100 % (140 °C), 3) 67.77 kDa, 3.8 and 100 % (180 °C), 4) 5.23 kDa, 1.3 and 64.5 % (180 °C), and 5) 3.86 kDa, 1.5 and 66.8 % (180 °C). The five fractions were obtained in sufficient quantity to be dried using both freeze drying and spray drying. The solids were stored for further processing into biodegradable films.

Chapter 6 focused on the formation of biodegradable films whose main ingredient was the purified solid fractions of hemicelluloses and pectins obtained in Chapter 4 and 5. The residual pulp obtained from the two hydrothermal treatments (140 and 180 °C) applied in Chapter 4 was studied as an additive in the films. A small percentage of residual pulp addition (< 5 %) decreased the oxygen permeability through the film (up to 29 %) but increased the water vapor permeability and worsened the tensile properties. Higher residual pulp content (5-25 %) allowed the film to regain properties similar to those of the reference film (the one without residual pulp) and significantly improved the surface hydrophobicity by increasing the water contact angle from 79.9° (0 % residual pulp) to 125.8° (25 % residual pulp). Glycerol was used as a plasticizing agent in the films at a content of 35 %. The influence of the molecular weight (67.77-102.75 kDa) and the composition of the purified hemicellulose and pectin fractions on films containing 1 % residual pulp was studied. Higher molecular weight decreased oxygen permeability (from 48.18 to 41.14 $cm^3 \cdot \mu m/m^2/kPa/day$), increased water vapor permeability (from 21.56 to 24.01 $g \cdot mm/m^2/kPa/day$), and decreased hydrophobicity (from 86.84° to 71.10°). Tensile strength was higher for higher pectin content and lower molecular weight (from 1.13 to 2.84 MPa), and elongation was higher for higher hemicellulose content (from 5.92 to 15.28 %). The films had acceptable properties for application in the food packaging industry, their main strengths being: 1) 100 % origin from an agri-food waste, 2) production through environmentally friendly processes, 3) non-toxicity, 4) high hydrophobicity without the need for chemical modification, and 5) predictable high biodegradability due to no chemical modification.

Introduction

1. Environmental problems

1.1. Dependence on fossil sources

The world economy is heavily dependent on fossil sources for product and energy generation. Non-renewable sources will be exhausted in a few years, as their use is growing due to the constant increase in population. In addition to their depletion, the use and transformation of these sources into products leads to environmental problems resulting from pollution, such as global warming caused by greenhouse gas emissions.

1.2. Plastic

The concern about dependence on fossil sources has its most significant connotation in the plastics industry. Plastic is a widely used material with a production of 320 million tons/year and increasing demand (Asgher et al., 2020). The use of plastic materials has facilitated our way of life in many fields due to their versatility and good properties, especially their mechanical and barrier properties. The production of plastic has also been extensively studied and is economically efficient. Due to its properties, one of its main applications is in food packaging, where plastic protects by controlling moisture and reducing the flow of gases that can lead to oxidation and/or spoilage. In addition to the packaging sector, plastic is used in all industrial sectors. Some of the most commonly used materials are polyvinylchloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polyamide (PA), polystyrene (PS), and ethylene vinyl alcohol (EVOH) (Asgher et al., 2020).

The main problems associated with plastic are the depletion of fossil sources, the pollution associated with the extraction and transformation processes, the difficulty of recycling, and above all the non-biodegradability. The fact that plastic is produced in such large quantities and cannot be biodegraded at a reasonable rate leads to its accumulation in the environment. Plastic waste ends up colonizing everything from oceans, seas, forests, countryside and remains for many years. The extremely slow degradation results in the emission of greenhouse gases such as methane and carbon dioxide (Kisonen et al., 2015). The main problem of plastic dumping is microplastics. Microplastics are small plastic particles (< 5 mm) that are abundant in water and soil but are not easily distinguishable. These particles are passed on to living organisms through

accidental ingestion causing serious damage. These problems have led to increased social awareness of the need to make a change and reduce the indiscriminate use of plastic, especially single-use plastics.

1.3. Solutions and alternative sources for the production of products

To reduce the problems arising from the depletion of fossil fuels and the production of plastics, the search for alternative sources to be used as raw materials for energy and products is a priority. The bioeconomy, a term introduced by the European Commission, seeks to address the environmental problems affecting the planet. This concept has 5 goals: (1) ensure food and nutrition security, (2) manage natural resources sustainably, (3) reduce dependence on non-renewable and unsustainable resources, (4) limit and adapt to climate change, and (5) strengthen European competitiveness and create jobs (European Commission, 2018). To achieve these objectives, bioeconomy advocates the decentralization of production and the use of renewable sources as raw materials. This form of production aims high productivity, efficient waste recovery, investment in science and technological development, supportive policies, and new market opportunities (Brodin et al., 2017). A simple scheme of the bioeconomy concept can be seen in Figure 1.

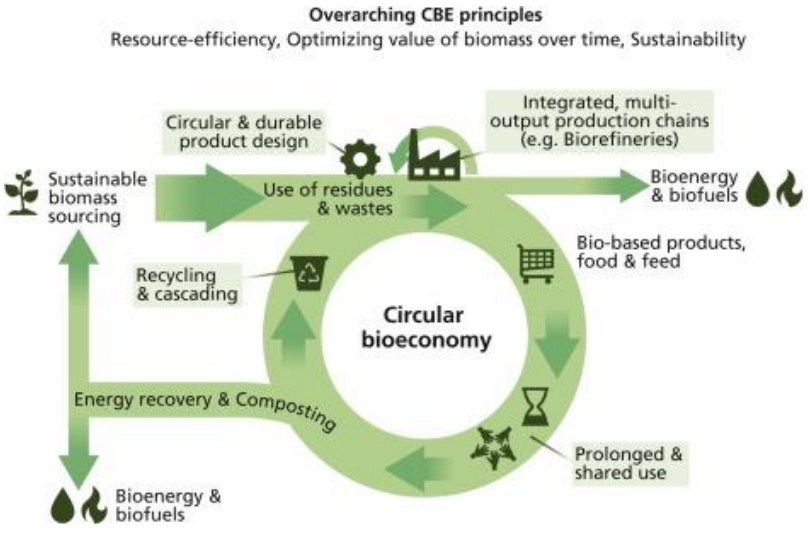

Figure 1. Circular bioeconomy scheme (Stegmann et al., 2020).

The European Commission has also adopted the new Circular Economy Action Plan (CEAP) in March 2020. This is one of the building blocks of the European Green Deal, Europe's new agenda for sustainability. This new action plan aims for initiatives along the entire life cycle of products, focusing on how products are designed, promoting circular economy processes, encouraging sustainable consumption, and aiming to ensure that waste is prevented and the resources used are kept in the European Union economy for as long as possible (European Commission, 2020). This plan introduces legislative and non-legislative measures targeting areas where action brings added value. On the issue of plastics, the European strategy for this problem is part of the European Union's circular economy plan. The objective of the strategy is to protect the environment and reduce marine litter, greenhouse gas emissions and dependence on imported fossil fuels. The strategy actions are based on several points: (1) new rules on packaging to improve the recyclability of plastics and increase the demand for recycled plastic, (2) improving the separate collection of plastic waste, (3) a Directive on single-use plastic products and fishing gear, (4) measures to restrict the use of microplastics in products and address and reduce the unintentional release of microplastics into the environment, (5) measures on bio-based, biodegradable and compostable plastics, (6) new rules on port reception facilities to tackle sea-based marine litter, (6) and scaling up support for innovation to develop smarted and more recyclable plastics materials (European Commission, 2020).

The key points that a feedstock has to meet from a bioeconomic point of view are to be abundant, sustainable and cheap, and ideally to be a side stream or a waste and therefore has no immediate alternative use. Natural waste includes waste from agricultural practices, with an overall production of 5 billion tons of biomass waste each year (Naidu et al., 2017). These wastes include biomasses such as straw, stems, stalks, leaves, husks, shells, peels, seeds, pulp or stubble from fruits, legumes or cereals (rice, wheat, corn, sorghum, barley, etc.), bagasse generated from sugarcane or sweet sorghum milling, spent coffee grounds, brewer spent grains, among many others (Fortunati et al., 2016). Unfortunately, the waste is often transported to dump sites to be burned or to decompose. These actions are not the most adequate from a bioeconomy point of view. The burning of agricultural waste generates carbon dioxide

among other atmospheric pollutants such as dioxins and aromatic hydrocarbons, and decomposition occupies space that could be used for other purposes.

Due to their high availability and carbon-based compositions, there is a great interest in the valorization of these wastes, both for economic and environmental reasons. This waste valorization refers to any industrial processing activities intended for reusing, recycling, and/or producing useful products or sources of energy (Loow et al., 2017a). Eventually, instead of disposing of biomass as residue, the management of these wastes ensure a reduction of the detrimental impact of them on the environment, but most important, conversion of these wastes into value-added products with industrial and commercial potential.

2. Biorefinery of lignocellulosics

2.1. Implementation of the biorefinery concept

The biorefinery concept is based on the conversion of economically achievable biomass into energy, chemical compounds, and high value-added materials or products through biochemical, chemical, physical, or thermo-mechanical processes according to the bioeconomy philosophy (Santos et al., 2017). The concept is similar to that of a petroleum refinery, but instead of using a fossil source as feedstock, biomass is used. This biomass can be of forest, agricultural, industrial and urban origin. The processes to be applied to obtain products must also be environmentally friendly, involving as far as possible the consumption of only renewable resources and minimizing pollution. Using biomass as a raw material instead of oil has enormous advantages associated with its low cost, availability, biodegradability, non-toxicity and efficiency (Edhirej et al., 2017).

Lignocellulosic biomass is the most abundant source of renewable carbon on Earth, and therefore it is considered one of the most promising biorefinery feedstock providing alternatives to the function of modern industrial societies (Loow et al., 2017b). In petroleum refineries, the processing of the feedstock always starts with the fractionation into different components, as in the case with lignocellulosic biomass. The feedstock in lignocellulosic biorefineries is a complex structure consisting on three main polymeric fractions: cellulose, hemicellulose and lignin, in addition to minor compounds such as pectin (biopolymer), ash (inorganic materials) and extractives (non-structural

carbohydrates, nitrogenous materials, chlorophylls, and waxes, etc.) (Santos et al., 2017). A scheme of the polymeric structure can be seen in Figure 2. The high heterogeneity of this feedstock mainly depends on the type of biomass and its origin, but also on other factors such as type of tissue, planta age, and conditions of growing and storing (Santos et al., 2017).

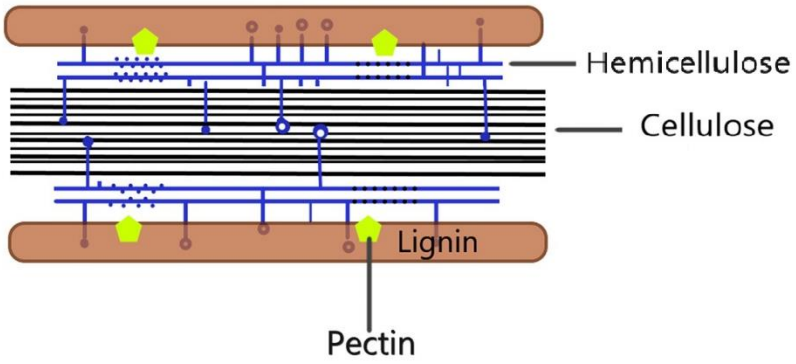

Figure 2. Structure of lignocellulosic biomass (Martins et al., 2020).

Due to the large variations of the chemical compositions of residual biomass, it is important to choose an effective method of biomass fractionation. This process mainly consists in the disruption of the lignocellulosic structure by using different treatments to enhance the separation of its main constituent components. Fractionation is probably the major challenge to the biorefinery development because of the great impact on the yield and efficiency of the downstream processes and the quality of the final products (Fortunati et al., 2016). The multiple streams obtained in a complete fractionation process should contain compounds in concentrations that allow their separation, purification, transformation, and utilization in an economically sustainable way (Steinbach et al., 2017). The current research concerning biomass pretreatment has studied several processes with the aim of getting good yield and cost-efficient pathways for the valorization of residual biomass (Fortunati et al., 2016). Figure 3 shows an overview of the several origins of lignocellulosic biomass and the possible treatments for its fractionation.

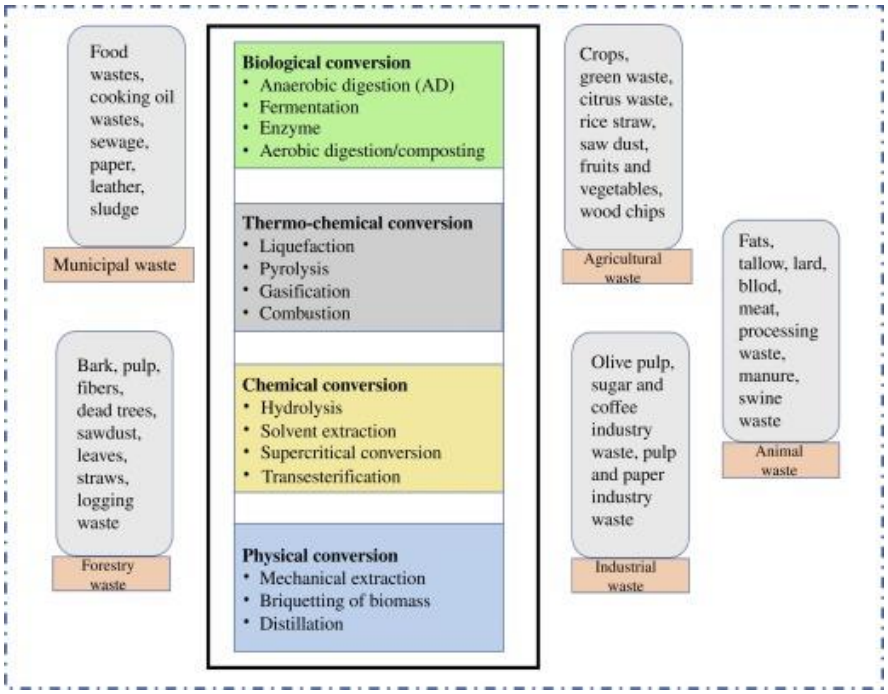

Figure 3. Examples of waste sources for biorefinery approach and their various conversion processes (Yadav et al., 2021).

One of the main products resulting from a biorefinery aims to solve the plastic problem: biomaterials. Food packaging is one of the sectors that consumes the most plastic, so, taking advantage of the non-toxicity of biomass, the production of biodegradable films for the food sector would be a very good application. The most common single-use plastics are those used in food packaging and are therefore also the main contributors to the presence of microplastics in the environment (Kalogerakis et al., 2017). The biodegradability of films produced from biomass would allow them to merge with the environment and living beings without generating problems. Just as plastics are made of polymers, bioplastics are made of the biopolymers present in biomass (cellulose, hemicellulose and lignin in lignocellulosics) once the fractionation and downstream processes of the raw material have taken place.

2.2. Cellulose

Cellulose is the most abundant biopolymer in nature. It is formed by linking glucose monomers by glycosidic bonding. Cellulose is present in the cell wall of plants and forms stiff microfibers embedded in a matrix of hemicelluloses, lignin and pectin (Rajinipriya et al., 2018). These microfibers in turn are held together by non-covalent hydrogen bonding between their -OH groups (Padayachee et al., 2017). The bonding of the

microfibers by lateral alignment allows fibers to form into crystalline or amorphous regions, depending on the hydrogen bonds formed between the molecules. The resulting fibers, especially the crystalline regions, are insoluble in water and able to resist high tensile strength providing support to the plant cells (Padayachee et al., 2017).

Methods of production of cellulose from biomass can be classified as physical, chemical, and enzymatic. Physical methods are generally based on the application of homogenization and mechanical milling to dilute cellulosic wood pulp-water suspensions. The disadvantage is the considerable energy consumption for the mechanical disintegration of the fibers into nanofibers. Among the chemical methods, the oxidation treatment disintegrates the cellulose into nanocellulose. Steam explosion is another pretreatment for obtaining cellulose nanofibers. The material is saturated at high pressure and temperature, followed by a sudden release of pressure forming steam and thus breaking the material, obtaining nanofibers. The hemicelluloses and lignin are released from the raw material leaving the cellulose isolated. Among the chemical methods, strong acid-hydrolysis stands out, which eliminates the amorphous regions of the cellulose, producing cellulose nanostructures as nanocrystals. It is usually performed in two steps, first removing the non-cellulose compounds, and then removing the amorphous region of the cellulose. The alkaline hydrolysis treatment allows partial separation of the cellulose fibers improving their physical and chemical properties. The treatment is usually applied with dilute NaOH or concentrated NaOH at low temperatures. Biological processes are usually based on multicomponent enzyme systems that hydrolyze specific compounds in the biomass, typically starch, pectins, hemicelluloses and lignin. Enzymes can also hydrolyze cellulose to oligosaccharides or cellobiose (Fortunati et al., 2016).

Once the cellulose is isolated from the biomass it can be presented in three different structures: cellulose microfibers or microfibrillated cellulose, cellulose nanofibers or nanofibrillated cellulose, and cellulose nanocrystals or nanowhiskers (Fortunati et al., 2016). Cellulose microfibrils are long and flexible and are related to the amorphous phase. They have a diameter of nanometers and a length of micrometers. By means of mechanical processes such as microfluidization, high-pressure homogenization, milling, and intense ultrasonication it is possible to obtain cellulose in this morphology

(Fortunati et al., 2016). Cellulose in the form of nanofibers consists of aggregates of cellulose with a diameter of nanometers and smaller than microfibers, and with a length of micrometers. Nanofibers include both amorphous and crystalline parts and are characterized by their high hydrogen-bonding ability, which allows for possible interlinking (Fortunati et al., 2016). They are therefore widely used in the preparation of transparent nanoporous films. Cellulose nanocrystals are characterized by their acicular structure and by being the morphology with the highest crystallinity, taking into account their diameter and length of nanometric dimensions (Fortunati et al., 2016).

Cellulose in the form of microfibers, nanofibers and/or nanocrystals has a high elastic modulus and high stiffness. It is a good material to act as reinforcement in the manufacture of materials, with the advantage that it is also biodegradable, biocompatible and non-toxic. Cellulose can be used in biomedical devices and food packaging applications (Fortunati et al., 2016). Other applications of interest are as adsorbent material and aerogels (Liu et al., 2019). Although most of the cellulose used industrially comes from wood or cotton, it would also be interesting to obtain it from vegetable waste from a sustainability point of view.

2.3. Hemicelluloses

After cellulose, hemicelluloses are the most abundant biopolymers in lignocellulosic biomass. They are amorphous heteropolysaccharides with β-(1→4) bonds between pentoses (xylose, arabinose), hexoses (glucose, galactose, mannose, fucose), and uronic acids (glucuronic acid, galacturonic acid), depending upon the source. Given the monosaccharides that can form hemicelluloses, three groups of amorphous biopolymers stand out as base structures: xylans, mannans and galactans. These groups can exist separately as single components or collectively as a mixture of them and other sugars (Steinbach et al., 2017). The hemicellulose chains contain acetyl, formyl, and other ester groups. These ester groups can be hydrolyzed under mild conditions, forming organic acids that promote the autocatalysis of the hemicellulose hydrolysis (Steinbach et al., 2017). Unlike cellulose, hemicellulose consists of shorter chains of 500-3000 sugar units in contrast with 7000-15,000 glucose units per polymer in cellulose. Moreover, while cellulose is crystalline, strong, unbranched, and resistant to hydrolysis, hemicelluloses have a branched, random and amorphous structure which allows them

to be water soluble, and consequently, their hydrolysis is easier when compared to cellulose. Their detailed structure and abundance vary widely between different sources and types of tissue (Fortunati et al., 2016). Hemicelluloses contribute to strengthening the cell wall by forming bridges with lignin and cellulose fibers creating a rigid structure of cellulose-hemicellulose-lignin (Santos et al., 2017).

Some of the most common methods of hemicellulose extraction are organosolv, alkaline, acid, and hydrothermal. Organosolv treatment is typical in the pulp and paper industry. Alkaline treatment is one of the most widely used because it allows the obtaining of hemicelluloses in their polymeric form, but it has environmental connotations and requires an important separation process. Acid treatment has a high yield because it favors the hydrolysis of the hemicelluloses but gives rise mainly to monosaccharides, thus losing a large part of the polymeric form of the hemicelluloses. Hydrothermal treatment has the advantage that it requires only water and biomass as reagents, and depending on the reactor configuration, hemicelluloses can be extracted in their polymeric and high molecular weight form.

In the extraction process, hemicelluloses move from the solid to the liquid phase and are partially depolymerized depending on the severity of the method. This causes their molecular weight to decrease to a greater or lesser degree. Extracted hemicelluloses can be presented, individually or collectively, in three general forms: monomeric (1 sugar unit), oligomeric (2-9 sugar units) and polymeric (more than 9 sugar units). Industrial applications are different depending on the form. Characterization of the extracted hemicelluloses is essential to obtain a subsequent suitable final product. Most of the applications depend on the molecular weight as explained next.

2.3.1. Monomeric form

Monomeric hemicelluloses can be used in the food, chemical and energy industries. Regarding the food industry, production of both xylitol from xylose (Canilha et al., 2003; Silva and Roberto, 2001; Yi and Zhang, 2012) and sugars (Otieno and Ahring, 2012; Peng and She, 2014) have been studied. Chemical industry applications have been focused on the production of sugar-based chemicals such as ethanol (Hari Krishna and Chowdary, 2000; Hu et al., 2008; Taherzadeh and Karimi, 2007), acetone (Peng et al., 2012a), butanol (Peng et al., 2012a), lactic acid (Koivula et al., 2011; Naidu et al., 2017), ferulic

acids (Koivula et al., 2011), and furanic compounds (Gürbüz et al., 2013; Koivula et al., 2011; Peng et al., 2012b; Sangarunlert et al., 2008). Monomeric hemicelluloses in the energy industry are researched to produce biofuels (Hu et al., 2008; Kenealy et al., 2007; Koivula et al., 2011; Olcay et al., 2013; Peng et al., 2012b; Peng and She, 2014; Taherzadeh and Karimi, 2007).

2.3.2. Oligomeric form

Food, feed, pharmaceutical, and agricultural industry are the sectors where hemicelluloses in their oligomeric form have received considerable attention as a promising raw material. Regarding food industry, hemicelluloses can be used in the production of food additives (Garrote et al., 2004; Parajó et al., 2004; Vázquez et al., 2001; Vegas et al., 2005); in pharmaceutical industry hemicelluloses are used as solubilizers, and as active and preventive agents against certain diseases (Damonte et al., 1996; Stone et al., 1998; Vázquez et al., 2001). Both in food and pharmaceutical industry, hemicelluloses can be used as gelling, stabilizing and viscosity-enhancing additives (Ma et al., 2012). In the agricultural industry oligomeric hemicelluloses have applications such as growth stimulator and accelerator, ripening agent and yield enhancer (Vázquez et al., 2001).

2.3.3. Polymeric form

In food industry, they can be used as barrier films for food packaging (Edlund et al., 2010; Goksu et al., 2007; Gröndahl et al., 2004; Hansen and Plackett, 2008; Hartman et al., 2006; Mikkonen et al., 2009), including hemicelluloses chemically modified (Gröndahl et al., 2006). Applications of hemicelluloses in food packaging have been also patented (Albertson and Edlund, 2012). In both pharmaceutical and cosmetic industry, hemicelluloses can be used as hydrogels (Gabrielii et al., 2000; Karaaslan et al., 2011; Lindblad et al., 2001; Sun et al., 2013), films or film-former substances (Gatenholm et al., 2008; Hansen and Plackett, 2008; Ruiz et al., 2013), thickeners, adhesives, emulsifiers, stabilizers, binders, etc. (Al Manasrah et al., 2012). Applications in pharmaceutical and medical sectors such as drug delivery and drug carrier have been researched (Oliveira et al., 2010; Sun et al., 2013; Zhao et al., 2015). In papermaking industry, polymeric hemicelluloses can be used as wet strength additives (Kataja-aho et al., 2012), strengthening agents (Lee et al., 2015) and flocculant agents (Kataja-aho et

al., 2012). New biopolymers from modified hemicelluloses have been obtained, for example thermoplastic hemicellulose derivatives (Jacobs et al., 2003), long-chain alkyl ester derivatives (Laine et al., 2010; Sun et al., 1999, 2001), hydrophobized hemicelluloses (Farhat et al., 2017) and cationic biopolymers (Ebringerová et al., 1994; R. Filho et al., 2013).

2.4. Pectins

Pectins are heteropolysaccharides from the primary cell wall and middle lamella of higher plants. They consist of α-galacturonic acid units liked by α-(1→4) bonds and a variable content of other monosaccharides (arabinose, galactose, rhamnose, and others) and methyl esters in the acid group (Babbar et al., 2016). The most abundant region consists of galacturonic acid chains, called homogalacturonan. Other regions that may be present are rhamnogalacturonan I, rhamnogalacturonan II, and xylogalacturonan (Mohnen, 2008). Homogalacturonan is a homopolymer partially methylesterified at the C-6 carboxyl, may be O-acetylated at O-2 and O-3, and may contain other crosslinking esters (Mohnen, 2008). Rhamnogalacturonan I pectin also contains rhamnose in its structure, while rhamnogalacturonan II is the most complex, containing up to 12 different sugars with around 20 different types of bonds (Mohnen, 2008). Structural studies suggest that pectins may be bound to certain hemicelluloses (Mohnen, 2008). Pectins are responsible for providing tensile strength and increasing the flexibility of the stiff cellulose (Padayachee et al., 2017). Their gelling properties may have an important influence on cell-cell linking. During ripening or microbial digestion of plant material, enzymes are responsible for the degradation of pectin leading to the disintegration of the middle lamella (Padayachee et al., 2017).

The most common method of pectin extraction is dilute acid extraction, using for example hydrochloric acid, nitric acid, or sulfuric acid. The main advantage of the method is that these acids are cheap and the yield is good, however, they are toxic and harmful to the environment (Jafari et al., 2017). The main variables influencing the extraction are the type of acid, extraction temperature, time, pH, and solvent to sample ratio (Raji et al., 2017). More environmentally friendly alternative extraction methods are enzyme-assisted extraction, ultrasound-assisted extraction, and microwave-assisted extraction (Dranca and Oroian, 2018).

At the industrial level, pectins are currently used in the food industry as additives for thickening, gelling, stabilizing, and emulsifying agent in jams, jellies, soft drink, fish, meat, fruit juice, and milk products, as well as a fat replacer in spreads, salad dressing, ice cream, and emulsified meat products (Raji et al., 2017). For all these applications, pectins are required to contain at least 65 % galacturonic acid (Schmidt et al., 2017). In the pharmaceutical industry, they are also used for lowering cholesterol levels, reducing blood pressure, restoring intestinal function, and weight reduction (Raji et al., 2017). Pectins can also be used as an encapsulating agent. In their oligomeric form, pectins are starting to be used as prebiotic agents (Babbar et al., 2016). At the industrial level, pectins are extracted from fruit peel (waste from the juice industry), mainly from citrus fruits (lemon, orange, lime) (Grassino et al., 2018). Other biomasses also contain pectin in smaller concentration but sometimes of higher quality, such as olive pomace, berry pomace, potato pulp, and carrot pulp (Grassino et al., 2016).

2.5. Lignin

Lignin is a non-polysaccharide aromatic biopolymer present in plant cells whose function is to maintain cell structural integrity by providing rigid strength, as well as to assist in water permeability into the cell (Padayachee et al., 2017). It is a complex cross-linked phenolic polymer with an amorphous structure consisting mainly of three units of phenylpropane (C9): p-hydroxyphenyl, guaiacyl and syringyl (Collard and Blin, 2014). The bonds in lignin are mainly β-O-4 ether linkages as well as carbon-carbon (C-C) and ester (C-O-C) (Banik et al., 2017). Lignin is found in layers between interactions of the polysaccharides cellulose and hemicellulose (Padayachee et al., 2017).

Lignin is usually classified according to the raw material and the extraction method used. The lignin from industrial processes, e.g. from by-products of pulping industry and bioethanol production, is of particular importance (Fortunati et al., 2016). In pulping processes, Kraft and sulfite methods are used, while in bioethanol production acid hydrolysis is normally applied (Fortunati et al., 2016). Derived from the pulping industry, highlights the Kraft lignin (or sulfate lignin, derived from the Kraft process), alkali lignin (or soda lignin, derived from the soda-AQ pulping), and lignosulfonates (derived from the sulfite pulping) (Fortunati et al., 2016).

Lignin, due to its aromatic character, has the potential to act as a substitute source in any product obtained from the petrochemical industry (Fortunati et al., 2016). Applications for lignin include: dyes, synthetic floorings, adhesives, sequestering, emulsifiers, binding, dispersal agents, and paints (Fortunati et al., 2016). In addition, chemical compounds such as hydrocarbons, alcohols, polyols, ketones, acids and phenol-derivatives can be obtained from lignin-based polymers, synthesized from lignin-modified monomers (Fortunati et al., 2016). Specific potential applications of lignin include: 1) valorization for the production of biofuels and energy, 2) high molecular mass applications like polymers, wood adhesives, and carbon fibers, and 3) production of polymer building blocks or aromatics monomers including benzene, toluene, xylene, phenol, and vanillin (Fortunati et al., 2016).

2.6. Extractives

Other important components of lignocellulosic biomass are extractives. They are defined as those components that are present in a non-structural form, and can therefore be extracted with the appropriate solvent. Extractives can be polar or non-polar (Sluiter et al., 2008). Within the polar ones, there are water-soluble extractives and ethanol-soluble extractives. Water extractives include free sugars and sugar acids, nitrogenous materials and inorganic materials. Ethanol-soluble extractives include waxes, chlorophyll, terpenes (phenols, hydrocarbons), and other minor compounds. Apolar extractives present in the biomass can be extracted with hexane, and include compounds such as terpenoids and fats.

The most comprehensive method for the removal of extractives, which is used for quantification, is Soxhlet extraction. The process takes about 24 hours and is based on evaporating the solvent so that it circulates through the biomass sample extracting the extractives, then condensing the extract and evaporating the solvent again, repeating the extraction cycle multiple times.

3. Biorefinery processes

Our concept of biorefinery is based on the generation of high-value bioproducts from biomass feedstock. To achieve this goal several processes are selected.

3.1. Separation of extractives

Soxhlet method is the most comprehensive method for the separation of extractives at lab scale, ideal for quantification and analysis.

Separation of extractives at a biorefinery scale is usually performed through more economical and larger scale processes. For water-soluble extractives, it is sufficient to apply water extraction at a temperature close to 100 °C, while hemicelluloses require a temperature higher than 120 °C.

For non-polar extractives, supercritical CO_2 is a more environmentally friendly option than the use of organic solvents. CO_2 is an inert solvent, non-toxic and cheap, but also very easily separable from the extracted compounds only by changing the pressure and temperature conditions.

The supercritical state in a fluid is reached when the critical point of pressure and temperature is exceeded, which generally gives rise to a state which has gas like compressibility, lesser density and high solvating power like liquids. Due to its high diffusivity, supercritical CO_2 can easily penetrate the solid and dissolve the soluble components. In this pretreatment it is very important that the fluid reaches and maintains pressure and temperature conditions during operation. Although the presence of moisture has traditionally been a drawback in extraction with volatile organic solvents, in the case of supercritical CO_2 the presence of some moisture would lead to the formation of carbonic acid which would not necessarily be an undesirable compound, as it could initiate hydrolysis of hemicellulose (Badgujar et al., 2021). Once the supercritical CO_2 is pumped and passes through the biomass, it is conducted to a separator tank where the conditions are such that the CO_2 is no longer supercritical, leaving the separator in the gas phase and precipitating the extracted components which can then be decanted. The gas-phase CO_2 leaving the separator can be recirculated and conditioned back to the supercritical state.

3.2. Fermentation of free sugars

Fermentation in lignocellulosic biomass is normally performed using hydrolysis pretreatment that cleavages cellulose and hemicellulose into their monomers, which are food for bacteria or yeast in the production of biochemicals that can then be separated by distillation (Karagoz et al., 2019).

In cases where the biomass has a high content of water extractives, it is interesting to apply fermentation to this easily separable fraction. This fermentation is favored in the case of certain vegetables due to the presence of nutrients in their composition such as vitamins and minerals. Moreover, it has the advantage of not needing to apply hydrolysis or enzymatic processes, so the number of steps is reduced and the presence of fermentation inhibiting agents is null. Obtaining the free sugars from the biomass could be performed as mentioned above by extraction with hot water (approximately at 100 °C) or by separation of the juice if it is a vegetable with a high percentage of moisture. In many plant residues of food origin, fermentation is characterized by a succession of homo- and hetero- fermentation by autochthonous lactic acid bacteria, with or without yeasts. The yeasts that pass into the medium or are added are responsible for the co-production of ethanol while the autochthonous bacteria produce lactic acid (Di Cagno et al., 2008). The addition of additives such as NaCl can reduce ethanol production and favor lactic acid production, while the addition of yeast rapidly increases ethanol formation (Di Cagno et al., 2008).

3.3. Formulation of bioactive extractives

Some bioactive compounds from biomass can be obtained from polar extractives and/or apolar extractives. Fractionation of these compounds such as polyphenols or carotenoids requires formulation to ensure proper preservation and use of the ingredient. For this reason, such materials are often subjected to a drying process either using freeze drying or spray drying.

Within the drying process, the encapsulation process stands out. In the encapsulation, small particles of the material to be dried are surrounded by a thin layer of a material called encapsulating agent. This material has a protective function for the active substance, preventing oxidative degradation reactions or the evaporation of volatile

compounds (Mahfoudhi and Hamdi, 2015). The most common encapsulating agents are starches, gum Arabic, methylcellulose, gelatin, whey protein, corn syrup, maltodextrins, disaccharides, pullulan, and sodium caseinate (Mahfoudhi and Hamdi, 2015). These agents must meet several properties such as being soluble in solvent, non-toxic, and biodegradable. For the use of active ingredients in food and pharmaceutical applications, it is necessary that the formulated compound can dissolved or form a stable suspension in water or ethanol.

3.4. Extraction of biopolymers

After the removal of extractives, the most easily extractable components are the biopolymers hemicelluloses and pectins. The main extraction treatments are:

- Acid treatment. This method can be performed in concentrated mode but is mostly done in dilute mode, with sulphuric acid being the usual agent. Depending on the severity of the treatment, biopolymers may be recovered in their oligomeric form, or they may be hydrolyzed to monomers. In the case of pectins, this method in its dilute mode is the most used. Depending on the subsequent application it is often necessary to carry out a neutralization post-treatment. One of the disadvantages of this operation is the possible corrosion of the equipment as well as the degradation of the biopolymers to organic acids and furan derivatives (Peng et al., 2012b)
- Alkaline treatment. It is based on the use of sodium or potassium hydroxide for the removal of hemicelluloses from the biomass, and sometimes also lignin and cellulose. During the alkaline treatment uronic and acetic esters are hydrolyzed as well as α-ether and ester linkages between lignin and hemicelluloses (Peng et al., 2012b). Subsequent neutralization treatment and separation of the hemicelluloses from the co-extracted compounds is very important. The alkaline agent must be separated because of its toxicity.
- Organosolv treatment. It is a typical method of the pulping industry based on the use of mixtures of water and organic alcohols or acids to fractionate the biomass. The extract contains lignin and hemicelluloses. Precipitation of the lignin results in the lignin in solid-state and a liquid solution containing the hemicelluloses.

Dimethyl sulfoxide (DMSO) is the most common solvent that has been applied to extract hemicelluloses (Peng et al., 2012b).

- Ionic liquids. This method is based on the use of salts with a very low melting point, so that at room temperature they are in a liquid state. Ionic liquids can dissolve cellulose among other biomass components (Anugwom et al., 2012). The main disadvantage is that ionic liquids are very expensive and still little-known materials.
- Biological treatment. Specific enzymes and microorganisms can degrade cellulose or lignin increasing the concentration of hemicellulose. It is an environmentally friendly process but generally slow and requires monitoring (Peng et al., 2012b).
- Ammonia fiber expansion. It is based on subjecting the biomass in contact with water (0.1-2.0 g/g dry biomass). Then anhydrous ammonia is added (0.3-2.0 g NH_3/g dry biomass) and the mixture is heated to the reaction temperature (60-180 °C). After the completion of the residence time, a rapid depressurization takes place and the gas-phase contents a suitable chemical fume hood (Chundawat et al., 2020). The treatment allows the removal of lignin and hemicelluloses, although the operating cost is considerable due to the need to recover the ammonia.
- Microwave treatment. The heating produced by the microwave action contributes to depolymerization of the material. Microwave-assisted water extraction has been reported as an efficient method for hemicelluloses extraction, requiring a shorter time and less solvent (Peng et al., 2012b).
- Steam-explosion. This treatment employs high pressure saturated steam (160-260 °C, 7-48 bar) followed by rapid depressurization. Depending on the severity, hemicelluloses and pectins can be converted to monosaccharides and a considerable amount of degradation by-products can be produced. Lignin may also be partially degraded, whereby cellulose may be isolated (Peng et al., 2012b).
- Hydrothermal treatment. It is based on extraction with water at temperatures between 120-240 °C, maintaining the liquid state by pressurizing the system. Depending on the severity of the operating conditions, hemicellulose, pectin,

cellulose, and part of the lignin could be extracted. Hydrothermal treatment is the most advantageous as the only reagents are water and biomass, with no need to neutralization and desalination treatment and no corrosion problems in the equipment. The heating of the water above 120 °C leads to the formation of hydronium ions in the water, which causes an acidification of the medium that allows the extraction of the biopolymers. In the extraction, depolymerization takes place to a certain degree, releasing organic acids which in turn autocatalyze the hydrolysis of the biopolymers in a process known as autohydrolysis. The extracted biopolymers generally have a broad molecular weight distribution. In case of water soluble extractives are not pre-extracted from the biomass, their extraction will also take place in the hydrothermal treatment together with the biopolymers. To recover the hemicelluloses and pectins in their polymeric form, further treatment is necessary to obtain a defined molecular weight by separating the biopolymers into different fractions and, above all, to achieve a good degree of purity by removing the co-extracted compounds.

3.5. Concentration, separation, and purification of biopolymers

One drawback of autohydrolysis is that is not specific. Unfortunately, many compounds can be co-extracted together with hemicelluloses and pectins, like free sugars, organic acids, lignin derivatives such as phenolic compounds, and degradation compounds such as 5-HMF and furfural.

When the target are the hemicelluloses or pectins, purification is necessary to reduce the presence of these components improving the quality of the extracts. Also, given the wide molecular weight distribution of the extracted biopolymers, separation steps are necessary for molecular weight fractionation. As seen previously, the applications of biopolymers are different depending on whether they are in their monomeric, oligomeric or polymeric form, and within their polymeric form their properties vary depending on their molecular weight.

Molecular weight distribution does not only refer to the average molecular weight, because as hemicellulose and pectins are polymers, their size distribution also involves concepts such as polydispersion, shape, or interaction properties. The average

molecular weight is not sufficient to characterize the fractions, as samples with the same average value can have very different distributions. The characteristic parameters are: the number-average molecular weight (Mn), which can be related to colligative properties; the weight-average molecular weight (Mw), which can be related to properties such as melt viscosity; the peak-average molecular weight (Mp), which is the molecular weight of the maximum peak in the molecular weight distribution; and the Z-average molecular weight (Mz), which can be related to properties such as toughness. The Mw/Mn ratio is known as polydispersity, and is an indicator of the broadness of the molecular weight distribution, i.e. it characterizes the shape of the distribution. The lower the polydispersity of a polymer, the higher the strength and toughness. However, the higher the polydispersity, the easier the polymer is to be processed.

In addition to purification and separation, it may be of interest to increase the concentration of the biopolymers in the extract if they are very dilute and drying is desired. There are different methods for the concentration, separation and purification of hemicelluloses and pectins. The most commonly used are chromatography, ethanol precipitation and ultrafiltration. In general, chromatography and ethanol precipitation result in more defined and homogeneous biopolymer fractions, however, ultrafiltration is the most investigated method due to its many advantages.

3.5.1. Ultrafiltration and diafiltration

Ultrafiltration is a type of membrane separation along with microfiltration, nanofiltration and reverse osmosis. It is characterized by the retention of macromolecular compounds. Water and dissolved compounds of low molecular weight pass through the membrane. The most important parameter defining an ultrafiltration membrane is the molecular weight cut-off (MWCO). This standard parameter defines the molecular weight above which 90 % of the solutes are retained, i.e. do not pass through the membrane. Ultrafiltration is based on pumping the solution to be treated through the membrane, which has two outputs: the retentate stream, with the components that have not managed to pass through the membrane, and the permeate stream, with the components that do pass through the membrane. Typically, the retentate is recirculated to the feed during operation, and in this way the solution is concentrated and the lower molecular weight compounds are removed through the

permeate. An important parameter in the ultrafiltration operation is the feed volume reduction, which is the percentage of the feed volume that is reduced in the operation, this volume being removed in the form of permeate.

Normally, the component of interest remains in the retentate and the operation continues until the desired concentration, and ideally, purity, is achieved. However, it is common for the lower molecular weight components that should pass through the membrane to do so only to a certain degree, and never in their entirety. Fouling of the membrane can result in a slower process due to lower permeate flux, and also because the mass transfer is hindered by phenomena such as concentration polarization, making it difficult for components to pass through the permeate. One method to improve purification is the process called diafiltration. Diafiltration is usually performed after ultrafiltration, but can also be performed alone or before ultrafiltration. It is based on the addition of a known volume of water to the feed tank for dilution. The ultrafiltration process then starts with the usual recirculation of the retentate. This process is maintained until the volume of water added to the feed is removed through the permeate. This stage is considered a diafiltration cycle. Although diafiltration does not increase the concentration of the retained compounds, it does increase their purity due to the entrainment of the lower molecular weight compounds into the permeate. The diafiltration cycles can be repeated until the feed (or retentate) reaches the desired purity, or equivalently until the diafiltration waters are extremely dilute and do not purify further. Since the only reagent required in diafiltration is water, it is a very economically and environmentally efficient purification process. Figure 4 shows a diagram of the ultrafiltration and diafiltration processes.

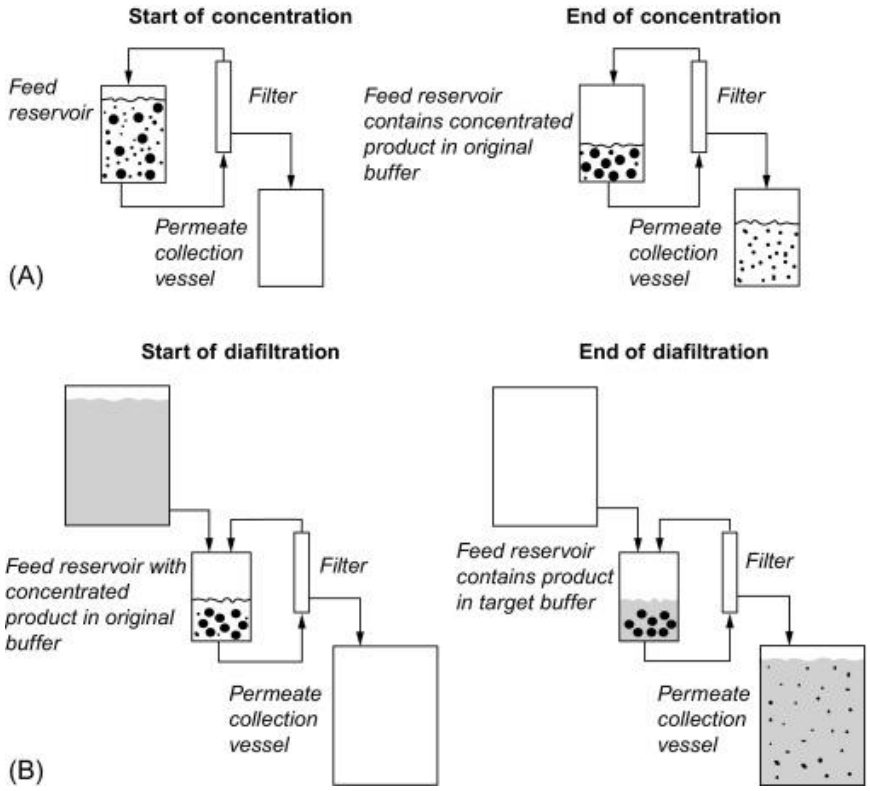

Figure 4. Diagram of the A) ultrafiltration and B) diafiltration processes (Liderfelt and Royce, 2018).

Ultrafiltration membrane operation is characterized by low energy consumption (close to ambient conditions, pumping at low pressure), low chemical consumption (cleaning only), scalability and flexibility of operation. The main difficulty with this method is the time required for operation, which depends on the size of the filtration area and the fouling of the membrane. The fouling can be reduced by different pretreatments. Apart from diafiltration, strategies to reduce fouling include pretreatment of the feed by heating or viscosity reduction, as well as microfiltration to remove microparticles.

3.5.2. Multistep ultrafiltration/diafiltration processes

Due to the wide molecular weight distribution of the extracted compounds, it is common to use more than one ultrafiltration membrane with different MWCO values to maximize the recovery of the biopolymers. These membranes can be used in cascade with decreasing MWCO values, with the permeate from one membrane feeding the next membrane. Another way of working is by mixed configuration, using a membrane of intermediate MWCO and then two membranes in parallel, the one with lower MWCO

treating the permeate from the first membrane, and the one with higher MWCO treating the retentate.

A reverse cascade configuration from low MWCO to high MWCO membranes would also be possible, but as the concentration of the retained compounds in each membrane increases, very high values of biopolymer concentration could be reached, which would make the process inoperable. If the extract already has an adequate concentration of biopolymers and purification is all that is desired, only diafiltration could be applied, thus separating out the compounds that are not to be retained.

3.6. Drying of biopolymers

3.6.1. Freeze drying

Freeze drying is the most suitable method for drying heat-sensitive compounds as it takes place at a low temperature, keeping the properties of the material almost intact (Lina F. Ballesteros et al., 2017). The process is based on the initial freezing of the product and then subjecting it to vacuum where the water is removed by sublimation. The duration of the drying depends on the sample volume, but generally varies between 12 hours and 5 days.

3.6.2. Spray drying

Spray drying is considered faster and simpler than freeze drying. It operates at medium to high temperature and the process takes place in a single step consisting of the evaporation of the solvent, usually water. Injection temperatures are between 150 to 200 °C, with drying temperatures from 85 to 120 °C. The solution to be dried is pumped into the drying chamber where it enters in atomized form thanks to an atomizer which produces fine droplets that are evaporated almost instantaneously on contact with the hot air in the chamber. The evaporation of the water from the small droplets allows the precipitation of microparticles of the solute to be dried, which are recovered through a cyclone. Given the mode of operation, it is about 4-5 times more economical than freeze drying, and requires much shorter periods of operation (Shishir and Chen, 2017). Another advantage of spray drying is the reduced contact time (5-100 s), which generally generates a low degradation of nutrients, colors, and flavors (Shishir and Chen, 2017). The product obtained after drying is much more stable due to the low moisture content

(2-5 %) and the low water activity (Shishir and Chen, 2017). The disadvantage of spray drying is that high air temperature or oxygen can degrade certain sensitive compounds.

3.7. Characterization of biopolymers

The characterization of both the extracted biopolymers and those that have been separated and purified is very important to evaluate them for potential applications. The biopolymers are characterized at least from a chemical, molecular and structural point of view. Chemical characterization is based on the determination of their chemical composition, which includes the degree of purity of the biopolymer. Molecular characterization is based on the determination of the molecular weight distribution, characterized mainly by the parameters MW and polydispersity, but also by the distribution curve. Structural characterization may include the determination of the chemical bonds forming the material, the visualization of the structure at the microscopic level, and the analysis of thermal degradation.

3.8. Biopolymer transformation for specific applications

The aim of the extraction, concentration, separation, purification, drying and characterization of biopolymers is their transformation into high value-added products. Both hemicelluloses and pectins have applications as food additives thanks to their physicochemical properties, but obtaining them in a purified form and with a high molecular weight allows the possibility of other new applications, among which the field of biomaterials stands out.

Although hemicelluloses and pectins have adequate properties in terms of composition, purity and molecular weight, one of their main drawbacks is their hydrophilicity. Therefore, several authors have applied chemical modification processes to reduce the hydrophilicity of biopolymers in the formation of biomaterials. One of the modification methods is often esterification (Peng and She, 2014). Another option for functional biopolymers is etherification, whereby an amphoteric biopolymer can be obtained (Peng and She, 2014). The fact that a biopolymer is amphoteric, i.e. possessing anionic and cationic groups, provides suitable properties for applications related to biomaterials, adsorbents, cosmetics and biomedicine. Another method of enhancing biopolymers is cross-linking with another biopolymer. One of the main applications of all

hydrophobized biopolymers is in biomedicine. They can be used as wound dressing, as they have the ability to maintain a high moisture environment at the wound-dressing interface, remove exudate, allow the exchange of gases and vapor and be impermeable to microorganisms and water (Farhat et al., 2017). Another application is drug delivery. Drug carriers offer the possibility of improving drug performance in terms of efficacy, safety and patient compliance. The biopolymer can be degraded completely by the action of natural enzymes in the human body. The form of biopolymers in drug delivery systems aims to protect the active substances and are therefore often found in the form of liposomes, microparticles, nanoparticles, polymeric micelles and nanocrystals (Farhat et al., 2017).

Biomaterials can also be formed without the need for chemical modification of hemicelluloses or pectins. Hydrogels and films have been successfully produced. Hydrogels are three-dimensional, hydrophilic polymeric structures, usually consistint of more than one polymer, capable of imbibing large amounts of water or biological fluids. Hydrogels can include acrylic acid or acrylamide for grafting to form cross-linked structure. They have wide applications in tissue engineering, drug delivery, and environmental protection (Peng and She, 2014). The functional groups of hydrogels can be modified to increase their selectivity towards the capsule of certain compounds and can be applied for the removal of toxicants or pollutants.

Films formed from hemicelluloses and pectins are generally known to have a very good oxygen barrier, which makes them interesting for food packaging applications. Films can be formed either on the basis of a single biopolymer combined with a plasticizer agent or by combining several biopolymers together with the plasticizer agent. In addition to the oxygen barrier, water vapor barrier and mechanical properties are important in films. Hydrothermally extractable biopolymer films are often reinforced with another biopolymer, usually cellulose nanofibers.

It is also worth noting the use of hemicelluloses in coating applications, especially in the pulp and paper industry where paper coatings plays a crucial role in the improvement of paper quality, such as reduction in ink absorbency (Farhat et al., 2017). Coated papers give better printing results, and the paper is more resistant to water and grease permeability (Farhat et al., 2017).

4. Agricultural and agri-food waste

4.1. The problem of agricultural and agri-food waste

Annually around one third of the world's food production ends up as waste, constituting 1.3 billion tons of biomass (Rajinipriya et al., 2018). In the European Union, this waste amounts to 100 million tons per year, which represents 35 % of total production (Marić et al., 2018). Waste from the food industry is distributed between drink industry (26 %), fruit/vegetable production and processing (14.8 %), cereal processing and manufacturing (12.9 %), meat product processing and preservation (8 %), manufacturing and processing of vegetable and animal oils (3.9 %), fish product processing and preservation (0.4 %), and others (12.7 %) (Nayak and Bhushan, 2019). Consequently, within food waste, as well as agricultural and agri-food waste, the predominant type of residue is vegetable waste. Vegetable waste amounts to 24 million tons per year in Europe and is generated at all stages of the value chain, from cultivation to sale to the consumer (Heredia-Guerrero et al., 2014).

The most prominent vegetable residues come from tomatoes, potatoes, and carrots, among others (Rajinipriya et al., 2018). Examples of where waste occurs are damage to plantations, damage in processing, lack of storage space, rejection by sellers, or waste in the market due to reaching the expiry date (Rajinipriya et al., 2018). In the case of food, the consumption of food itself also always generates residues associated with the inedible parts of the plant. Some figures of the residues generated in the production process reach values of 30-50 % for mango, 20 % for banana, 40-50 % for pomegranate, and 30-50 % for citrus fruits (Banerjee et al., 2017). The problem of vegetable waste is not only economic but also environmental, due to the problems arising from its generation and disposal. Vegetable waste is characterized by a high moisture content, which means that its incineration is a treatment with reduced efficiency and is associated with the emission of greenhouse gases (Banerjee et al., 2017). The handling of this type of organic waste is delicate, and there are currently not clearly advantageous alternatives for its use. The most common is landfill or sale for animal feed (Surbhi S et al., 2018), as well as possible use as fertilizer (Christiaens et al., 2015). Applications such as composting and biogas production have also been explored (Christiaens et al., 2015). Inappropriate landfilling of vegetable residues causes environmental problems such as

toxicity to aquatic life, pollution of surface and ground waters, altered soil quality, phytotoxicity, colored natural waters and odors (Nayak and Bhushan, 2019). Overloaded landfills generate methane, a potent greenhouse gas 30 times more harmful than carbon dioxide. Global greenhouse gas emissions from food waste are the third largest contributor to greenhouse gas emissions after China and the USA (Banerjee et al., 2017). For this reason, current legislation worldwide is focused on the use of these wastes with the establishment of increasingly restrictive policies and policies that favor the search for alternatives (Nayak and Bhushan, 2019). Although the best option for waste is to prevent its generation, vegetable waste has a good potential for use due to its abundance, renewable and biodegradable nature. Its valorization and transformation for the production of products and energy has been outlined by the waste management hierarchy under the European Union (Nayak and Bhushan, 2019). Most of the industries in charge of vegetable production as well as those in which waste is produced are in general relatively small or medium-sized and technologically simple companies and therefore do not usually apply circular economy concepts. However, the situation is changing and both large and small corporations are showing interest and demanding solutions for these wastes.

Due to the particularity of vegetable waste because of its high moisture content and the type of the most abundant compounds, it is necessary to study and segregate it with a view to its valorization through a specific biorefinery. Vegetable waste as a raw material in biorefinery stands out for being inexpensive and abundant. Among the compounds present are carbohydrates (e.g., polysaccharides, oligosaccharides), aromatic compounds, essential oils, and phytochemicals (polyphenols, glucosinolates, carotenoids), free sugars, proteins, lipids, flavor compounds, etc. Biorefinery focuses on the valorization of these components through their extraction by means of biochemical, chemical, thermal and/or physical processes. Once extracted, the components can undergo several downstream stages for their purification, followed by their formulation and/or transformation into products. It is key that the processes applied in biorefinery are environmentally friendly and also economically efficient so they can be applied in both small and large companies (Surbhi S et al., 2018). Many of the components present in vegetable residues, once extracted and transformed or conditioned, can have

applications as food additives, nutraceuticals, therapeutics, cosmetics, plastic substitutes, etc. (Nayak and Bhushan, 2019). There is no doubt about the potential of these compounds in the aforementioned industries from an environmental and economic point of view, but also in society as the natural origin of products and their sustainable production are increasingly valued (Martins and Ferreira, 2017)

4.2. Agricultural and agri-food waste in Castilla y León region

The government of Castilla y León region through the Consejería of Agriculture and Livestock of the Junta de Castilla y León established a "Plan to Promote the Agri-food Bioeconomy for a Competitive Rural Environment for the period 2014-2020". It established a series of strategic objectives including the use and valorization of by-products from agricultural and livestock production and industry, and the development of new treatment and extraction processes focused on obtaining sustainable bioproducts and clean energy. The fulfilment of these strategic objectives is established through specific lines of action, including the integrated valorization of by-products and the sustainable production of bioproducts and bioenergy (Palacios Cuesta et al., 2020).

Agricultural by-products have a series of characteristics that condition their use (Palacios Cuesta et al., 2020):

- Their production is seasonal and can be very voluminous at certain times. This aspect influences the dimensioning of treatment and processing plants for these materials.
- Most of these by-products, especially those derived from vegetables, have a high moisture content of more than 85 %. The high moisture content makes these products unstable and easily degradable, and pathogens are relatively easily present during their production and treatment, making it necessary to recover them quickly. The high percentage in humidity also increases transport costs, so logistics is a key factor in valorization

In Castilla y León, 37.6 % of the surface area is arable land, divided between dray land with more than three million hectares and irrigated land with more than half a million hectares. The 25 % of the surface are meadows and pastures, mainly dry land. About 31 % of the area is forest land. These figures show the importance of land use in the regional

economy and environment. The remaining land, about 6 %, is used for other purposes, including urban use (Palacios Cuesta et al., 2020).

Between 2013-2017, an annual average of 15,410 tons of horticultural crops were produced in Castilla y León. The most abundant were carrots (38.0 %), onion (15.4 %) and leek (9.7 %). Horticultural production in the region generated an average of 23.5 % of the volume in by-products, 80.7 % of which were generated in the agri-food industry (processing stage) (Palacios Cuesta et al., 2020).

When managing this important percentage of agricultural and agri-food by-products, it is necessary to consider some considerations:

- The management of by-products, so-called “residues”, is an obligation for the entities that generate them, irrespective of the profitability that can be achieved by this process.
- In some cases, the revenues obtained from waste management do not cover the costs or only provide the energy supply for the operation. In this situation, the use of public funds to finance the necessary investments becomes necessary.
- When assessing a project related to the management of these by-products, it is necessary to consider environmental as well as economic issues, which is why collaborations with research teams close to the area of bioeconomy are very useful
- There are different alternatives depending on the level of investment to be made. The cheapest alternative for the valorization of this type of by-products is composting, followed by the production of animal feed, energy, biofibers and functional foods, and biomaterials.

4.3. Case studies: biomass used in this thesis

Two residual biomasses were studied in this thesis.

First, spent coffee grounds resulting from the preparation of coffee beverage or from instant coffee production industries (agri-food waste). Second, discarded carrots as a waste from the agricultural and agri-food industry of carrot processing. These case studies are explained next.

5. Spent coffee grounds

5.1. Spent coffee grounds as residue

Spent coffee grounds is one of the main organic wastes from the agri-food industry. It is produced in the coffee beverage brewing process, but mainly in industries involved in the production of soluble coffee. To obtain soluble coffee, the coffee is extracted with hot water or steam and the extractives are released into the aqueous phase. The extract is then dried to formulate the product in solid form for sale and consumption, thus facilitating its transport, storage, use, and reducing its degradation. The production of soluble coffee is enormous, with 50 % of the total coffee crop being used for this purpose. Consequently, the production of spent coffee grounds reaches a worldwide figure of around 6 million tons per year (Ballesteros et al., 2014).

Due to the extraction process, coffee grounds are a residue that, like other vegetable residues, contains a high percentage of moisture. This high moisture makes it difficult to dispose of the waste in one of the most common ways, which is to incinerate it to obtain energy. The need for a prior drying process and the emission of greenhouse gases makes this process an unsuitable option. The other way to dispose of spent coffee grounds is to dump it in landfills, with the associated disadvantages of i) occupying land that could be used for other purposes (e.g. cultivation), ii) the emission of odors due to fermentation processes, and iii) the discharge of toxic components such as caffeine and tannins into the environment (Melo et al., 2014). Due to these drawbacks, the best alternative is the application of the biorefinery concept for the valorization of the components of this material. Spent coffee grounds have compounds of significant interest, are very abundant and have no other application competing with their use in biorefining.

The main components of spent coffee grounds from soluble coffee production are (on dry basis): water extractives (9.84 % w/w ± 0.41), ethanol extractives (2.40 % w/w ± 0.24), hexane extractives (20.00 % w/w ± 0.04), glucose oligo/polysaccharides (11.97 % w/w ± 0.05), mannose oligo/polysaccharides (17.46 % w/w ± 0.09), galactose oligo/polysaccharides (0.58 % w/w ± 0.03), lignin (26.90 % w/w ± 0.10), proteins (11.11 % w/w ± 0.77), and ash (0.60 % w/w ± 0.04) (Ramos-Andrés et al., 2019). Water extractives are not very abundant due to the prior extraction process performed to

obtain soluble coffee. The extractives in ethanol include other polar components that could not be extracted with water, and the extractives in hexane include the apolar compounds of the spent coffee grounds, among which the abundant oil stands out. Glucose in polysaccharide form may be part of both cellulose and hemicellulose. In the case of mannose and galactose, both are known to be part of hemicellulose, being of the glucomannan, galactomannan and/or galactoglucomannan type. The most abundant extractives are oil and hemicelluloses, so the valorization of both was studied in this thesis.

Figure 5. Spent coffee grounds (Karmee, 2018).

5.2. Spent coffee oil extraction

Spent coffee oil is characterized by being a highly saturated vegetable oil. The study of its applications focuses on its use in cosmetics as an additive, in the bioproduction of polyhydroxyalkanoates, and in the manufacture of biodiesel (Campos-Vega et al., 2015). In addition, the oil contains compounds of interest such as tocopherols, cafestol, and kahweol, which can be used as additives in the food, cosmetic and pharmaceutical industries (Battista et al., 2020).

The valorization of spent coffee oil is interesting because it is present in a considerable percentage in the raw material (approximately 20 %), but also because its extraction facilitates the subsequent extraction of the biopolymer hemicellulose, resulting in a more open structure. The extraction of oil, as a non-polar substance, is usually performed with organic solvents such as hexane. This has clear disadvantages as these solvents are expensive, toxic to human and harmful to the environment. The moisture

content of spent coffee grounds is an important parameter in oil extraction, as the higher the moisture content, the lower the extraction yield (Battista et al., 2020).

In recent studies, the extraction of oil from spent coffee grounds using different methods has been developed. Toda et al. (2021) studied the kinetics of extraction with an organic solvent, using absolute ethanol and hydrated ethanol. Hydrated ethanol gave worse results, but under pressure conditions and at a temperature of 150 °C the polarity of ethanol decreased and yields were high (up to 96.8 %). Colucci Cante et al. (2021) extracted the oil in an alternative way using hydrofluorocarbon Norflurane as solvent at 5-11 bar, obtaining good results with dry, wet and partially dry samples but requiring more extraction time the higher moisture content. Leal Vieira Cubas et al. (2020) sought to improve Soxhlet extraction efficiency by applying a pretreatment using a non-thermal plasma technology. They compared it with an ultrasound pretreatment. The pretreatment with plasma more than doubled the extraction yield compared with the Soxhlet extraction alone and improved by 30 % over the ultrasound pretreatment.

Regarding the use of supercritical CO_2, Araújo et al. (2019) combined it with ethanol acting as a co-solvent, and extracted the oil using a semi-batch process. A higher yield was obtained using supercritical CO_2 and ethanol combined than using each one individually. The maximum extraction percentage achieved was 15.9 % using a ratio of $scCO_2$:EtOH (2:1), 80 °C, 20 MPa, and 25 min extraction time. The use of ethanol increased the presence of antioxidants in the extract. Bitencourt et al. (2020) also extracted the oil accompanied by phenolic compounds under conditions of 333 K and 40 kPa, with pure supercritical CO_2, with ethanol, and with a mixture of supercritical CO_2 and ethanol. Coelho et al. (2020) performed the extraction with supercritical CO_2 at different temperatures and pressures, using isopropanol, ethanol, and ethyl lactate as co-solvents, which allowed reaching the maximum extraction yield in a shorter time than using supercritical CO_2 alone. The antioxidant capacity of the oil was increased up to 12.5 times with the use of co-solvents.

The oil extraction method selected for this thesis was the supercritical CO_2 treatment. It is advantageous because CO_2 is a cheap gas, could be considered a waste, and is non-toxic. Since the potential products produced in the spent coffee grounds biorefinery may have applications in the pharmaceutical and/or food industry, avoiding the use of toxic

compounds in the process is of great interest. The supercritical CO_2 extraction process also allows operation at low temperatures, thus reducing the risk of thermal degradation of some of the components extracted or present in the defatted solid. One of the most important advantages of the oil extraction in this thesis was to facilitate the subsequent extraction of hemicelluloses. Not using co-solvents for the extraction of antioxidant compounds results in these components remaining in the solid, so they could be partially co-extracted together with the hemicelluloses.

5.3. Spent coffee oil valorization

The transformation of the spent coffee oil was not one of the goals of this thesis. Nevertheless, we performed its chemical characterization for potential transformations.

Several authors have studied the production of biodiesel from spent coffee oil through direct transesterification into fatty acid methyl esters with short-chain alcohols such as methanol. The disadvantage is that the spent coffee oil is highly saturated, presenting high levels of free fatty acids that can hinder the transformation process leading to soap by-products due to the neutralization that can develop in the alkaline process. This phenomenon causes catalyst deactivation and forms unwanted emulsions. The high viscosity of the spent coffee oil also hinders its handling and the separation stages of the biodiesel formed. To solve this problem, in the work of Al-Hamamre et al. (2012) an extra acid-catalyzed stage was implemented before the alkali-catalyzed stage. In certain cases the biodiesel obtain by this double stage did not meet the requirement set in the NP EN 14214:2009 standards, but it can be blended with others. Tuntiwiwattanapun et al. (2017) produced biodiesel directly from spent coffee grounds without oil extraction, obtaining a yield of 77 %. A methanol wash of the feedstock reduced the presence of free fatty acids. The methanol was separated by evaporation and the biodiesel was separated from the glycerol by adding hexane, from which it was then separated by evaporation.

Other applications of the spent coffee oil are the production of polyhydroxyalkanoates, a class of polyesters which are primarily synthesized by species of Gram negative and/or Gram-positive microorganisms, anaerobic photosynthetic bacteria and archaea as intracellular carbon and energy source. It has been shown that coffee oil can be easily used as food and converted into PHA by microorganisms, being these biopolymers

completely biodegradable and with good properties as a thermoplastic material (Battista et al., 2020). Spent coffee grounds oil can be used for biosurfactant production, although there is still little work on this. Biosurfactants are substances with hydrophilic and hydrophobic zones with applications in various sectors such as petrochemicals, mining, metallurgy, fertilizers, agrochemicals, foods, pharmaceuticals, and cosmetics, among many others (Battista et al., 2020). Such biosurfactants would be produced by bacteria fed with the oil as a carbon source.

5.4. Valorization of the solid

5.4.1. Extraction of hemicelluloses

During the roasting process, some depolymerization and debranching of the hemicelluloses present in the coffee grounds occurs, which increases their solubility in water in the process of obtaining soluble coffee (Ballesteros et al., 2017). Despite this, around 70 % of the hemicelluloses remain in the coffee grounds matrix after the coffee brewing process or during the production of soluble coffee (Zabaniotou and Kamaterou, 2018). As mentioned above, these hemicelluloses are also more easily extractable once the oil has been fractionated from the raw material. Also, the process of obtaining soluble coffee results in the extraction of a large percentage of the free sugars present in the coffee, so the purity of the extracted hemicelluloses is much higher than if the hemicelluloses were extracted directly from the coffee rather than from the spent coffee. All these characteristics are advantages in terms of the application of the biorefinery concept to this defatted residue.

Regarding hemicelluloses from spent coffee grounds in literature, Mussatto et al. (2011) subjected spent coffee grounds to dilute acid hydrolysis for the recovery of monosaccharides from hemicelluloses using various operating conditions. The optimal conditions allowed obtaining 100 % of galactan, 77.4 % of mannan, and 89.5 % of arabinan, thus an efficiency of 87.4 % in the hydrolysis of hemicelluloses. The aim was therefore to obtain an extract rich in sugars whose one of its applications could be the production of mannitol from mannose. Getachew and Chun (2017a) studied the influence of microwave and ultrasound pretreatments on the hydrothermal extraction of spent coffee grounds at temperatures between 180-240 °C and pressures between 20-60 bar. The hydrolyzate was characterized by its content of phenolic compounds,

flavonoids and free sugars, so the extracts had good antimicrobial properties. Ravindran et al. (2018) led to the extraction of polyphenols and flavonoids. It also removed a considerable amount of lignin and then applied enzymatic hydrolysis to the solid in order to release the sugars. Pedras et al. (2019) performed extraction with ethanol and H_2SO_4 for semicontinuous extraction by hydrothermal treatment at different temperatures, pressures, and water flow. They achieved a carbohydrate yield of 33.7 g/100 g dry spent coffee grounds and phenolic yield of 4 g/100 g dry spent coffee grounds at 200 °C, 70 bar and 10 ml/min. The phenolic compounds were extracted before the carbohydrates, which were mostly recovered as oligosaccharides. Most of the work focusing on extraction has sought to obtain the hemicelluloses in their monomeric form or at most in their oligomeric form.

The extraction method of this thesis was hydrothermal treatment in a pilot-scale reactor. The reactor was operated in flow-through mode, so that the spent coffee grounds were loaded inside the reactor with a cartridge and the water circulated continuously entering from the top of the reactor and exiting from the bottom. This mode of operation causes the extract to continuously flow out of the reactor and fresh water to flow in, so that the mass transfer is considerably higher than in a batch extraction, resulting in a high extraction yield. Moreover, as the liquid time in the reactor is much shorter than in the batch operation, the extracted hemicelluloses undergo less depolymerization in the liquid phase and can reach very high molecular weight in the extract, in contrast to most of the works in the literature. Compared to continuous extraction in which a suspension of water and biomass is pumped, flow-through extraction simplifies pumping as only water is pumped, and it is possible to work with the desired amount of solid as long as there is space in the reactor, without any limitation regarding pumping. Once the water reached the desired operating temperature, it started to be circulated through the reactor and extraction took place. Over time, liquid samples were taken at the reactor outlet to study the evolution of the composition and molecular weight distribution of the hemicelluloses. The extract is collected in a jar for further concentration, separation, and purification treatment.

5.4.2. Concentration, separation, and purification of hemicelluloses

After extraction of hemicelluloses from spent coffee grounds, especially by hydrothermal treatment, the presence of impurities and a wide molecular weight distribution can be revealed. In the literature, no work has been found in which ultrafiltration or diafiltration is applied to biopolymers extracted from spent coffee grounds. However, biopolymers have been transformed into high value-added products with or without other purification methods. Fortunati et al. (2016) elaborated films from glucomannan from spent coffee grounds, with good properties such as gas barrier, flexibility and mechanical strength, being suitable for food packaging. Voepel et al. (2009) produced hydrogels for drug-delivery applications also from galactoglucomannan. Takao et al. (2006) purified hemicelluloses from spent coffee grounds using carbon chromatography. The hemicelluloses were obtained in their oligomeric form using hydrothermal treatment at 220 °C and were found to have good effects reducing abdominal and subcutaneous fat accumulation in humans when administered daily. Getachew and Chun (2017) reduced the molecular weight of hemicelluloses to their oligomeric form during hydrothermal treatment in a semicontinuous reactor at 180 and 220 °C, in order to improve their antioxidant capacity. The hemicelluloses were previously extracted from spent coffee grounds by alkaline treatment, 5 % of them being in monomeric form.

Thuvander and Jönsson (2016) applied a concentration and purification treatment using ultrafiltration with similar galactoglucomannnan hemicelluloses (they did not used spent coffee grounds hemicelluloses). The hemicelluloses were obtained from thermomechanical pulp mill with spruce as the main raw material. Ultrafiltration allowed to increase the concentration of hemicelluloses from 0.85-1.5 g/L to 25-52 g/L, with a feed volume reduction of up to 98 %. Subsequently, the same authors incorporated diafiltration to purify the hemicellulose, but did not achieve the desired effect due to the presence of lignin in the extract, which had a molecular weight similar to that of the retained hemicelluloses (Thuvander et al., 2016).

To condition the hemicelluloses for potential applications, ultrafiltration membranes were used in this thesis to concentrate, separate and purify. Three cascade membranes with MWCO of 30, 10 and 5 kDa were used. The feed was therefore fractionated into

three different fractions to which several diafiltration steps were applied for purification. Part of the diafiltration water was reused in smaller MWCO diafiltrations to recover certain entrained biopolymers and optimize.

6. Discarded carrots

6.1. Discarded carrots as residue

Carrot (*Daucus carota* L.) is one of the most widely cultivated vegetables with a global production of around 36 million tons (Encalada et al., 2019). Its production has increased by 30 % in the last decade (Varanasi et al., 2018). It is number 6 in the ranking of the 22 most popular vegetables (Jafari et al., 2017). The countries where most carrots are grown are China (more than 45 %), Russia (4.9 %), USA (3.7 %), Uzbekistan (3.4 %), Poland (2.5 %), Ukraine (2.4 %), and the UK (2 %) (Encalada et al., 2019). The remaining 36 % is distributed among more than 100 countries.

From a nutritional point of view, carrots are a source of nutrients including carotenoids, vitamins (A, B, C, D,E, and K), minerals (calcium, phosphorus, sodium, iron, and potassium), bioactive compounds, and biopolymers (Jabbar et al., 2014). Among the nutrients, carotenoids are responsible for the characteristic orange color of carrots which is the main criterion for acceptance or rejection by consumers. Carotenoids are essential in the human diet due to their key antioxidant activity (de Andrade Lima et al., 2018). Their functions include their transformation into vitamin A after consumption, participation in visual physiological metabolism, protecting cells from oxidation, properties related to the prevention of certain cancers, beneficial properties for the skin, and preventive properties against certain degenerative diseases (Ma et al., 2015). The most common carotenoids in carrots are β-carotene (60-80 %), α-carotene (10-40 %), lutein and lycopene, as well as their cis-isomers (Riganakos et al., 2017). The carotenoid content of 100 g of carrots is between 6-15 mg (Riganakos et al., 2017). Considering that the recommended intake of β-carotene for adults is 4.8 mg, carrots are an ideal source of this nutrient (Hiranvarachat and Devahastin, 2014).

Carrots are consumed in fresh form, as juice, in dried from or in the form of pre-cooked preparations. In juice production, significant amounts of waste are generated in the form of carrot pulp, which makes up 30-50 % of the initial weight of the carrot (Varanasi

et al., 2018). In addition to the pulp, about 11 % of the initial weight of carrots is lost as waste in the form of tubers, attached flesh and peels at the carrot cultivation stage (de Andrade Lima et al., 2018). However, the main waste derived from carrot cultivation and consumption is the so-called discarded carrots. Discards are those carrots that are not consumed due to defects related to their color, size, diameter, length or shape (e.g., they are intended to be straight to facilitate peeling). These discards constitute between 25-30 % of the total carrots grown (Encalada et al., 2019). Carrots may also be discarded in markets and homes because they have reached their expiry date and have deteriorated over time. As it is a vegetable waste, its handling is delicate due to it is an organic matter with up to 95 % moisture (Ramos-Andrés et al., 2020), so the main uses are landfilling, dumping in the field, and use for animal feed (Surbhi S et al., 2018). Only 15-20 % of the discards are destined for animal feed, so most of them end up causing some problems due to their lack of management (Clementz et al., 2019). The abundance of nutrients in the carrots means the discards use to rot quickly, causing bad odors, the presence of insects and other organisms.

Due to the abundance of this waste and the compounds it contains, it is interesting to apply the biorefinery concept to obtain high value-added products. The chemical characterization of the discarded carrots (pulp on dry basis) includes as main components sucrose (30.20 % w/w ± 1.44), glucose (19.44 % w/w ± 0.73), fructose (9.11 % w/w ± 1.53), water soluble pectin (5.12 % w/w ± 0.84), cellulose (10.71 % w/w ± 0.40), hemicellulose (8.43 % w/w ± 0.02), pectins (5.78 % w/w ± 0.34), proteins (2.26 % w/w ± 0.44), hexane extractives (1.20 % w/w ± 0.79), and lignin (7.75 % w/w ± 0.24) (Ramos-Andrés et al., 2020). Discarded carrots usually possess composition and therefore properties virtually identical to fresh carrots (Aimaretti and Ybalo, 2012). In the present thesis, almost all carrot components have been valorized: free sugars (sucrose, glucose and fructose), cellulose, hemicellulose, pectins, carotenoids (part of hexane extractives) and lignin.

Figure 6. Discarded carrots of the company Muñozval (Valladolid, Spain).

6.2. Discarded carrots in Castilla y León region

In Castilla y León, between 2013-2017 an average of 334,372 tons of root and bulb vegetables were produced, including 178,959 tons of carrots (Palacios Cuesta et al., 2020). The production of carrots resulted in the generation of only 2 % of its volume of by-products from cultivation, corresponding to leaves and root residues. Once the plant has been harvested, the stones and soil are removed, and the carrots are washed and sorted. If the product is destined for the fresh marked, the heads and tails are removed and incorporated into the soil. In this process, unsuitable carrots are also separated due to defects. If they are not in a bad state due to rotting or pest attacks, are destined for canning or freezing. In total, around 20 % of carrots are discarded in Castilla y León at the processing plant. Consequently, of the 178,959 tons of carrots grown, 3579 tons are by-products of cultivation, and 40,266 tons are by-products of the processing stage. The destination of carrot by-products in Castilla y León is mainly (Palacios Cuesta et al., 2020):

- Incorporation back into the field together with the soil resulting from the washing process. This is done just for the smallest pieces or broken pieces of small size.
- Use as feed for horses, cattle or sheep. The operator who carries out this work has containers and vehicles to transport the by-product and takes it directly to the farms, remaining in the plant for the minimum time possible, generally less than one day.

- In some cases, the discards have also been used for juice production, but this initiative has not been successful. Most juices are made from different raw materials to obtain a mixture that is more attractive to the consumer, more stable and easier to preserve. For this reason, carrots for juice have to be shipped to processing plants, and most of them are far away from the region.

6.3. Separation of juice and pulp

In the case of discarded carrots, the extractives in water are very abundant, so their separation is essential. Since the moisture content of this raw material is very high (approximately 95 %), separation of the juice is a way to separate the water soluble compounds and to increase the subsequent biopolymer extraction yield.

Carrot juice is less consumed than other juices due to its high sugar and low acid content, making it an easily fermented material in 1-2 days. Therefore, it is usually subjected to sterilization, blanching and pH lowering treatment (its original value is close to 6) to extend its shelf life (Zhang et al., 2016). For the valorization of discarded carrot juice, both sugars and carotenoids are the major compounds in the juice, so obtaining this juice by physical processes is a good method for the fractionation of these compounds separately from the carrot biopolymers present in the pulp. The physical separation of the juice it is simple and allows for no degradation of the compounds present, such as carotenoids, which could be partially damaged in an extraction with water at temperatures close to 100 °C. The carotenoid content of the juice is estimated to be 50 % of the total carotenoid content in carrot, but this value may vary depending on the separation process (Sharma et al., 2012). Since carotenoids are insoluble in water, it is assumed that their content is directly related to the solids content of the juice which are present in the form of micro and nanoparticles (Grimi et al., 2007). In contrast, free sugars are totally dissolved in the juice.

Once the juice has been separated, the pulp can be subjected to high temperatures for the extraction of biopolymers. The juice can also be valorized.

6.4. Valorization of the juice

Improving the stability of carrot juice to avoid its degradation represents most of the work in the literature. The application of lactic fermentation to develop a probiotic food with interesting properties for the food industry is also well studied.

Since the most abundant components of the juice are sugars and carotenoids, which are very different from each other, in this thesis the juice was subjected to a separation process for the valorization of each of these two components. The separation was performed with ultrafiltration membranes using diafiltration. The repetition of several diafiltration cycles improved the separation and resulted in purification of the retained fraction (rich in carotenoids) by reducing the sugar content. In each diafiltration cycle, a known volume of water was added to the feed tank and subjected to ultrafiltration until the feed recovered its original volume. In this way, the water carries away the dissolved components (sugars and other nutrients such as vitamins and minerals) in each cycle, while the undissolved solids (pulp microparticles where carotenoids are present) do not pass through the membrane. Before the application of diafiltration, microparticles larger than 50 μm were removed from the juice. At the end of the separation, the sugar rich fraction is that formed by the diafiltration waters and can also be obtained in the normal ultrafiltration process of the juice in the form of permeate. Each of the two fractions obtained can be valorized in different ways: fermentation of sugars and formulation of carotenoids,

6.4.1. Fermentation

The carotenoid-free fraction of the juice obtained after membrane separation can reach a very high sugar content of more than 100 g/L. It is therefore a perfect broth for fermentation. It also has the aforementioned advantages of having nutrients and no fermentation inhibiting compounds.

Fermentation experiments in the literature have shown that the autochthonous microorganisms in carrot are lactic acid bacteria, whose characteristics vary depending on the quality of the vegetable, the climate and the growth conditions (Gardner et al., 2001). However, fermentation has usually occurred in a hetero form with co-production of ethanol by yeasts (Di Cagno et al., 2008). Rakin et al. (2007) studied juice improvement using brewer's yeast autolysate as an additive. They demonstrated that

the nutrient content of carrot juice was beneficial for lactic fermentation. Gardner et al. (2001) developed lactic acid fermentation with different lactic acid bacteria applied to cabbage, carrot, and beet vegetable products. Their results showed the two-stage fermentation, with lactic fermentation taking place first, followed by ethanol fermentation by yeast. Avoiding co-fermentation to ethanol would be desirable in certain applications, and this is what Di Cagno et al. (2008) achieved due to the addition of NaCl an additive. In their work, they identified autochthonous microorganisms of carrots among other vegetables, confirming the presence of lactic acid bacteria and developing a suitable fermentation protocol. The operation with autochthonous microorganisms was characterized by a marked decrease in pH, rapid carbohydrate consumption, and prevention of invasion by Enterobacteriaceae and yeasts. Sharma and Mishra (2014) studied the lactic acid fermentation kinetics of the typical fermenting microorganism in vegetables, *Lactobacillus plantarum*.

Regarding the fermentation of carrots to obtain biochemicals other than lactic acid, Aimaretti and Ybalo (2012) produced bioethanol from discarded carrots using yeast discards, and obtained a yield of 0.408 g/g and productivity of 0.4 g/L/h without the need to make any modifications to the broth, except for pH adjustment. The culture broth was carrot must. In the study of Clementz et al. (2019), bioethanol was produced using two different culture broths from discarded carrots. One was the juice, and the other was a hot water extract. The main difference between the two broths was that the juice contained carotenoids and fibers, while the hot water extract did not, as these compounds are not extractable in water. Both broths were suitable for fermentation as they had a good C:N ratio and adequate P and K concentration. Zhang et al. (2020) produced volatile fatty acids, intermediates in the fermentation process, by applying anaerobic conditions to vegetable residues including carrots. The fermentation of carrots was characterized by a marked decrease in pH which ultimately inhibited the production of volatile fatty acids. Salvañal et al. (2021) extracted carotenoids from discarded carrots and fractionated two products: dietary fibers and sugar-rich syrup. Th sugar-rich fraction was fermented with two strains of *Rhizopus* spp. to obtain lactic acid. The maximum lactic acid concentration (22.18 g/L) was obtained by supplementing the syrup with additives. Survase et al. (2013) applied ABE fermentation with *Clostridium*

acetobutylicum DSM 792 using carrot waste as fermentation supplement. Supplementation with 5 % carrot residues resulted in a maximum product concentration of 9.96 g/L, while the control concentration was 7.43 g/L. Khoshkho et al. (2022) produced bioethanol by fermenting carrot pulp with *Saccharomyces cerevisiae* and beet molasses. A maximum ethanol production of 40.63 g/L was obtained.

In the present thesis, the three most typical modes of fermentation were studied: autochthonous microorganisms, lactic acid bacteria, and yeasts. The effect of NaCl as additive in low concentration for the inhibition of invading yeasts during fermentation was also studied.

6.4.2. Formulation of carotenoids

Carotenoids are known to degrade on exposure to heat, light and oxygen. Its formulation in encapsulated form and in solid phase as a powdered product improves storage conditions as it allows significant resistance to degradation by microorganisms, lipid oxidation, and certain enzymatic activities (Shishir and Chen, 2017). In addition, its morphology and coating with an encapsulating agent makes it more suitable for industrial applications as it improves its solubility and increases its bulk density (Shishir and Chen, 2017).

The most common methods of solid-phase carotenoids formulation are freeze drying and spray drying. Freeze drying occurs at very low temperatures minimizing thermal degradation. Spray drying takes place in much shorter times, but the high temperature joined to the contact with oxygen can lead to partial degradation of the carotenoids. On the other hand, spray drying in the encapsulation of vegetable substances sometimes has the disadvantage of stickiness due to sugars and acids, which have a low glass transition temperature (Guldiken et al., 2019). This is the temperature at which the solid changes from a glassy state to a rubbery state, which causes the solid to tend to stick to the walls of the dryer. The encapsulating agent not only protects the carotenoids but also minimizes potential stickiness problems. The fact that the carotenoids are obtained with a very low sugar content is also an advantage.

Carotenoids from carrots have been extracted by innovative techniques compared to the use of organic solvents, such as Elik et al. (2020) who applied microwave extraction

with flaxseed oil as solvent to discarded carrot pulp. By this method they were able to recover 77.48 % of the total carotenoids. The resulting extract had very good properties such as high antioxidant capacity due to the presence of phenolic derivatives. As an edible oil was used as solvent, the product obtained could be used almost directly in the food industry. In addition to chemical synthesis and carotenoid extraction, it is possible to synthesize carotenoids by applying biotechnology. Kaur et al. (2019) started from a variety of vegetable residues (orange, carrot, and papaya peels) and applied solid-state fermentation with *Blakeslea trispora* (+) MTCC 884, obtaining a maximum carotenoid concentration of 0.127 mg/mL.

Concerning the formulation of carotenoids and other active compounds from carrots, Guldiken et al. (2019) used spray drying to obtain coated and uncoated liposomes with encapsulated back carrot extract, whose main ingredient was anthocyanins. The stability of the active compound improved with physical and chemical encapsulation. Also from back carrot, Espinosa-Acosta et al. (2018) encapsulated using spray drying acidified alcoholic extracts rich in anthocyanins. They tested different encapsulating agents and operating conditions in search of an optimal way. Valorizing carrot juice, Janiszewska-Turak et al. (2017) encapsulated the juice using spray drying, employing different encapsulants (maltodextrin, gum Arabic, mixtures of both, and whey protein isolate). Characterization of the microcapsules obtained showed that gum Arabic was the best encapsulant for carotenoid retention and stability, and reduced stickiness problems associated with the high sugar content of the juice. The solid-phase formulation of the juice extended the shelf life of the juice by preventing fermentation, and the encapsulation reduced the degradation of the carotenoids present in the juice. Elik et al. (2021) performed the encapsulation of carotenoid enriched flaxseed oil using spray freeze drying employing sixteen different emulsion formulations based on amilodextrin, pectins and wax as encapsulating agents. This technique consists of atomizing the emulsion and then flash freezing the microdroplets in liquid nitrogen, followed by freeze drying. The spray freeze drying technique had a lower encapsulation efficiency than normal spray drying. Kaur et al. (2021) performed the encapsulation of β-carotene from carrot with sucrose as encapsulating agent and using a different method: co-crystallization. The encapsulation efficiency was 77.58 % and the antioxidant activity was

68 %. Šeregelj et al. (2021) compared freeze drying and spray drying methods in the encapsulation of carotenoids from carrot processing waste using sunflower oil as solvent. They studied the effect of three encapsulants, i.e. whey protein, maltodextrin and inulin. Haas et al. (2019) studied the impact of structure on carrot concentrate powders obtained using spray drying and freeze drying. Spray drying powders, with a low surface to volume ratio, showed higher carotenoid retention during storage (76-77 %) compared to freeze drying powders, characterized by a porous structure and resulting in rapid carotenoid degradation (69-93 %). Two different concentrates were studied, one containing crystalline carotenoids (carrot concentrate) and the other containing carotenoids dissolved in lipid droplets (carrot concentrate emulsion).

In contrast with the use of organic solvents, in the present thesis carotenoids were recovered by a physical separation method (diafiltration) embedded in microparticles of discarded carrot pulp. This method also allowed their separation from the sugars present in the juice, thus reducing stickiness problems in the drying process and increasing the purity in the solid product. Although they are not soluble in water, their formulation by encapsulation makes the carotenoids suitable for potential applications in the food and pharmaceutical sector. Drying and encapsulation were studied using the two most typical methods, spray drying and freeze drying.

6.5. Valorization of the pulp

6.5.1. Extraction of biopolymers

Hemicelluloses and pectins are the most easily extractable biopolymers and can be fractionated using environmentally friendly methods such as hydrothermal treatment. Carrot hemicelluloses are of the arabinogalactan type, consisting of a long chain of linked galactoses, with arabinose and galactose present in different branched forms. Immerzeel et al. (2006) studied the structure and indicated that arabinogalactan hemicelluloses are bound to pectins in carrots. The pectins in carrots, as in other vegetables, consist mostly of galacturonic acid unis constituting the structure called homogalacturonan. It was found that the presence of galacturonic acid favors some binding between arabinogalactan and homogalacturonan.

Encalada et al. (2019) obtained antioxidant pectin enriched fractions using high power ultrasound-enzyme assisted extraction applied to discarded carrots. First, ultrasound

treatment and then enzymatic digestion were applied. Pectins were co-extracted with antioxidants (carotenoids) by the use of ultrasound, resulting this product very interesting for the food industry. Sucheta et al. (2020) performed extraction of pectins from black carrot pomace in hot acidic water (pH 2.5) using microwave at 110 °C/5 min, ultrasound at 70 °C/30 min and conventional heating at 110 °C/90 min. Microwave was the most effective extraction method, followed by conventional heating and finally ultrasound. Idrovo Encalada et al. (2019) obtained pectin-enriched fractions by applying high-power ultrasound pretreatment and sodium carbonate to discarded carrots, with interesting applications in food. Christiaens et al. (2015) studied various vegetable residues as a source of pectins, including discarded carrots. Significant differences in pectins were found depending on the waste from which they are derived, making one or the other more suitable depending on the application. Jafari et al. (2017) performed optimization of pectins extraction as a function of operating conditions using acid extraction applied to carrot pomace. The maximum yield was 15.6 %, with good properties such as galacturonic acid content of 75.5 % and an emulsifying activity of 60.3 %. Mierczyńska et al. (2017) studied the chemical and rheological properties performance of pectin enriched fraction extractable using citric acid from various vegetables, including discarded carrots. The pectins showed good properties. Jayesree et al. (2021) applied carotene-pectin hydrocolloidal complexation for co-extraction of carotenoids and pectins from carrot peel waste.

In the present thesis, hemicelluloses and pectins from discarded carrots were extracted using hydrothermal treatment in flow-through reactors, as in the case of spent coffee grounds. Together with the hemicelluloses and pectins, a high concentration of free sugars was extracted, as these are the most abundant compounds in discarded carrot pulp. A single reactor was used to study the effect of time and temperature, and then several reactors were used to obtain sufficient extract to produce solid purified fractions for processing into products. The way to work with several reactors was through hydrothermal treatment in cycles, which involved three flow-through reactors operating in series at the same time. As the operating time of one reactor expired, that reactor was deactivated and a new reactor was added to the sequence, a process that was

repeated until the desired amount of discarded carrots was processed. The hydrothermal extract was recovered for further conditioning.

6.5.2. Concentration, separation, and purification of biopolymers

The extract containing hemicelluloses and pectin from discarded carrots is normally characterized by a broad molecular weight distribution, especially due to the abundant presence of free sugars.

As far as is known, there is no known work to date on the concentration, separation and purification of biopolymers extracted from discarded carrots. However, membrane treatments have been applied to biopolymers extracted from other biomasses. Silva et al. (2017) conditioned xylan from alkaline extraction of bleached pulp. Precipitation followed by washing was used as purification method and was applied to the filtered extract (not concentrated) and to the filtered extract concentrated by ultrafiltration. Concentration applying ultrafiltration allowed a considerable reduction in precipitating agent consumption. Compared to the hemicelluloses obtained using alkaline treatment, it is worth mentioning the work of Buruiana et al. (2017), who conditioned hemicelluloses obtained applying hydrothermal treatment of corn stover. Oligomers were obtained at a concentration of 11.7 g/L, and were treated applying diafiltration, ultrafiltration and freeze drying, reaching a concentration of 21.94 g/L and a purity of 89 %. Similarly, Rico et al. (2018) extracted hemicelluloses and lignin derivatives with high antioxidant activity applying non-isothermal hydrothermal treatment. The hemicelluloses had a concentration of 9.8 g/L, and after several cycles of diafiltration, their purity reached a value of 72.4 %. The residual solid after extraction was also interesting because it was enriched in cellulose and lignin.

Thuvander et al. (2018) focused on increasing the permeate flux in membrane operation by applying air sparging. The treated solution was an alkaline extract from wheat bran. In a later work, Thuvander and Jönsson (2019) treated these alkaline-extracted hemicelluloses by trying to increase the permeate flux by prefiltration of the extract, air sparging, and also nitrogen sparging. Nitrogen sparging had no effect on the flux, which seems to indicate that the effect of air sparging was due to a partial oxidative degradation of the hemicelluloses, which decreased their molecular weight. Oriez et al. (2019) applied alkaline treatment to sugarcane bagasse to extract hemicelluloses, lignin,

phenolic monomers, and acetic acid. Purification was performed applying ultrafiltration, comparing 7 different membranes. In all membranes was achieved the separation of lignin and hemicellulose oligomers on the one hand, and salts, phenolic monomers, and acetic acid on the other hand. The highest retention of lignin and hemicelluloses was 85 and 90 %, respectively. Thuvander and Jönsson (2020) performed a study of the techno-economic impact of the application of prefiltration and air sparging prior to ultrafiltration/diafiltration of hemicelluloses obtained by alkaline treatment. The flow rate increased from 52 L/m^2/h to 151 L/m^2/h during ultrafiltration, and from 46 L/m^2/h to 130 L/m^2/h during diafiltration, so the cost was significantly reduced. Al-Rudainy et al. (2020) treated spent sulphite liquor, a by-product of the sulphite pulping process of wood. This material contained polymeric hemicelluloses, so ultrafiltration was applied at pilot-scale and the results were compared with laboratory scale.

For pectin, Muñoz-Almagro et al. (2020) performed sunflower extraction with sodium citrate, and purified the pectins through alcohol precipitation, ultrafiltration + diafiltration, and microfiltration + diafiltration. The highest yield was obtained with the ultrafiltration + diafiltration method (13.3 %), as well as a purity higher than 90 %. Sabater et al. (2021) obtained pectins of a desired molecular weight applying enzymatic treatment and then purified by ultrafiltration, characterizing the retentate and the permeate. Oligosaccharides from di- to hexamers of galacturonic acid were obtained, as well as monomer units of galacturonic acid. The bioactivity of both fractions was studied. Neckebroeck et al. (2021) extracted a pectin-rich extract with nitric acid from onion flesh. The extract had a bimodal molecular weight distribution, so both fractions were separated by centrifugal ultrafiltration. The lowest molecular weight corresponded to galactans while the highest molecular weight was pectins. Shen et al. (2021) obtained pectins from leaves of *Aralia elata* (Miq.) seem. purified them by anion resin and sequence ultrafiltration columns. The fractions were structurally characterized in deep detail and showed bioactivity making them potential ingredients for the food and medical industry.

In the present thesis, hydrothermal extracts were subjected to concentration, separation, and purification treatment. Up to 4 ultrafiltration membranes were used in series (30-10-5-1 kDa), mixed (10-5-1 kDa; 10-30-1 kDa) and reverse (10-30 kDa)

configuration. Moderate volumes and high volumes of extract were processed for subsequent freeze drying or spray drying and transformation. In all cases, several diafiltration cycles were used to improve the separation and purification of the biopolymers, and part of the diafiltration water was reused as in the spent coffee grounds.

6.5.3. Drying and characterization of biopolymers

The purified biopolymer fractions have sometimes been dried to facilitate characterization, storage, and further processing.

For hemicelluloses, Jacquemin et al. (2012) obtained extracts from wheat straw and wheat bran by alkaline treatment. The extracts were purified applying evaporation and ethanol precipitation, and dried using freeze drying. As an alternative method, ultrafiltration, anion exchange chromatography and spray drying were combined. The purity of the solids was not high, 36.5 % in the first case and 24.6 % in the second. Rivas et al. (2020) performed a compete biorefinery process to obtain a solid product with an oligosaccharide purity of 90 %, obtained using freeze drying. These oligosaccharides were obtained from poplar applying hydrothermal treatment and purified with membranes.

Regarding pectins, Monsoor (2005) applied freeze drying, spray drying, and vacuum oven drying to hull pectin, comparing the methods. FTIR characterization showed no significant structural differences as a function of drying method, nor did any method have negative effects. Huang et al. (2017) studied the effect of different drying conditions applied to pectin obtained from sugar beet pulp. These conditions were hot air drying (40, 50, 60 °C), vacuum drying (40, 50, 60 °C), freeze drying, and spray drying (160, 190, 220 °C). No important differences in the structure of the obtained pectin were shown, but some differences in apparent viscosity and activation energy were observed.

In the present thesis, the hemicellulose/pectin fractions purified using ultrafiltration/diafiltration were dried. When the hydrothermal treatment was performed in a single flow-through reactor system, the hemicelluloses and pectin fractions were dried using freeze drying in order to characterize the fractions. Some of these fractions were in sufficient quantity to study their transformation into

biodegradable films. The purified fractions resulting from large-scale hydrothermal extraction with three reactor cycles in series were larger in volume and were spray-dried. These fractions were also characterized and then used in the formation of biodegradable films.

6.5.4. Residual pulp

Residual pulp is the pulp resulting from the extraction of hemicelluloses and pectins, in this case using hydrothermal treatment.

Guimarães et al. (2016) produced a suspension of microfibrillated cellulose from carrots by mechanical defibrillation, to be applied as reinforcement in starch edible films. The reinforcement resulted in lower water vapor permeability and higher tensile strength. Higher number of passages through the mechanical defibrillator resulted in lower fiber aggregation. Siqueira et al. (2016) also produced cellulose nanofibers from carrot pulp. They proposed various applications for these fibers, such as strong nanopapers with excellent mechanical properties. Otoni et al. (2018) started from carrot minimal processing waste and hydroxypropyl methylcellulose and high-pressure microfluidized cellulose fibers and formed biocomposites. The material properties were studied as a function of the content of each of the three ingredients and modelling was performed based on the results. The scale-up process allowed for continuous production by casting of 1.56 m^2 of biodegradable biocomposite every hour. Teh et al. (2019) applied life cycle assessment to study the production of nanocrystalline cellulose from defatted fruit bunch. The study compared three methods of obtaining nanocrystalline cellulose: acid hydrolysis I (chlorine bleaching), acid hydrolysis II (chlorine-free bleaching) and TEMPO-oxidation. The results showed that acid hydrolysis II had the lowest environmental impact, but acid hydrolysis I was more optimal from a technical and economic point of view. Berglund et al. (2020) applied a combination of life cycle assessment and life cycle costing to study nanofiber production from carrot waste. The treatment consisted of mechanical nanofibrillation via ultrafine milling and direct bleaching prior to nanofibrillation. The process was optimized to reduce the environmental impact.

In the present thesis, extraction in cycles of three reactors in series resulted in significant quantities of this residual pulp. The material was dried using freeze drying and was chemically characterized, showing that its main characteristic is the high cellulose

content and the presence of lignin in considerable quantities. It is therefore a material with potential applications in obtaining biomaterials where cellulose and lignin are suitable due to the properties they possess, such as strength and hydrophobicity, respectively. After characterization, the residual pulps obtained were further treated for use in the formation of biodegradable films together with purified solid fractions of hemicelluloses and pectins.

6.5.5. Biodegradable films

6.5.5.1. Need for biodegradable films

A sustainable concept for the production and the use of plastics is needed. Plastic has excellent mechanical and barrier properties, but it takes many years to degrade, and this entails a significant risk related to the presence of this material in the environment. Plastic also includes ingredients such as additives, colorants, stabilizers, processing aids, etc. (Asgher et al., 2020). Plastic producers are not obliged to indicate all the ingredients included in their material, so consumers are unaware of the compounds to which the food and beverage they consume are exposed.

Biorefineries would address the problem through the manufacture of biomaterials. Specifically, food packaging is one of the sectors that consume the most plastic, which is normally single use, so it is a field in which biomaterials are of great importance. The non-toxicity of films made from biomass, as well as their biodegradability, are the main advantages. Biodegradability means that they decompose in a relatively short period of time and in an environmentally non-toxic way.

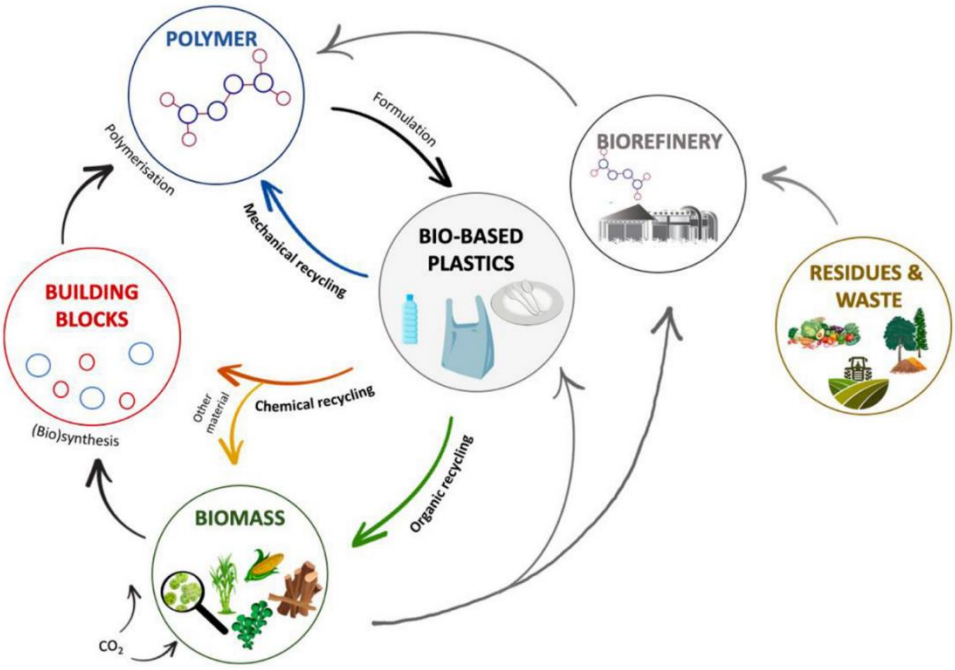

Figure 7. Production and recycling of bio-based plastics (Patrício Silva, 2021).

The objective of biopolymers is having sufficient quality relative to the main properties required of a material in food packaging: mechanical properties, hydrophobicity, water vapor permeability, and oxygen permeability, among others. The mechanical properties are evaluated in terms of the maximum tensile stress, which is the maximum stress a material can stand before it breaks; the maximum tensile strain, which is the maximum ratio of extension to original length; and the Young's modulus, which is the parameters expressing the stiffness of the material. Another important parameter is to consider the possible change in the characteristics of bioplastics during the period when the material interacts with the food (Asgher et al., 2020).

The main current difficulties in the production of bioplastics can be summarized as follows (Palacios Cuesta et al., 2020):

- The best-known bioplastics are obtained from agricultural products (maize, sugarcane, potato…), not from by-products, which may increase competition in land use with food, animal feed or energy use. According to the European Bioplastics Association, by 2024, it is estimated that 2 % of the usable agricultural land will be in demand for the production of bioplastics.

- The term “bioplastic” is often used incorrectly for any biodegradable material, although there are biodegradable plastics obtained from non-renewable sources which are therefore not bioplastics.
- Further study is needed on the biodegradation of bioplastics, as some of them fragment rapidly but the fragments take time to degrade, and vice versa. The effect of their biodegradation on the environment has yet to be studied.
- The cost-effectiveness of bioplastics production is often still lower than that of petroleum plastics, so optimization steps are still required, not to mention the influence of the high or lower price of a barrel of oil.

In Castilla y León, the use of plant material for the manufacture of bioplastics and fibers is mainly oriented towards packaging and the production of coverings for agriculture, the latter being an application in which these bioplastics can be quickly incorporated into the soil (Palacios Cuesta et al., 2020). Some research is still needed for further industrialization of the production process. The further industrialization of bioplastics is associated with greater contact between companies and academia, as it is a relatively new field and certain points of research are of great importance; i) identifying the compounds of interest and the form in which they are present in biomasses, ii) studying the effects that these compounds have on the environment or on humans, iii) optimizing extraction processes at laboratory scale and proceeding to scale-up at pilot and industrial level.

Films are made of biopolymers of various origins. Biopolymers of microbial origin (polyhydroxyalkanoates and polylactic acids), lignocellulosic biopolymers (cellulose, hemicellulose, pectins, starch and lignin), and protein type biopolymers (gelatin, keratin, gluten, soy protein, and whey protein isolates) stand out (Asgher et al., 2020). Specifically, the most widely used bioplastics are polylactic acids from corn or sugarcane and polyhydroxyalkanoates from microorganisms (Palacios Cuesta et al., 2020). The raw materials most commonly used for the production of bioplastics are: starch from banana, maize, sugarcane, potato, sunflower, rapeseed, nut shells and other fruit shells, wheat husk, peanut, bamboo, rice husk, cassava starch and corn starch (Palacios Cuesta et al., 2020).

In the present thesis, the biopolymers used were those present in discarded carrot pulp: hemicelluloses, pectin, cellulose, and lignin. The hemicellulose and pectins were as described above extracted using hydrothermal treatment, conditioned by multiple stages of ultrafiltration and diafiltration, and dried using freeze drying or spray drying. Cellulose and lignin biopolymers were recovered through the residual solid remaining after hydrothermal treatment of the discarded carrot pulp.

Both in discarded carrot pulp and in any lignocellulosic material, the matrix is composed of a combination of cellulose, hemicellulose, lignin, and pectin. Each biopolymer has different characteristics, so in the formation of biodegradable films or any biomaterial, it is interesting to imitate nature's strategy and combine the same ingredients, in greater or lesser proportion and after treatment. Polymer blending is the method used in this thesis, based on mixing more than one polymer to obtain materials. It is a simple process that does not require the establishment of chemical modifications. One of the advantages associated with its simplicity is that the biodegradability of the biopolymers is usually maintained after the blending process, as these biopolymers do not undergo significant changes and it is known that biodegradability does not depend so much on the origin of the polymer as on the type of chemical bond it presents. The interactions established between biopolymers in the blending process can be hydrophilic/hydrophobic, electrostatic, or hydrogen bridging (Asgher et al., 2020).

6.5.5.2. State of the art

Arabinogalactan hemicellulose

There is little work in the literature on films made from arabinogalactan. Lucyszyn et al. (2016) prepared films by combining residual bacterial cellulose mechanically defibrillated with arabinogalactan and xyloglucan. Arabinogalactan was extracted from *Pereskia aculeata* leaves and xyloglucan from *Guibourtia hymenaefolia* seeds. The incorporation of xyloglucan improved the stability of the films in water due to the good affinity of xyloglucan for cellulose. The incorporation of arabinogalactan resulted in a film with potential biomedical applications as biocompatible wound dressing. Gheribi et al. (2019) valorized *Opuntia ficus-indica* peels, an agricultural by-product, by extracting the mucilage. The mucilage was composed of 97 % carbohydrates, mainly of the arabinogalactan type, which had good water solubility and emulsifying capacity. Films

were produced from arabinogalactan with a high water contact angle (91°), high solubility (42 %) and grease proof character. The mechanical properties were decent for food packaging. Oliveira et al. (2019) extracted arabinogalactan from *Pereskia aculeata* leaves and formed films with concentrations of arabinogalactan between 1.5-2 % in water, and glycerol between 20-25 % (w/w). The elongation reached values of 46.10 %, maximum load of 13.8 N, and water vapor permeability of 8.288 g·mm/day/m^2/kPa.

Other hemicelluloses

There is a multitude of works focused on the formation of films from different types of hemicelluloses. Most of them have resorted to chemical modification of the hemicelluloses or the addition of a reinforcing agent to improve the mechanical properties of the film. In other works, however, native hemicelluloses were the only biopolymer in the formation of the films.

By chemically modifying the hemicelluloses, Guan et al. (2016) produced drug-loading films by the cross-linking reaction of quaternized hemicelluloses and chitosan with epichlorohydrin as crosslinker. The mechanical properties were very good, with a tensile strength up to 37 MPa. The loading behavior of the film was investigated by adding ciprofloxacin, which reached a maximum loading concentration of 18 %. Works in which a fortifying agent was added are worth mentioning. Pereira et al. (2017) produced films based on wheat straw hemicelluloses combined with cellulose nanocrystals and citric acid. The reinforced films had better mechanical properties and higher water barrier. Citric acid had a dual effect as a plasticizing agent and as a crosslinker. The optimum conditions were 5.9 % (w/w) of cellulose nanocrystals and 30 % (w/w) of citric acid. Rao et al. (2019) incorporated graphene oxide into quaternized hemicelluloses for film formation. The films had a tensile strength of 43.83 MPa, so the graphene oxide had a good effect as a reinforcing agent. In addition, the films had a high sensitivity to humidity, bending spontaneously in high humidity and stretching in low humidity conditions. Xu et al. (2019) prepared plasticized films based on hemicelluloses/chitosan reinforced with cellulose nanofibers. The tensile strength was increased by 2.3 times with the addition of 5 % cellulose nanofibers. Among various plasticizing agents, glycerol gave the highest tensile strain at break (7.80-18.53 %). Mugwagwa and Chimphango (2020) made films from acetylated hemicellulose reinforced with nanocellulose and

coated with polycaprolactone. The films were evaluated as active packaging for aqueous, alcoholic, fatty and acidic food. The films were also doped with polyphenols. The hydrophobicity increased from a water contact angle of 24.29° to 82.48°, and the solubility was reduced by 82.8 %. Weerasooriya et al. (2020) produced hemicellulose-based films with addition of carboxymethyl cellulose. The hemicellulose was extracted by alkaline treatment of defatted fruit bunch. The best mechanical properties were obtained with a hemicellulose content of 60 %, and structural analysis showed no modification in the structure of carboxymethyl cellulose by mixing with hemicellulose. The films were suitable for food packaging. Sutay Kocabaş et al. (2021) valorized the waste bulgur bran from bulgur production by extracting hemicelluloses. They formed hemicellulose-based films and to improve the properties they added commercial cellulose nanocrystals and cellulose nanofibers. The addition of citric acid was also investigated as a plasticizing agent. The incorporation of cellulose nanocrystals and cellulose nanofibers improved the tensile strength of the films but decreased the water vapor permeability and biodegradability.

Without any chemical modification or reinforcing agent, Mendes et al. (2017) formed films with glycerol and galactomannan-type hemicelluloses extracted from *Caesalpinia pulcherrima* and xyloglucan-type hemicelluloses extracted from *Tamarindus indica*. The hemicelluloses had a very high molecular weight, and their solutions showed non-Newtonian behavior. Galactomannan films had higher water vapor permeability than xyloglucan films. In general, the films showed good thermal stability and beneficial properties for food packaging. Chadni et al. (2020) studied the influence of the method of hemicellulose extraction on film formation. Arabinoglucuronoxylans and galactoglucomannans were extracted from spruce sawdust pre-soaked in water or NaOH by steam explosion, microwave assisted extraction, and high voltage electrical discharges pretreatments. Steam explosion gave the highest extraction yield and microwave treatment the highest molecular weight. The plasticized films had a good oxygen barrier in the extraction of the material pre-soaked in NaOH due to the intermolecular bonding of hemicelluloses and lignin.

Pectins

There are many studies in which films have been formed from pectins. In these cases, unlike hemicelluloses, chemical modification and the addition of reinforcing agents have not usually been used, but the incorporation of bioactive agents has been studied in order to give the films specific properties both in packaging and in biomedicine.

Gouveia et al. (2019) aimed to improve the properties of pectin-based films. They produced thermoplastic pectin by thermo-compression molding using a natural deep eutectic solvent as plasticizer. Glycerol and choline chloride as plasticizers were also used for comparison. An increase in compression time resulted in a decrease in tensile strength. The films were suitable for food packaging in the case of foodstuffs of a low moisture content. Spatafora Salazar et al. (2019) aimed to improve the water vapor barrier of orange and mango peel pectin films by adding silicon dioxide nanoparticles. The WVP was reduced by 30-60 %. Chaichi et al. (2019) tried to improve the mechanical properties of pectin-based films by crosslinking with calcium ions in two steps as well as studying the influence of glycerol content. Ca^{2+} ions reduced the swelling degree and elongation at break, and increased the tensile strength. The optimum Ca^{2+} and glycerol content was determined.

Norcino et al. (2020) prepared pectin films including copaiba oil nanoemulsion as active ingredient. The nanoemulsion was suitably dispersed in the film with good interaction with pectin. The properties changed considerably, decreasing the elastic modulus and tensile strength, and increasing the roughness, elongation at break, and antimicrobial activity. Meerasri and Sothornvit (2020) incorporated gamma-aminobutyric acid as a bioactive ingredient in a percentage between 5-15 % into pectin films. The mechanical properties were similar to those of the reference film, while the film solubility and water vapor permeability decreased. The active agent not only acted as an antioxidant but also had plasticizing effects similar to those of glycerol in the reference film. Younis et al. (2020) modified the properties of apple pectin-based films through the incorporation of chitosan/pectin fibers. The fibers were needle, spindle or whisker shaped, and their incorporation was concentrated on the top side of the films. The small proportion incorporation resulted in improved water barrier, higher thermal stability and better mechanical properties. Guo et al. (2021) studied the effect of ultrasound treatment on

films formed by watermelon peel pectins. Ultrasound treatment decreased the particle size and turbidity of the film-forming solution and changed the rheological properties. A 10 min treatment dispersed the pectin molecules and increased hydrogen bonding interactions, so that oxygen permeability, water vapor permeability and tensile strength decreased, and density and elongation at break increased. A longer treatment of 15 min was no beneficial.

The films produced in this thesis had as main ingredient the purified fractions of hemicelluloses and pectins corresponding to the highest molecular weight (> 30 kDa). Although an attempt was made to make films with the fractions obtained in the 10, 5 and 1 kDa membranes, these fractions did not form films. Since hemicelluloses and pectins are characterized by their hydrophilicity and relatively poor mechanical properties, they are usually chemically modified or combined with other polymers. In the present thesis, the process led to fractions of sufficient quality not to require chemical modification or combination with other materials. However, the addition of the fraction of discarded carrots abundant in cellulose and lignin in different percentages was studied. Within the study range, films were also produced with the purified hemicellulose and pectin fraction as the only ingredients, and films with the cellulose and lignin rich fraction as the only ingredient. In all cases, glycerol was used as a plasticizer agent. The plasticizer agent reduces intermolecular interactions giving a smoother and flexible material. The molecular weight distribution and the hemicellulose/pectin ratio were the two main parameters of the purified biopolymer fractions obtained, so their influence on the quality of the films formed was also studied. The quality of the films, as they are intended for use in food packaging, was evaluated in terms of mechanical properties, hydrophobicity, oxygen barrier, water vapor barrier, and structure. For the use of the cellulose and lignin rich fraction in this thesis, the residual pulp fraction was subjected to a multistep treatment, without the use of chemical agents or chemical reaction, which allowed obtaining cellulose nanofibers combined with lignin by mechanical treatment (milling and high-pressure homogenization).

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Aims and contents

The main objective of this doctoral thesis is the application of the biorefinery concept to agricultural or agri-food waste for the valorization of all the components present in the material with a view to their transformation using environmentally friendly processes into products.

This overall objective is translated into a number of partial objectives:

1. Application of the biorefinery concept to spent coffee grounds, fractioning them into (i) oil, (ii) hemicelluloses hydrolyzed, and (iii) cellulose and lignin-rich solid. For this purpose, the following tasks have been carried out in Chapter 1:
    a. Chemical characterization of the spent coffee grounds.
    b. Extraction of spent coffee oil with supercritical CO_2 and chemical characterization of the extracted oil.
    c. Study of the influence of temperature (140, 160 °C) and time on the extraction of hemicellulose from spent coffee grounds applying hydrothermal treatment in a flow-through reactor system.
    d. Concentration, separation, and purification of the extracted hemicelluloses applying ultrafiltration and diafiltration resulting in purified fractions of low, intermediate and high molecular weight.
2. Application of the biorefinery concept to discarded carrots by separating the juice from the pulp and valorizing the pulp components.
    For this purpose, the following tasks have been developed in Chapter 2:
    a. Chemical characterization of discarded carrot pulp.
    b. Extraction of the biopolymers hemicellulose and pectin from discarded carrot pulp applying hydrothermal treatment in a flow-through reactor system, studying the influence of temperature (140, 160, 180 °C) and reaction time.
    c. Chemical and structural characterization of residual pulp after hydrothermal treatment.

3. Application of the biorefinery concept to the juice of discarded carrots, valorizing the carotenoids and free sugars.

   To this end, the following tasks have been carried out in Chapter 3:

   a. Separation of discarded carrot juice into a carotenoid-rich fraction and a free sugars-rich fraction applying multiple diafiltration cycles.
   b. Formulation of carotenoids from discarded carrot juice via encapsulation and drying.
   c. Transformation of free sugars from discarded carrot juice through different types of fermentation to obtain lactic acid and ethanol.

4. Conditioning of hydrothermal extracts obtained at different temperatures from discarded carrot pulp applying ultrafiltration and multistage diafiltration.

   To this end, the following tasks have been developed in Chapter 4:

   a. Determination of the composition and molecular weight distribution of hydrothermal extracts (140, 160, 180 °C).
   b. Concentration, separation, and purification of hemicelluloses and pectins by means of several membranes in cascade or mixed configuration, in both cases applying multiple diafiltration cycles.
   c. Drying of the obtained purified fractions.

5. Application of pilot-scale biorefinery concept to discarded carrot pulp to obtain fractions in sufficient quantity for processing into products.

   To this end, the following tasks have been carried out in Chapter 5:

   a. Hydrothermal treatment of discarded carrot pulp in cycles of several flow-through reactors in series operating at the same time, at low and high temperature (140, 180 °C).
   b. Conditioning of the two extracts obtained using several membranes in mixed configuration, applying ultrafiltration and multiple diafiltration cycles.
   c. Study of fouling in membrane operation.
   d. Drying of the purified fractions obtained using freeze drying and spray drying.
   e. Chemical, molecular weight, and structural characterization of purified fractions

6. Processing of large-scale discarded carrot pulp fractions into biodegradable films for food packaging applications.

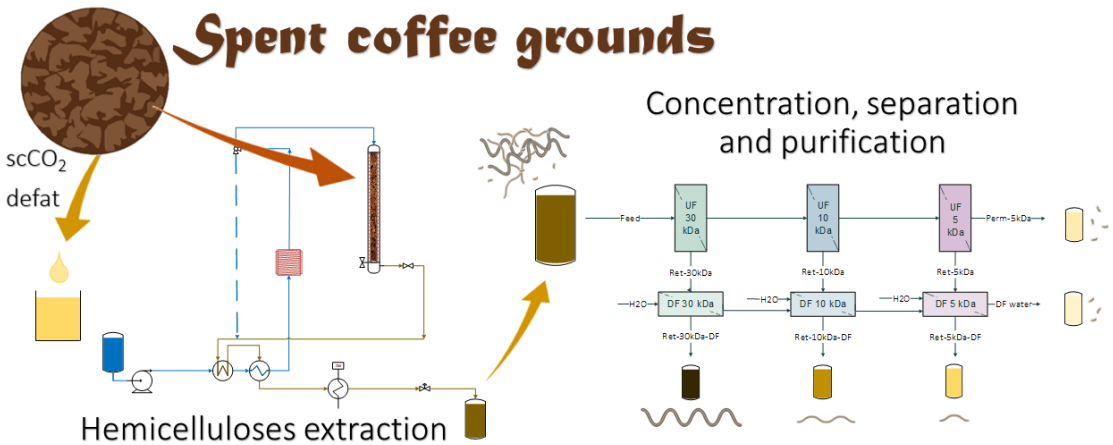
   For this purpose, the following tasks have been developed in Chapter 6:

   a. Chemical characterization of residual pulps resulting from large-scale hydrothermal treatment at 140 and 180 °C.
   b. Application of mechanical treatment to residual pulps to obtain lignin-containing cellulose nanofibers.
   c. Formation of biodegradable films based on purified, highest molecular weight hemicellulose/pectin fractions obtained on a large scale.
   d. Comparison of both residual pulps (140, 180 °C) in terms of the properties they contribute to films based on hemicellulose/pectin.
   e. Study of the influence of higher or lower residual pulp content as an additive on the properties of hemicellulose/pectin-based films.
   f. Study of the influence of molecular weight and composition of the hemicellulose/pectin fraction on film properties.

Chapter 1

Production of molecular weight fractionated hemicelluloses hydrolyzates from spent coffee grounds combining hydrothermal extraction and a multistep ultrafiltration/diafiltration[1]

Spent coffee grounds are a huge residual stream from instant coffee makers. The production of spent coffee oil and molecular weight fractionated hemicellulose hydrolyzates via supercritical CO_2 and a hydrothermal treatment followed by concentration, separation, and purification through cascade ultrafiltration/diafiltration (30-10-5 kDa) was studied. Hemicelluloses extraction yield reached 3.49 g/100 g of dry defatted spent coffee after 40 min at 160 °C. The ultrafiltration system allowed concentrating up to 5-fold certain groups of hemicellulose, being most of them retained in the first membrane. Hemicellulose concentration and molecular weight of the feed exerted a great influence on the mass transfer through the membrane due to the formation of aggregates. However, purification through diafiltration allowed both to decrease by-products retentions from 45.6 % to 8.7 %, increasing the molecular weight of each fraction. Six hemicellulose products were obtained with purities between 83.7 – 97.8 % (w/w) and weight-average molecular weights between 1641 and 49,733 Da.



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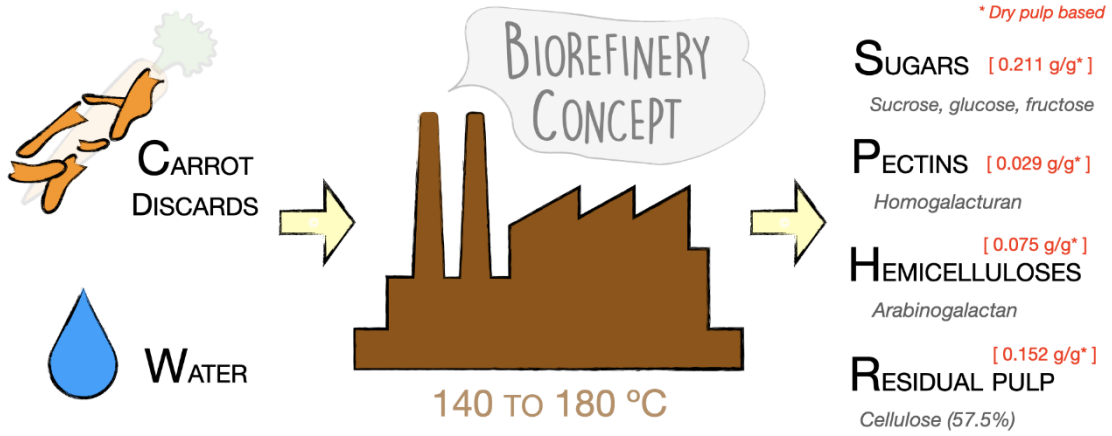
Chapter 2

Hydrothermal production of high-molecular weight hemicellulose-pectin, free sugars and residual cellulose pulp from discarded carrots[1]

Discarded carrots account for 30 % of the total production ending up in landfills, land, or a small part as cattle food. Valorization of discarded carrot pulp was studied by hydrothermal treatment, fractionating free sugars, hemicellulose and pectin in the liquid phase and residual pulp in the solid phase. Extraction took place in flow-through mode at 140, 160 and 180 °C, achieving recoveries up to 211.0 g/kg dry pulp of free sugars, 29.13 g/kg dry pulp of homogalacturonan pectin, and 70.45 g/kg dry pulp of arabinogalactan hemicellulose. The residual pulp reached a cellulose content of 57.5 % (w/w) while before the treatment it was 10.7 % (w/w). Most of the free sugars were extracted in the preheating stage in batch, so they could be obtained separately from the biopolymers. The flow-through extraction allowed to obtain hemicellulose and pectin of molecular weights > 30 kDa. The evolution of different ranges of molecular weight was studied in detail for a better understanding of the phenomenon of autohydrolysis and the link between hemicellulose and pectin. The ample molecular weight distribution in the hydrolysate allows for a subsequent fractionation via ultrafiltration membranes, to obtain a high molecular weight fraction for applications such as film-forming (in combination with the residual pulp).



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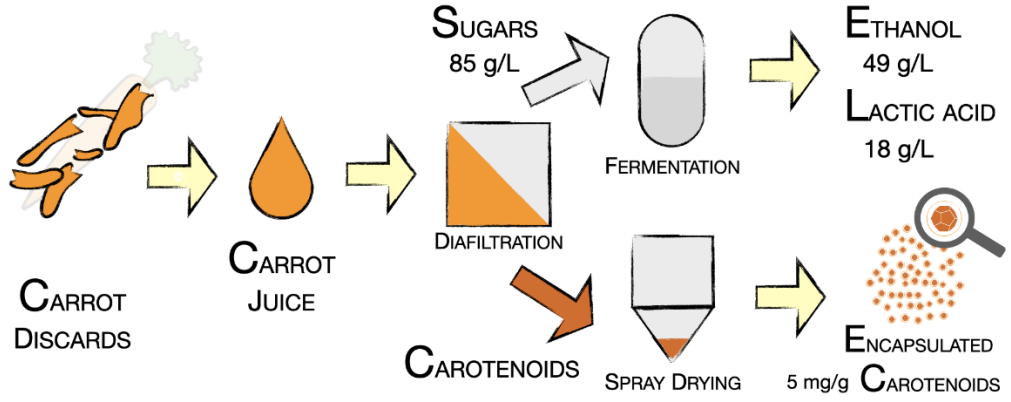
Chapter 3

Biorefinery of discarded carrot juice to produce carotenoids and fermentation products[1]

Discarded carrots are a major food waste that is produced from cultivation to sale. This waste has high humidity, which generates not only economic but also environmental problems, requiring a specific biorefinery for its valorization. The pulp and the juice were separated and received a different treatment. The juice was subjected to a process of separation and purification using several diafiltration cycles (30 kDa) giving rise to a fraction rich in carotenoids (4996.4 µg/g) and a fraction rich in sugars (84.83 ± 3.26 g/L) and nutrients. The carotenoids have potential applications in the food and pharmaceutical industry; therefore, they were formulated through encapsulation with gum Arabic using spray drying and freeze drying. Encapsulation using spray drying was effective as it improved stability in water and reduced degradation by 51.9 % compared to unencapsulated carotenoids. The sugar-rich fraction was valorized to obtain lactic acid and ethanol through three types of fermentation: with autochthonous microorganisms, with lactic acid bacteria, and with yeast, resulting in obtaining mostly lactic acid (17.64 ± 1.54 g/L) or ethanol (49.46 ± 0.28 g/L). The addition of 6 % (w/v) of NaCl to the medium allowed the production of pure lactic acid with both autochthonous microorganisms and lactic bacteria. Sugar consumption was high (92.4 - 97.5 %) except in cases with autochthonous microorganisms (23.3 %) and lactic bacteria (43.8 %) where a pH control seems to be necessary for total sugar consumption.



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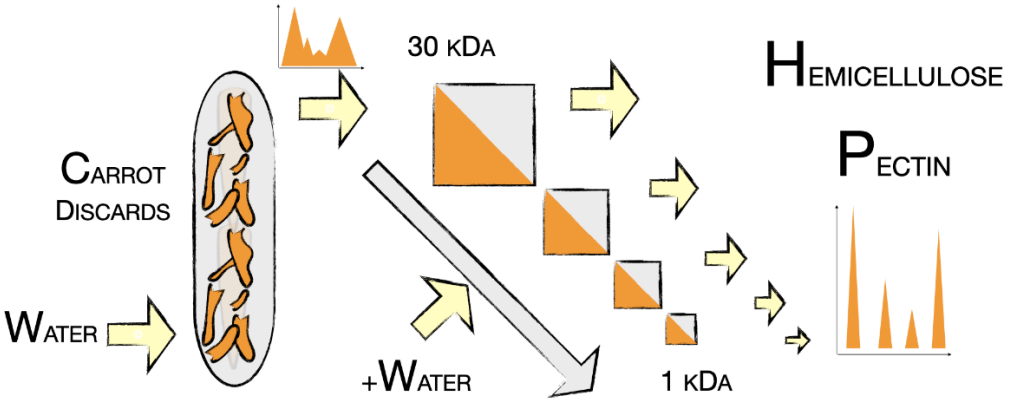
Chapter 4

Production of purified hemicellulose-pectin fractions of different molecular weight from discarded carrots by hydrothermal treatment followed by multistep ultrafiltration/diafiltration[1]

Hemicelluloses and pectins are good candidates as biopolymers for the formation of products such as packaging films. Purified freeze-dried fractions of hemicelluloses and pectins, of different molecular weights, were obtained by treating hydrothermal extracts (140, 160, and 180 °C) of discarded carrots with ultrafiltration membranes (30, 10, 5, and 1 kDa). After each ultrafiltration, several cycles of diafiltration with partial water reuse were applied, obtaining a better separation and purification. A cascade configuration (30-10-5-1 kDa) was used in the 140 and 160 °C extracts, and a mixed configuration (5-10-1 kDa) in the 180 °C extract. High molecular weight hemicelluloses increased in concentration by a factor of 5 in the cascade configuration and by a factor of 16.67 in the mixed configuration. A high removal of free sugars (98.9-99.5 % w/w) and by-products (94.4-99.2 % w/w) through 1 kDa permeate and diafiltration waters was obtained. The system allowed moving from feeds with molecular weight, polydispersity, and purity in the ranges 9.02-18.83 kDa, 16.2-31.6, and 30.12-33.51 % (w/w) to fractions with values in the ranges 2.59-102.75 kDa, 1.2-4.0, and 73.1-100 % (w/w).



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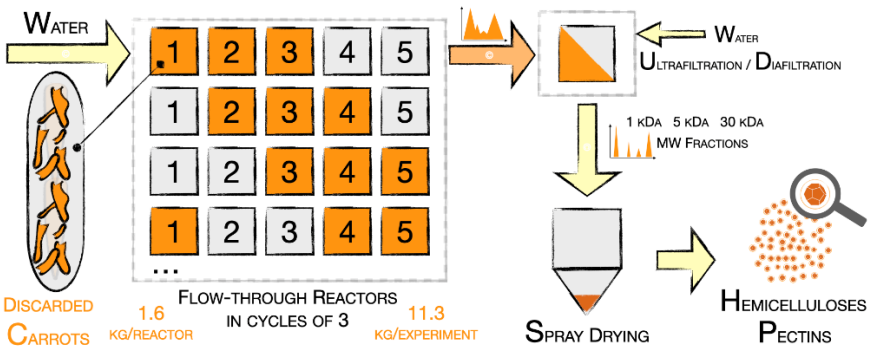
Chapter 5

Pilot-scale biorefinery for the production of purified biopolymers based on hydrothermal treatment in flow-through reactor cycles[1]

Purified solid fractions of hemicellulose and pectin biopolymers with different molecular weight were produced on a pilot-scale from discarded carrots. The pulp was subjected to hydrothermal extraction on a pilot plant operating in cycles of three flow-through reactors in series at 140 and 180 °C. Two operational modes of start-up (with and without water pre-filling) were tested, showing that it influences the final hydrolysate concentration and the stability of the system. Free sugars, arabinogalactan hemicellulose and homogalacturonan pectin were produced at maximum recoveries of 379.5 g/kg dry pulp, 81.0 g/kg dry pulp and 5.35 g/kg dry pulp, respectively, with the extraction of arabinogalactan reaching a yield of 96.1 % (w/w). The extracted biopolymers were separated and purified through ultrafiltration and diafiltration cycles using a multi-membrane system (30, 10, 1 kDa). Ultrafiltration and especially diafiltration allowed going from extracts with molecular weight, polydispersity and purity values of 14.77 kDa, 19.2 and 22.2 % w/w (140 °C extract) and 8.08 kDa, 18. 2 and 14.9 % w/w (180 °C extract), to fractions with values of 80.36 kDa – 67.77 kDa - 9.85 kDa – 5.23 kDa and 3.86 kDa (molecular weight), 1.3 – 3.8 (polydispersity), and 64.5 – 100 % w/w (purity). The five fractions were freeze-dried and spray-dried. The pilot-scale system allowed the production of purified biopolymer fractions of high purity, different average molecular weight, and in sufficient quantity for their subsequent transformation into products such as biodegradable films.

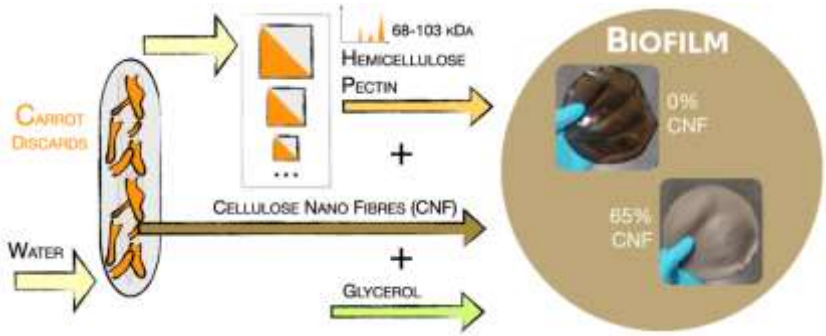


[1] Ramos-Andrés, M., Díaz-Cesteros, S., Majithia, N., García-Serna, J., 2022. Pilot-scale biorefinery for the production of purified biopolymers based on hydrothermal treatment in flow-through reactor cycles. Chemical Engineering Journal. 135123, https://doi.org/10.1016/j.cej.2022.135123

Chapter 6

Biofilms from purified hemicellulose and pectin fractions and residual pulp from hydrothermally treated discarded carrots[1]

Biodegradable films were produced from discarded carrot fractions. The main ingredient was purified high molecular weight fractions of hemicelluloses and pectins previously obtained by hydrothermal treatment and ultrafiltration/diafiltration. As an additive agent in the films, a fraction of lignin-containing cellulose nanofibers was used. This fraction was obtained from a mechanical treatment applied to the residual solid of the hydrothermal treatment. The influence of residual pulp content on film properties was studied, showing that a small residual pulp content (< 5 %) improved oxygen permeability (up to 29 %) but worsened water vapor permeability and tensile properties. A higher residual pulp content (5-25 %) allowed to recover of the properties of the reference film (without residual pulp) and provided a higher hydrophobicity increasing the water contact angle from 79.9° to 125.8°. The study of the influence of the molecular weight (67.77-102.75 kDa) and composition of the hemicellulose and pectin fraction in films containing 1 % residual pulp showed that a higher molecular weight decreased oxygen permeability (from 48.18 to 41.14 $cm^3 \cdot \mu m/m^2/kPa/day$), increased water vapor permeability (from 21.56 to 24.01 $g \cdot mm/m^2/kPa/day$), and decreased hydrophobicity (from 86.84° to 71.10°). Tensile strength was higher with higher pectin content and lower molecular weight (from 1.13 to 2.84 MPa), while elongation was higher with higher hemicellulose content (from 5.92 to 15.28 %). The films obtained had acceptable properties for food packaging and high hydrophobicity, with the great advantage of being 100 % from agri-food waste by applying environmentally friendly processes and without the need for chemical modification.



[1] Ramos-Andrés, M., Hu, L., Xu, C., Grénman, H., García-Serna, J., 2022. Biofilms from purified hemicellulose and pectin fractions and residual pulp from hydrothermally treated discarded carrots. In the process of being submitted to a journal.

1. Introduction

1.1. Plastic problem

Global concerns about environmental issues and declining petroleum resources are the main problems currently facing the plastics industry. The global production of plastics is about 320 million tons/year, with increasing demand due to population growth (Asgher et al., 2020). Some of the most commonly used plastics are polyvinylchloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), polyethylene (PE), polyamide (PA), polystyrene (PS), and ethylene vinyl alcohol (EVOH) (Asgher et al., 2020). These materials are noted for their excellent barrier and mechanical properties, as well as their cost-effectiveness. Plastic is a crucial material in packaging because it extends the shelf life of the food by controlling moisture and gas flow and reducing food oxidation. In addition to their use in packaging, plastics are present in most consumer products and are used in all industrial sectors. Although the development of plastic materials has helped to facilitate our way of life, there are many problems associated with their use. Waste plastics end up in landfills but also in seas, forests, and municipal landscapes, degrading extremely slowly and emitting greenhouse gases such as carbon dioxide and methane (Kisonen et al., 2015). Small plastic particles or microplastics (< 5 mm) are increasingly abundant on land and in the ocean and can be passed to living organisms through accidental ingestion (Kisonen et al., 2015). The main problem with plastic is its non-biodegradable nature and the difficulty of recycling it, which has led to an increasing consumers' awareness concerning plastic consumption, especially with regard to single-use plastic (Luchese et al., 2018). Increasing environmental awareness has led to the need to create biodegradable materials that come from renewable sources and are produced at an equivalent cost to petroleum-based materials. The key point for the development of these new biomaterials is the use of sources that are suitable, abundant, sustainable and cheap, and ideally, that are side streams or waste so their use in the production of biomaterials does not compete with any other application.

1.2. Biorefinery concept

The biorefinery concept refers to the conversion of biomass into energy, chemical compounds, and high value-added materials. This biomass can originate from forestry,

agricultural, industrial, or urban waste. It is a concept analogous to the classic petroleum refineries but with abundant and renewable raw materials and environmentally friendly processes. The use of selected waste fractions in the production of new biomaterials has advantages such as low or even negative cost, low density, availability, biodegradability, nontoxicity, and eco-efficiency (Edhirej et al., 2017).

1.3. Discarded carrots

The biomass used in the current work was a waste product from the agri-food industry, namely discarded carrots. Carrot (*Daucus carota* L.) is one of the most important cultivated vegetables, with an annual global production of about 36 million tons (Encalada et al., 2019). The most abundant waste in the production of carrots is the so-called discarded carrots, those that do not reach the consumer for reasons of shape, color, size, etc. Discards can occur from cultivation to the sales stage and are estimated to be around 30 % of the carrots grown (Marić et al., 2018). Another important waste is carrot pulp, which is generated in the juice industry. The application of the biorefinery concept to discarded carrots must be based on their chemical characterization. Carrots are rich in nutrients such as carotenoids, vitamins (A, B, C, D, E, and K), minerals (calcium, potassium, phosphorus, sodium, and iron) and bioactive compounds (Riganakos et al., 2017). The main components present in the pulp of discarded carrots are sucrose (30.2 % w/w), glucose (19.4 % w/w), fructose (9.1 % w/w), water soluble pectin (5.1 % w/w), cellulose (10.7 % w/w), hemicellulose (8.4 % w/w), pectins (5.8 % w/w), proteins (2.3 % w/w), hexane extractives (1.2 % w/w), and lignin (7.8 % w/w) (Ramos-Andrés et al., 2020). The use of discarded carrot pulp in the formation of biomaterials in this work was based on the use of the four biopolymers it contains: cellulose, hemicellulose, pectins and lignin.

1.4. Hemicellulose and pectin-based biomaterials

Several studies have developed biodegradable materials using microbial polymers (PHA and PLA), lignocellulosic polymers (cellulose, hemicellulose, starch and lignin), and protein-based polymers (gelatin, keratin, gluten, soy protein, and whey protein isolates), for applications such as packaging films (Asgher et al., 2020). Hemicellulose is the second most abundant biopolymer in lignocellulosic biomass, and it is of great interest for the

development of biomaterials because it is bioactive, biocompatible, biodegradable, edible, has a dense macromolecular network and has good oxygen barrier properties (Huang et al., 2018). Pectins are biopolymers present in the primary cell wall and middle lamella of higher plants, and they consist mainly of α-galacturonic acid subunits. The fact that hemicellulose and pectin are water soluble and non-toxic makes them particularly suitable biopolymers for the food, pharmaceutical and biomedical industries (Mendes et al., 2017).

Hemicelluloses and pectins can be extracted from the plant cell wall using various methods including acid hydrolysis, hydrothermal treatment, alkaline treatment, and organic solvents. In addition to being extracted, hemicelluloses and pectins are partially degraded or depolymerized depending on the severity of the extraction method, which causes their molecular weight to decrease. The least aggressive and most environmentally friendly extraction method is hydrothermal treatment, where the only reagents are biomass and water. The extracted hemicelluloses and pectins are often accompanied by other components, such as free sugars or compounds from the autohydrolysis, especially in hydrothermal treatment. For the use of hemicellulose and pectins in the formation of biomaterials, it is necessary not only that they have an adequate molecular weight but also that their purity is high, which is why a concentration, separation and purification treatment is necessary after extraction. Ultrafiltration and diafiltration steps using selected membranes can be applied to the extract, resulting in hemicellulose/pectin fractions of suitable characteristics (Ramos-Andrés et al., 2021a).

Hemicelluloses and pectins can also have certain disadvantages in the formation of biomaterials, such as high brittleness, low mechanical strength, hydrophilicity, sensitivity to humidity, semi-crystalline structure and high hydroxyl group content (Weerasooriya et al., 2020). These characteristics can be influenced by using high quality hemicellulose/pectin, either in terms of molecular weight, polydispersity, or purity, as well as by using additives in the formation of the biomaterial, or by performing chemical reactions that modify the hemicelluloses or pectin, for example by increasing its hydrophobicity.

One of the main applications of biomaterials is food packaging because it is one of the sectors that consume large quantities of plastic. Biomaterials are a good alternative to plastic due to the properties already mentioned, especially their non-toxicity and biodegradability. Biopolymers based on hemicellulose/pectin stand out in food packaging because they generally provide good barrier properties against oxygen, but their main disadvantage is their hydrophilicity and their poorer mechanical properties compared to synthetic materials.

1.5. Polymer blending

In nature, cellulose is located within a matrix of hemicellulose, pectin and lignin. Cellulose is a polysaccharide made up of thousands of glucose monomers. Individual cellulose molecules are non-covalently bonded by lateral alignment to form semi-crystalline cellulose fibers, which are insoluble and able to resist substantial force and provide support to the plant cells (Padayachee et al., 2017). Cellulose fibers are held together by hydrogen-bonding through -OH groups to exclude water from between cellulose molecules by forming a firm microfibril (Padayachee et al., 2017). Hemicelluloses are branched and amorphous polymers, so they do not possess strength properties. They perform the role of bridging cellulose and pectin together providing a cross-linked network structure (Padayachee et al., 2017). Lignin is a heterogeneous set of polymers derived from hydroxycinnamic acids involved in the maintaining of the cell structural integrity imparting resistive rigid strength. In addition, lignin is related to the control of water permeability into the cell (Padayachee et al., 2017). Pectins have a block structure with two distinct domains of polysaccharides, called linear homogalacturonan (composed of D-galacturonic acid units) and branched “hairy” domain (composed of several monosaccharides) (Padayachee et al., 2017). In discarded carrots, pectins are mainly homogalacturonan. Pectin imparts tensile strength and increases the flexibility of the rigid cellulose (Padayachee et al., 2017). In the formation of biomaterials, a potential strategy is to apply the same strategy as in nature, thus achieving good properties by combining components in varying proportions. Polymer blending is a method for generating biomaterials based on mixing components. Its advantages include its simplicity and the lack of chemical modification (Mendes et al., 2017). Biodegradability is a property that depends not so much on the origin of the material as

on the type of chemical bonds present. Chemical reactions can cause the biopolymer to lose biodegradability even if the origin of the biopolymer is 100 % natural.

To improve the properties of the films based on hemicellulose and pectin, one of the main additives included in polymer blending is cellulose. The cellulose can be in the form of cellulose whiskers, cellulose nanofibers (CNF) or bacterial cellulose, and typically it improves the mechanical properties of the biomaterial (Peng et al., 2011). CNF is an entangled network with inter-fibrillary hydrogen bonding between nanofibers, and it can be produced by applying high-pressure homogenization to cellulose fibers in water, resulting in nanofibers of several micrometers in length and nanometers in diameter (Claro et al., 2019). CNF has unique characteristics such as good mechanical properties and high aspect ratio (Peng et al., 2011). Like cellulose and hemicellulose or pectin, the other component that is naturally present in the cell wall of plants is lignin. Therefore, following the polymer blending strategy, it is interesting to incorporate lignin in order to obtain a biomaterial with good properties. Several studies have elaborated films containing lignin that has been extracted together with hemicellulose. In the present study, lignin was incorporated together with CNF as it was part of the same material fraction. The main advantage of lignin is that it is a hydrophobic component so it would provide hydrophobicity to the film without the need to chemically modify the hemicellulose or pectin. The other purified biopolymer fractions used in the present work were composed of hemicelluloses and pectins. The use of pectins in the formation of biodegradable films represents an interesting point because they provide tensile strength and flexibility to hemicellulose-based materials (Padayachee et al., 2017). Finally, the use of plasticizers such as sorbitol, xylitol or glycerol is essential to ensure that the biomaterial formed by hemicellulose/pectin has the required plasticity and flexibility, reducing its brittleness (Chadni et al., 2020). An ideal plasticizer should facilitate molecular motion and decrease internal friction, as its function is to disrupt the hydrogen bonds between the polymer chains and increase the free volume and chain mobility (Xu et al., 2019b). In the present work, glycerol was used as a plasticizer.

1.6. Our previous work

In our previous work, we have focused on the valorization of discarded carrots through (1) hydrothermal extraction of free sugars, hemicelluloses and pectins (Ramos-Andrés

et al., 2020), (2) fermentation of sugars and encapsulation of carotenoids present in the juice (Ramos-Andrés et al., 2021b), (3) ultrafiltration/diafiltration treatment of the hydrothermal extracts to obtain purified fractions of hemicelluloses/pectins of different molecular weight (Ramos-Andrés et al., 2021a), and (4) production of purified solid fractions of hemicelluloses/pectins and solid fractions rich in cellulose and lignin (residual pulp) through pilot-scale operation cycles. In the present work, the purified hemicellulose/pectin fractions were used as the main biopolymers in the manufacturing of biodegradable films, and CNF with lignin were added as additives. The origin of all the compounds was from discarded carrots.

1.7. State of the art

In the formation of biodegradable films for food packaging, focus is placed on the tensile properties of the film as well as on its barrier properties, mainly oxygen permeability (OP) and water vapor permeability (WVP). The hydrophobicity of the surface is also important, and it is measured as the water contact angle. In previous research, films have been made from hemicelluloses, either alone or in combination with other similar biopolymers. Zhang and Whistler (2004) produced corn hull arabinoxylan hemicellulose films with different plasticizers. The films showed tensile strength values of 10-51 MPa, 365-1320 MPa elastic modulus, 6-12 % elongation, and 0.23-0.43·10^{-10} g/(m·Pa·s) of WVP. Péroval et al. (2002) developed films composed of arabinoxylan hemicelluloses from maize bran and glycerol as plasticizer. They added different hydrophobic components and studied their effect on barrier properties, film structure and mechanical properties. The hydrophobic agent selected was hydrogenated palm oil. Subsequently, an emulsifying agent was added to the mixture, and Phan The et al. (2002) showed that drying the film at a higher temperature caused a destabilization of the emulsion resulting in the formation of a bilayer that improved the barrier and mechanical properties. Liu et al. (2016) made films from bagasse hemicellulose mixed with chitosan and plasticizer. The higher the hemicellulose content, the better the tensile strength, which reached a value of 14.26 MPa. Drying at 55 °C instead of 25 °C improved the mechanical properties and the water vapor barrier.

In some cases, hemicelluloses or a similar biopolymer were blended with fibers, as in the present work with residual pulp. Edhirej et al. (2017) prepared films by mixing

cassava bagasse and sugar palm fiber with cassava starch as matrix and fructose as plasticizer. They studied the influence of different palm fiber loadings between 2-8 % and found that higher fiber content improved hydrophobicity but not thermal properties.

The addition of CNF to improve the properties of the hemicellulose-based film is very common. Xu et al. (2019b) made hemicellulose/chitosan films and added different percentages of CNF. A 5 % CNF content increased the tensile strength 2.3 times and improved the barrier properties. The glycerol plasticized films had good mechanical properties with a tensile strength of 31.02-38.56 MPa and tensile strain at break of 10.07-15.98 %. Peng et al. (2011) incorporated a CNF content of 0-20 % into xylan hemicellulose films. In the range between 5-20 %, the mechanical properties were improved, with a content of 20 % being optimal.

In addition to the incorporation of hemicelluloses and cellulose, in other studies, such as that of Hu et al. (2016), lignin was included. Films were made from dewaxed rice straw previously fractionated into cellulose-rich solid and hemicellulose/lignin-rich suspension. CNF was produced from the solid fraction and added to the films in different percentages. Young's modulus improved with higher CNF content between 10-30 %, while tensile strength and tensile strain had their optimum value at 20 % CNF. As for the WVP, the incorporation of 10 % CNF increased the permeability, while a higher content (20-30 %) decreased it.

In other works, hemicelluloses and/or CNF were chemically modified to increase the hydrophobicity of the film. In the work of Mugwagwa and Chimphango (2020), a higher content of acetylated CNF and a higher degree of acetylation resulted in an increase of the water contact angle from 24.59 to 82.48°. Gordobil et al. (2014) used corn cob hemicelluloses purified by ultrafiltration to obtain hydrophilic films (xylan, cellulose, glycerol) and hydrophobic films (acetylated xylan and cellulose). The addition of CNF always improved the mechanical properties, but these properties were better in the hydrophobic films (Young's modulus 2300 MPa, strength 44.1 MPa, strain at break 5.7 %).

1.8. State of the art of carrot films

Regarding the work related to carrots, Idrovo Encalada et al. (2016) prepared films by mixing commercial pectin with carrot fibers obtained after hot water treatment. Thanks to the carrot fibers content, the films had good antioxidant capacity. The higher bonding between the pectin chains and the carrot fibers resulted in lower elasticity. As for the WVP, the addition of carrot fibers did not improve it due to its mostly hydrophilic character. Rajinipriya et al. (2018) produced nanofibrillated cellulose and nanocrystalline cellulose from carrot pulp by means of non-conventional ball milling and acid hydrolysis, respectively. Both suspensions were separately cast into films and the optical properties, morphology and mechanical properties were evaluated. The mechanical properties were worse compared to the literature, which could be due to the different preparation method of the nanocellulosic films. Otoni et al. (2018) prepared biodegradable biocomposites from carrot waste combined with hydroxypropyl methylcellulose and high-pressure microfluidized cellulose fibers. The optimized formulation contained 33 % of carrot waste and had 30 MPa of tensile strength, 3 % elongation at break, and 2 GPa of Young's modulus, which are rather suitable properties for food packaging. Varanasi et al. (2018) produced nanofibers from carrot waste with a mechanical process without chemicals. Translucent and strong flexible films were prepared from the material using a filtration process. The value of WVP was 140 g/(m^2·day), tensile strength was 65.71 MPa and Young's modulus was 6842.3 MPa. Siqueira et al. (2016) redispersed carrot nanofibers from the juice production residue demonstrating their good mechanical properties and their reinforcing capacity applied to composite materials. In contrast to other nanofibers, carrot nanofibers redispersed after drying more easily and without the need for surfactants or chemical modifications. The produced nanopapers had strong and stiff networks. Sogut and Cakmak (2020) incorporated carrot fibers and microcrystalline cellulose into chitosan-based films with different loadings between 0-5 %. Mechanical properties and thermal stability improved with higher filler content. High concentrations of carrot fibers reduced WVP.

1.9. Novelty of the work

In this work, biodegradable films were made from components extracted from discarded carrots, water, and glycerol. In our previous work, purified high molecular weight solid fractions of hemicellulose/pectin and solid fractions of CNF/lignin were obtained from discarded carrots by environmentally friendly and chemical-free processes. In the present work, we studied (1) the influence of the CNF/lignin percentage and (2) the influence of the molecular weight and composition of the hemicellulose/pectin fraction on the properties of the films (barrier, tensile, structural, hydrophobicity) for their potential application in food packaging. For the first time, films were prepared based on hemicellulose and pectin extracted from discarded carrots, incorporating as an additive agent a fraction that also comes from carrots. The reinforcing agent in biofilms is usually based on cellulose nanofibers, but in this case, the additive also contained a considerable percentage of lignin. The films prepared had acceptable properties and were noted for their high hydrophobicity, with values comparable to those in the literature when chemical modification of the biopolymers is applied.

2. Materials and methods

2.1. Raw material processing and characterization

The biopolymers used in the formation of the films were purified samples of hemicelluloses and pectins of different composition and molecular weight, obtained from discarded carrot pulp. The extraction was performed in pilot-scale using hydrothermal treatment in a flow-through reactor system (Ramos-Andrés et al., 2020) or by cycles using several flow-through reactors. The hydrothermal treatment was performed at two temperatures: 140 and 180 °C. Conditioning of the extracted hemicelluloses/pectins was carried out applying concentration, separation and purification using multiple stages of ultrafiltration/diafiltration with 30, 10, and 1 kDa molecular weight cut-off (MWCO) membranes. Once the samples were purified, they were freeze-dried and/or spray-dried. Solid fractions of purified biopolymers were obtained using water and biomass as the only reagents. Table 1 shows the characterization results of the solid fractions used.

Table 1. Characterization results of the purified solid fractions of hemicellulose-pectin. MW: weight-average molecular weight, PD: polydispersity, AG: arabinogalactan, P: pectin, G: galactan, A: arabinan.

	MW (kDa)	PD	Purity (% w/w)	AG/(AG+P)	G/A
MW-1	102.75	4.0	98.9	0.585	1.74
MW-2	80.36	2.4	100	0.900	1.84
MW-3	67.77	3.8	100	0.732	1.63
MW-4	9.85	2.1	100	0.487	1.39
MW-5	5.23	1.3	64.5	0.803	1.73
MW-6	3.86	1.5	66.8	0.629	1.30

The cellulose-lignin fibers used as additive agent were residual pulp samples obtained from the hydrothermal treatment applied at 140 and 180 °C to discarded carrot pulp. The duration of the hydrothermal treatment was 45 min at 140 °C and 30 min at 180 °C in a flow-through reactor, with the biomass retained inside the reactor and the water circulating from top to bottom. The composition of the residual pulp was determined according to the standardized methods published by National Renewable Energy Laboratory (NREL). The samples were subjected to water Soxhlet extraction. The water extraction allowed determining the concentration of polar extractives, mainly residual free sugars. Polysaccharides and lignin were determined by two-step acid hydrolysis. The first step fractionated the biomass into acid-insoluble material (acid-insoluble lignin and ash) and acid-soluble material (cellulose, hemicellulose, pectin, and acid-soluble lignin). The second hydrolysis step allowed the composition of the polysaccharides to be determined through their controlled breakdown into monomers. These monomers were identified and quantified by HPLC according to a previously described method (Ramos-Andrés et al., 2020). Acid-soluble lignin was quantified by UV-Vis spectroscopy. Acid-insoluble lignin was measured by gravimetric method by recovering the solid resulting from the first acid hydrolysis step. Finally, ash was determined as the residue remaining in the solid after oxidation at 550 °C to constant weight. The composition on a dry basis of the residual pulps can be seen in Table 2. Glycerol used as a plasticizer in the formation of films was provided by Merck company.

Table 2. Chemical characterization results of the solid fractions of cellulose-lignin in dry basis.

	Residual pulp 140 °C (% w/w)	Residual pulp 180 °C (% w/w)
Water extractives	19.13 ± 4.67	16.07 ± 4.57
Cellulose	34.68 ± 3.91	42.82 ± 0.50
Hemicellulose	3.43 ± 1.27	2.13 ± 0.25
Pectin	1.07 ± 0.26	n.d.
Lignin	21.04 ± 3.66	32.02 ± 6.99
Others	20.65	6.96

2.2. Preparation and characterization of the CNF from residual carrot pulp

The residual pulps were subjected to a treatment consisting of water washing, freeze-drying, ball milling, hydration, and high-pressure homogenization. Washing was performed to reduce the content of free sugars that impregnate the material after hydrothermal treatment. For freeze drying, the residual pulps were frozen overnight at -25 °C and then freeze-dried under vacuum (0.180 mbar) for 96 h using a Telstar Lyoquest – 55 unit (Terrasa, Spain). The freeze-dried pulps were subjected to milling in a ball mill Retsch PM100 which operated for 4 hours in 1-minute periods with 15-minute breaks in between to avoid heating of the sample. In the film preparation process, the residual pulp powder was hydrated by keeping it under magnetic stirring in water overnight. The concentration of the suspension was 0.4 % (w/v). After hydration, the suspension was subjected to high-pressure homogenization (ATS-100D) under 2 cycles of 200 bar and 10 cycles of 1000 bar. CNF was produced from the residual carrot pulps previously obtained at 140 °C (CNF-140) and 180 °C (CNF-180).

Images of the residual pulp before and after homogenization were obtained using an optical microscope (Nikon, Japan). The obtained suspensions were dropped onto a glass sheet prior to the glass covered, and then were analyzed at different magnifications.

Structure of the residual pulp before and after homogenization was observed by transmission electron microscopy (TEM) with a JEM-1400 PLUS TEM microscope (JEOL Ltd., Japan) in bright field mode with an accelerating voltage of 80 kV.

2.3. Preparation of the films

Blends were prepared for film formation according to the composition shown in Table 3.

Table 3. Composition, size, and weight of the films. AG: arabinogalactan, P: pectin.

Film	AG-P sample	AG-P (% w/w)	Residual pulp (% w/w)	Glycerol (% w/w)	Diameter (cm)	Weight (g)
140-A5	MW-3	40	25	35	8.80	1.00
180-A5	MW-3	40	25	35	8.80	1.00
A0	MW-3	65	0	35	13.5	2.35
A1	MW-3	64	1	35	13.5	2.35
A2	MW-3	63	2	35	13.5	2.35
A3	MW-3	60	5	35	13.5	2.35
A4	MW-3	50	15	35	13.5	2.35
A5	MW-3	40	25	35	13.5	2.35
B1	MW-3	0	65	35	13.5	2.35
C1	MW-1	64	1	35	13.5	2.35
C2	MW-2	64	1	35	13.5	2.35

The preparation of the blends for film formation started by determining the appropriate volume of homogenized residual pulp suspension (0.4 % w/v) according to the desired residual pulp content in the film (Table 3). Once the volume was selected, the desired content of arabinogalactan and pectin solid sample (AG-P) was added and, if necessary, water to reach a total volume of approximately 157 mL. The blend was subjected to magnetic stirring and heated at 40-50 °C in a thermostatic bath for 3 hours. After this, the mixture was left in magnetic stirring overnight. The predetermined amount of glycerol dissolved in 3 mL of water was added. The final blend was kept under magnetic stirring at 40-50 °C in a thermostatic bath for 3 hours. Finally, the samples were poured into polystyrene Petri dishes (VWR) and placed in a ventilated oven at 40 °C for approximately 6 hours. After that time, the second part of the drying was performed in a room conditioned at 23 °C and 50 % relative humidity (RH).

2.4. Characterization of the films

2.4.1. Thickness

The thickness of the films was measured using a Lorenz Wetter paper thickness meter (L&M micrometer SE250, Sweden) at 10 random positions around the film, and the mean value was used in the calculations. Precision of the measurements was ± 1 µm.

2.4.2. Water vapor permeability (WVP)

Water vapor permeability (WVP) was measured gravimetrically according to the ASTM standard E96/E96M-05. The desiccant method was employed in which a quantity of anhydrous $CaCl_2$ (Honeywell Fluka^{TM}) was added in a dish covered with the test specimen while the edges were sealed with molten wax. The assembly was placed in a climate-controlled chamber (23 °C, 90 % RH) for 4 days. The top side of the film was exposed to the conditioned atmosphere. Water vapor transmission rate (WVTR) through the specimen was calculated by measuring the increase in the weight of the assembly over the duration of the analyses. The average value of the measurements was determined in g/m^2·h. WVP was determined by multiplying WVTR by the thickness and dividing by the partial pressure of water vapor, reporting the measurements in (g·mm)/(m^2·kPa·day). The films were conditioned at 23 °C and 50 % RH before the characterization.

2.4.3. Oxygen permeability (OP)

The oxygen transmission rate (OTR) was determined according to ASTM F1927 using an oxygen permeability analyzer with a coulometric sensor (Mocon Ox-Tran 2/21 MH/SS) at controlled conditions (23 °C, 50 % RH). The oxygen concentration was reported over the duration of the analyses. OTR was determined and reported in cm^{3}STP/m^2·day. Oxygen permeability (OP) was calculated by multiplying OTR by the thickness of the film and dividing by the partial pressure of oxygen, reporting the measurements in (cm^{3}STP·µm)/(m^2·kPa·day).

2.4.4. Water contact angle

Contact angle measurements were performed with an optical goniometer (CAM 200, KSV NIMA, Biolin Scientific Oy) and One Attension Theta 1.4 software. Three microliter

drops of 18.2 MΩ type-1 deionized distilled water were released onto the sample surface with the sessile drop measurement method. Three different measurements were performed per specimen.

2.4.5. Tensile properties

Tensile properties were determined at 23 °C and 50 % RH with an Instron 4465 universal testing equipment (Instron Corp., High Wycombe, England) with a load cell of 100 N. The initial grip distance was 50 mm and the rate of grip separation was 1 mm/min. The specimen width was 6 mm and the length was 75 mm. The thickness of the films was measured according to Section 2.5.1. Six replicate specimens of each film were measured. The maximum load, tensile stress at maximum load, tensile strain (extension) at maximum load, tensile strain (extension) at break (standard), and elastic modulus (E-modulus) were determined.

2.4.6. SEM

Films surface structure was observed by scanning electron microscopy (SEM) with an X-ray analyzing system (Thermo Scientific, Germany). Images were taken of both sides at various magnifications. Cross-sections of the broken films were also analyzed.

2.4.7. FTIR

Fourier transform infrared (FTIR) spectroscopy of the films was obtained using a Thermo Scientific Nicolet iS™ 50 FTIR spectrometer (USA). The spectra were collected with 64 scans and a resolution of 4 cm^{-1} in the 4000-400 cm^{-1} wavenumber range.

3. Results and discussion

3.1. Production of CNF from residual carrot pulp

Figure 1 shows the optical microscope images of the 180 °C residual pulp before and after high-pressure homogenization. Without homogenization treatment, residual pulp powder was distributed as elongated aggregates reaching a length between 100-200 µm and a variable width of around 25 µm. This arrangement indicates the need for the homogenization step. After homogenization, no aggregation was present, but a homogeneous distribution of the material in the form of a network can be seen. The

optical microscope images did not clearly show the presence of nanofibers, so TEM imaging was performed.

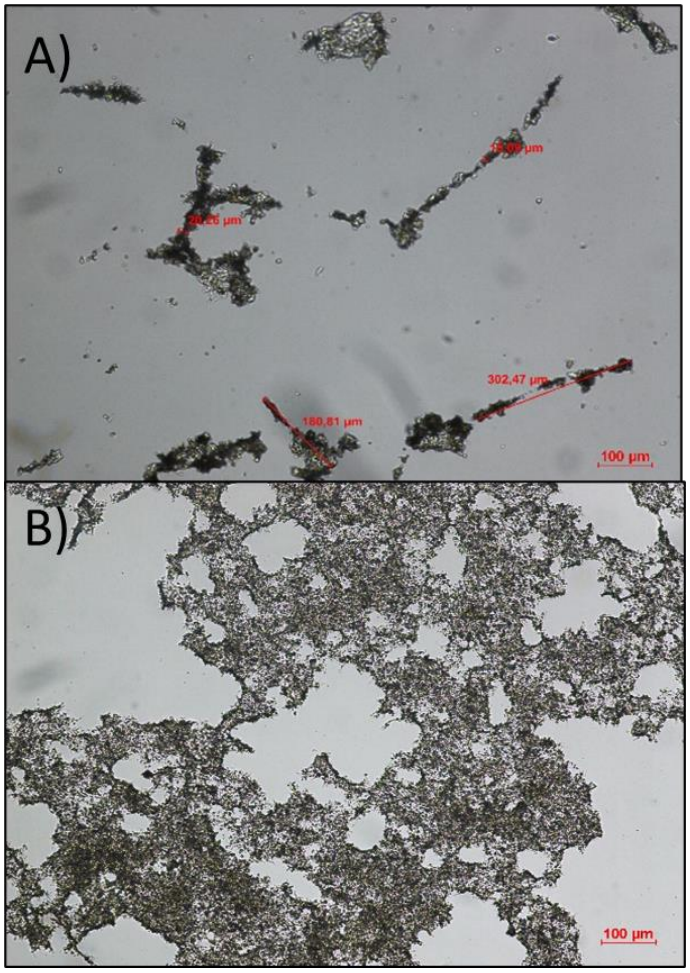


Figure 1. Optical microscope images of the 180-CNF A) before and B) after high-pressure homogenization treatment.

Figure 2.A and B shows TEM images of both residual pulps (140 and 180 °C). In both cases, the presence of fibers can be seen, so it can be concluded that the ball milling treatment resulted in obtaining CNF. These nanofibers had a length of around 2 µm and a diameter of a few nanometers. However, the fibers were found in the form of agglomerates probably due to the presence of substances other than cellulose as shown by the chemical characterization (Table 2). The main compounds accompanying the fibers were lignin and extractives. The presence of lignin may provide interesting properties to CNFs, such as higher hydrophobicity. Figure 2.C shows the result of the complete treatment including the homogenization of the CNF (sample 140-CNF). There was less presence of aggregates as well as nanoparticles, which were probably more

homogeneously distributed in the material. The dimensions of the nanofibers were similar before and after homogenization, but the dispersion increased with homogenization.

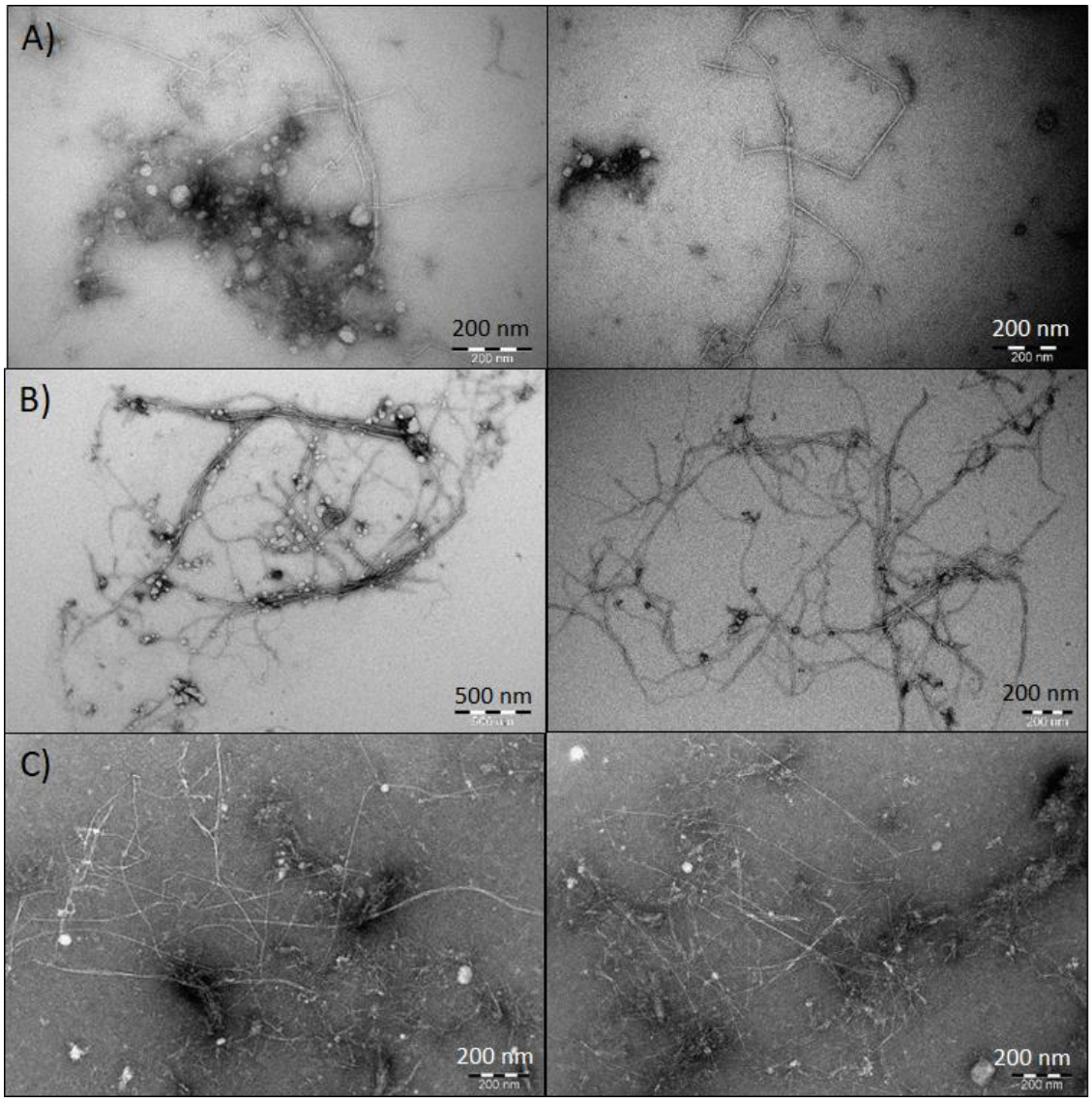


Fig. 2. TEM images of A) 140-CNF before homogenization, B) 180-CNF before homogenization, and C) 140-CNF after homogenization.

3.2. Effect of different CNFs (140-CNF and 180-CNF)

3.2.1. Water vapor permeability (WVP)

The WVP of 140-A5 and 180-A5 films was evaluated to compare the two residual pulps. The result was 24.14 (g·mm)/(m^2·kPa·day) for 140-A5 and 24.35 (g·mm)/(m^2·kPa·day) for 180-A5. Given the results, no difference in WVP can be established for the use of one residual pulp or the other.

In comparison with WVP values of other authors, it is important that the RH gradient used in the analysis is the same or similar. Among the authors who used the same or a similar gradient, Mikkonen et al. (2009) stands out, who produced arabinoxylan films without chemical modification. Plasticizing with a similar content of glycerol, the WVP was 78 (g·mm)/(m^2·kPa·day), higher than that obtained in the present work. On the other hand, Phan The et al. (2002) also produced arabinoxylan films without chemical modification and plasticized with glycerol, and the WVP was 11.94 (g·mm)/(m^2·kPa·day), lower than in the present work. With the addition of hydrogenated palm kernel oil as hydrophobic agent in the film, Phan The et al. (2002) reduced the WVP to 8.04 (g·mm)/(m^2·kPa·day).

3.2.2. Water contact angle

The surface hydrophobicity of the 140-A5 and 180-A5 films was evaluated to compare the two residual pulps. For this purpose, the contact angle of the water on the surface in contact with the Petri dish (bottom side) and on the surface in contact with the environment (top side) were studied. In both films, the bottom side had a glossy shade while the top side had a matt shade, as can be seen in Figure 3. This difference in shades was also reflected in the hydrophobicity of the surfaces. The bottom (shiny) side was found to be slightly hydrophobic in both films, with contact angles of 53.34° (140-A5) and 64.55° (180-A5). However, the top (matt) side was found to be markedly hydrophobic in both films, with 116.57 (140-A5) and 91.02° (180-A5) values. The film containing 140-CNF was more hydrophobic than the film containing 180-CNF even though the 180-CNF fraction had more lignin than 140-CNF (21.04 vs. 32.02 % w/w). The reason could be that a lower treatment temperature kept the lignin less altered and therefore its hydrophobic character less modified, or it could be due to a synergistic effect between lignin and other components towards hydrophobicity. The difference in hydrophobicity between one side and the other could be associated with different morphology of the surfaces in accordance with their different shine, as can also be seen in the SEM analysis. Considering the results and given that there is no difference in WVP between the two residual pulps, 140-CNF was selected for the next films because of its higher hydrophobicity.

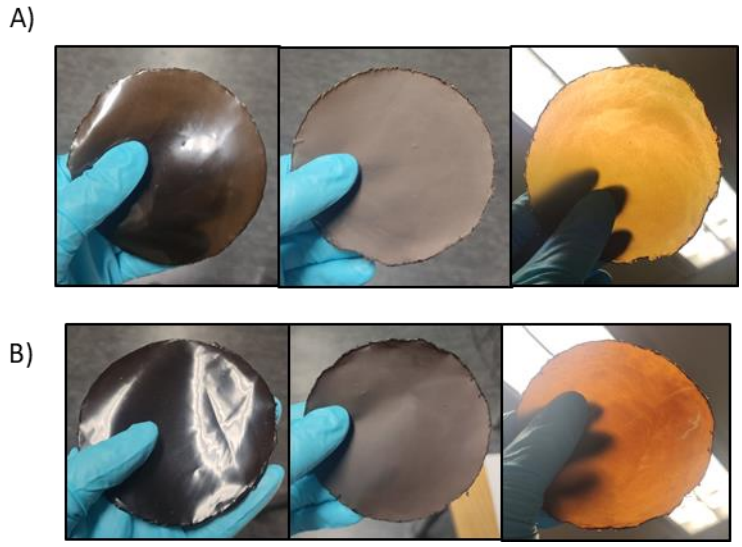


Figure 3. Images of the bottom side, top side, and surface against light background of the films A) 140-A5 and B) 180-A5.

Compared to literature values, chemical modification of hemicelluloses through transesterification performed by Zhang et al. (2020) resulted in a value of 120°, similar to the maximum contact angle obtained in the present work. Péroval et al. (2002) added lipids to the films and passed from contact angles of 70.8° in the reference film to a maximum of 94.4° in the film with hydrogenated palm oil. The contact angle values of Xu et al. (2019b) were between 83.4 – 101.1° in the case of CNF-reinforced hemicelluloses/chitosan films. Kisonen et al. (2015) produced films with CNF and the addition of chemically modified hemicelluloses. They obtained contact angles from 55° (CNF with unmodified hemicellulose) to 78° (combining CNF with both modified and unmodified hemicelluloses).

3.2.3. SEM

Both films 140-A5 and 180-A5 were structurally analyzed using SEM to find differences between the two residual pulps and to better understand the obtained morphology. As can be seen in Figure 4, the top (matt) side showed a very rough surface with the presence of some fibers. There were also some granules that could be associated with lignin. Both the roughness and the lignin could be factors in the high hydrophobicity of this side. The bottom side of both films shows the presence of a crystalline structure consistent with the morphology of calcium oxalate crystals. This salt is naturally present in carrots and has been obtained in similar work on the preparation of biodegradable

films from similar biomass (Habibi et al., 2009). The salt typically precipitates during drying and remain on the bottom side of the film, and may be responsible, together with the smooth morphology, for the abundant shine on this side. As can be seen in Figure 5, the cross-sectional images showed a homogeneous structure similar for both residual pulps. In both cases, there was a layer on the bottom side of the film, whose smoothness contrasts with the roughness of the top side. This is where the calcium oxalate crystals were deposited. To confirm the presence of the precipitate, the composition was evaluated using FTIR.

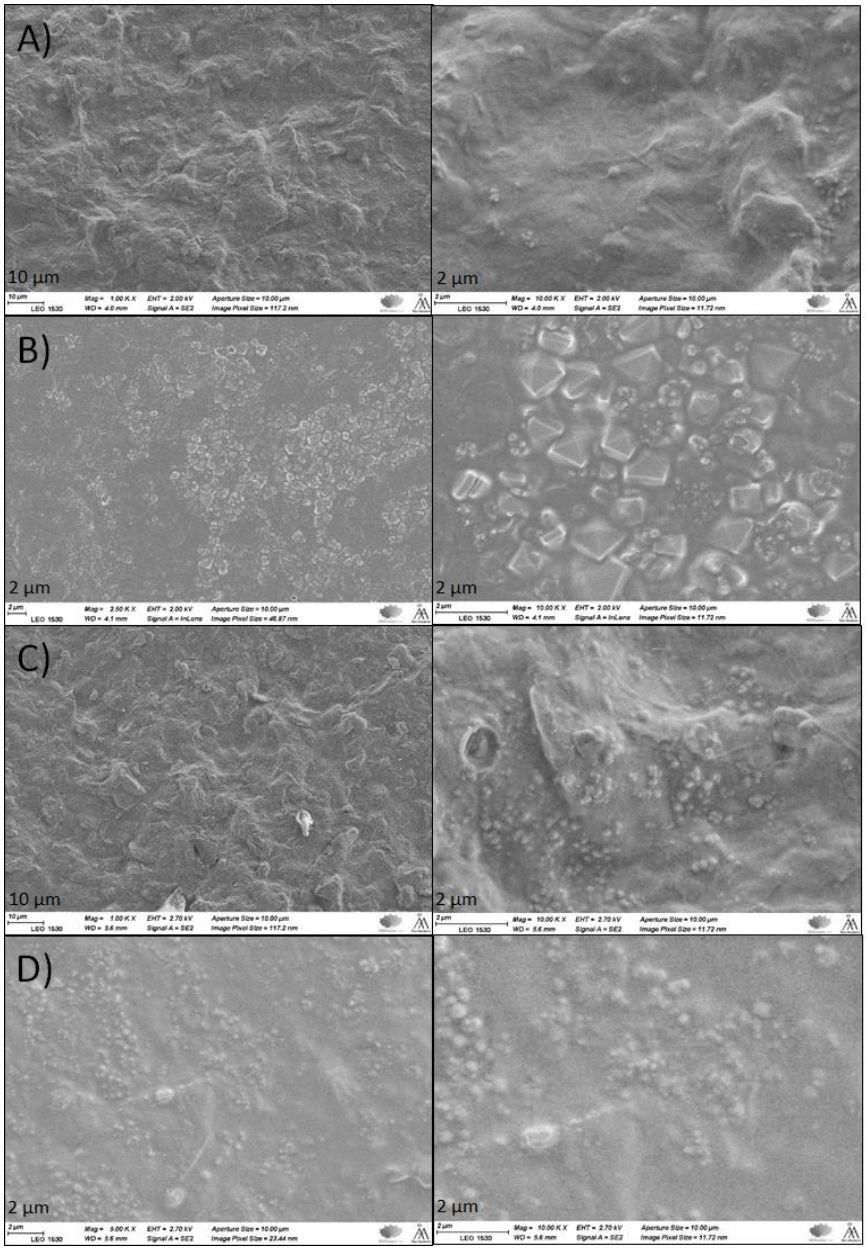


Figure 4. SEM images of the A) top side of 140-A5 film, B) bottom side of 140-A5 film, C) top side of 180-A5 film, and D) bottom side of 180-A5 film.

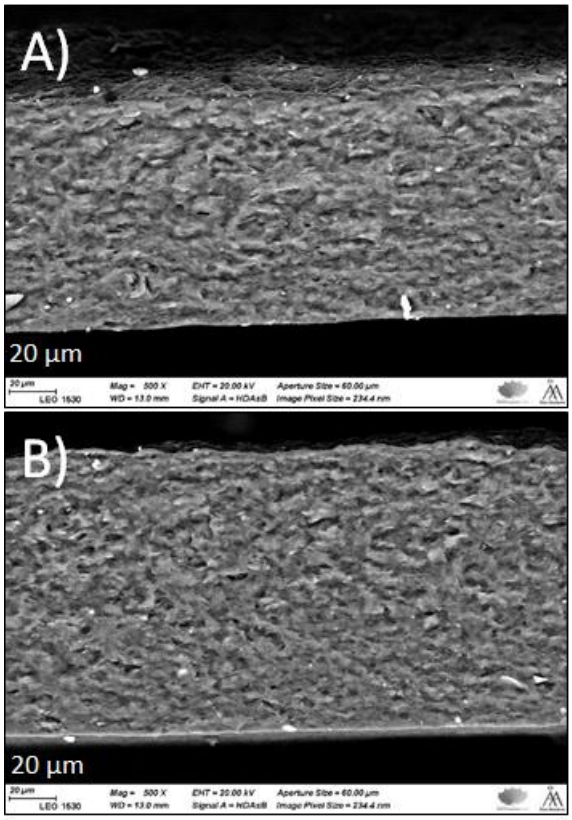


Figure 5. SEM images of the cross-sections of A) 140-A5 film and B) 180-A5 film.

3.2.4. FTIR

FTIR spectra of both sides of the 140-A5 film were determined to confirm the presence of calcium oxalate crystals on the bottom side. According to Figure A.1, the remarkable difference between the two spectra was the presence of the 1630 and 1320 cm^{-1} peaks. These peaks are associated with calcium oxalate based on the spectrum of this compound obtained from the NIST library.

3.3. Effect of different 140-CNF content (0-1-2-5-15-25 % w/w)

140-CNF was the material selected to act as an additive in the formation of AG-P based films and in the formation of a CNF based film without AG-P. According to Table 3, the presence of 140-CNF was studied for concentrations of 0 % (A0), 1 % (A1), 2 % (A2), 5 % (A3), 15 % (A4), 25 % (A5), and 65 % (B1). The seven films with potential applications in food packaging, were characterized by determining their oxygen permeability, water vapor permeability, water contact angle, tensile properties, and FT-IR spectra. Images of the films can be seen in Figure 6.

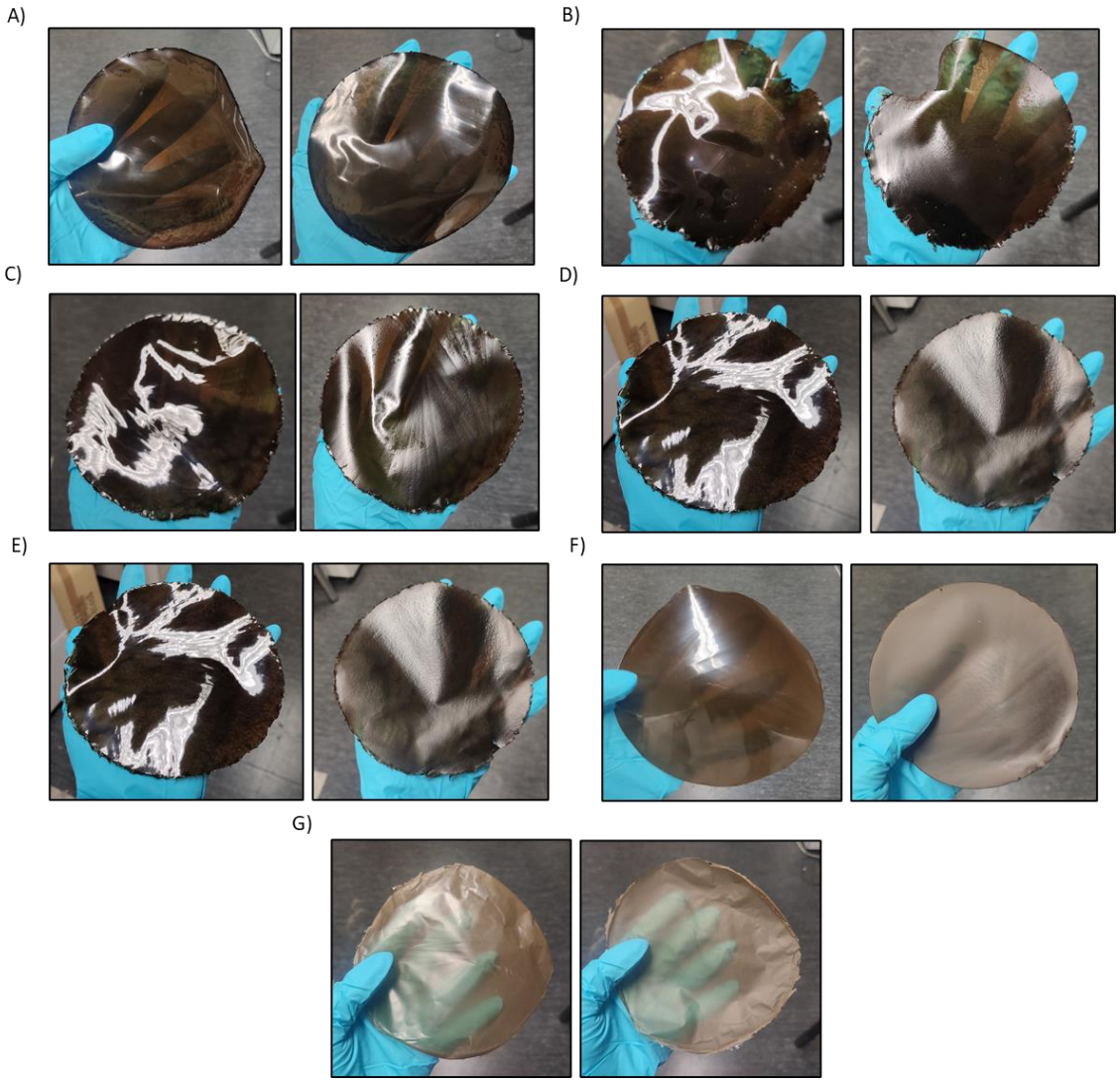


Figure 6. Images of the bottom side and top side of the films A) A0 (0 % 140-CNF), B) A1 (1 % 140-CNF), C) A2 (2 % 140-CNF), D) A3 (5 % 140-CNF), E) A4 (15 % 140-CNF), F) A5 (25 % 140-CNF) and G) B1 (65 % 140-CNF).

3.3.1. Oxygen permeability (OP)

The OP results of the films can be seen in Figure 7.A. The lowest OP value was 48.18 (cm^3STP·µm)/(m^2·kPa·day) for A1 (1 % 140-CNF). Considering that the OP of the film not containing CNF was 67.73 (cm^3STP·µm)/(m^2·kPa·day), the presence of a low percentage of fibers was beneficial in decreasing the OP value. The addition of a higher percentage of CNF also decreased the OP, although by a smaller percentage as the percentage of 140-CNF increased. This occurred for the approximate range of 1-5 % 140-CNF, as in the case of A4 (15 % 140-CNF) and A5 (25 % 140-CNF) the fibers no longer decreased the OP, but the values were 81.31 (cm^3STP·µm)/(m^2·kPa·day) for A4 and 73.65

($cm^3STP \cdot \mu m)/(m^2 \cdot kPa \cdot day$) for A5, the latter very similar to the original value of A0. The decrease of OP in A5 with respect to A4 could not be considered relevant. The high OP value of film B1, which did not contain AG-P but consisted only of 140-CNF and glycerol, is noteworthy. The permeability in this case was 239.83 ($cm^3STP \cdot \mu m)/(m^2 \cdot kPa \cdot day$), so clearly the fibers were a much worse material than AG-P as far as OP is concerned. However, fibers resulted only in a slight decrease in OP when they were present in low proportion. This may be due to the acquisition of synergistic properties between the two ingredients in that concentration range.

In food packaging, the requirements for OP depend on the application. If the food is high in polyunsaturated fat, an extremely low OP would be desired. However, if the film is applied to fresh fruits or vegetables, a certain OP and carbon dioxide permeability is desired to avoid anaerobic fermentation.

Comparing with literature, the OP value obtained by Zhang et al. (2020) with chemically modified hemicelluloses-based films was between 1.21 – 4.24 ($cm^3 \cdot \mu m)/(m^2 \cdot kPa \cdot day$), as expected lower than the value obtained in the present work. Xu et al. (2019a) also obtained a lower value, between 4.95 – 5.06 ($cm^3 \cdot \mu m)/(m^2 \cdot kPa \cdot day$), when making CNF-reinforced hemicellulose/chitosan films. Mikkonen et al. (2009) obtained a value of 7.4 ($cm^3 \cdot \mu m)/(m^2 \cdot kPa \cdot day$) for glycerol plasticized arabinoxylan films. The higher OP value in the present work with respect to literature values was found in both the films including residual pulp and the film without residual pulp (A0), so it could be associated with the hemicellulose/pectin fraction. The reason for the higher permeability could be related to the type of binding between hemicelluloses and pectins, perhaps less strong than the binding of hemicelluloses to each other in a film containing only hemicelluloses.

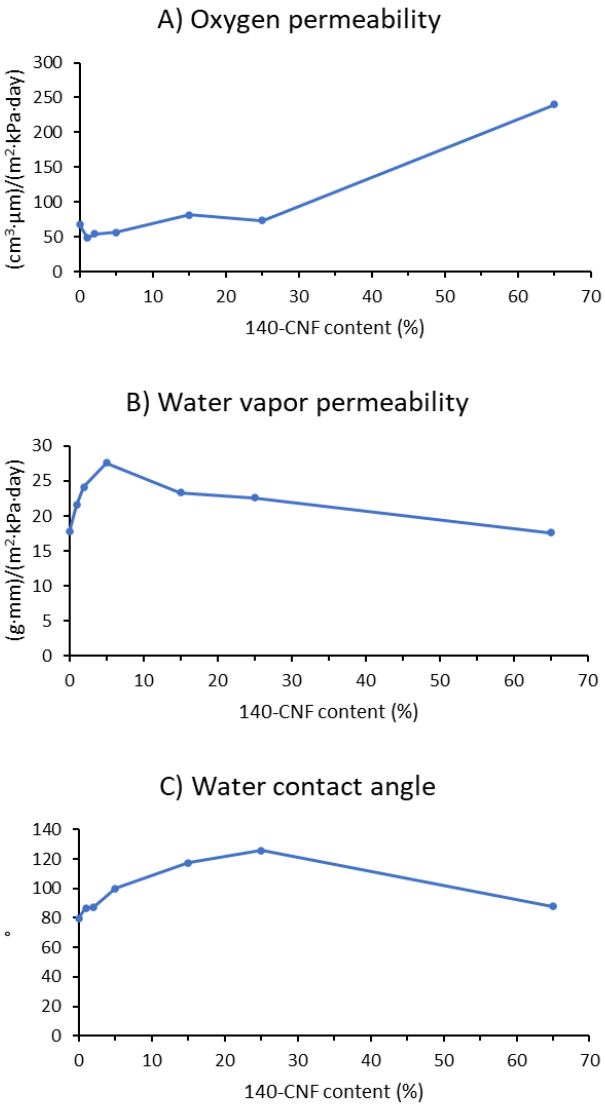


Figure 7. OP, WVP and water contact angle of the films A0 (0 % 140-CNF), A1 (1 % 140-CNF), A2 (2 % 140-CNF), A3 (5 % 140-CNF), A4 (15 % 140-CNF), A5 (25 % 140-CNF) and B1 (65 % 140-CNF).

3.3.2. Water vapor permeability (WVP)

Figure 7.B shows the WVP values for films A0, A1, A2, A3, A4, A5, and B1. The WVP value in the absence of CNF was 17.77 (g·mm)/(m^2·kPa·day). As the 140-CNF content increased the WVP also increased slightly, rising from 21.56 (g·mm)/(m^2·kPa·day) in A1 (1 % 140-CNF) to 27.56 (g·mm)/(m^2·kPa·day) in A3 (5 % 140-CNF). The A4 and A5 films differed in trend as in the OP case, with WVP values decreasing to 22.62 (g·mm)/(m^2·kPa·day) in A5. Finally, the film containing only 140-CNF (B1) had a WVP of 17.64 (g·mm)/(m^2·kPa·day), almost identical to the AG-P film. In view of the evolution, the

addition of a very small content of 140-CNF seems to be of interest because it did not modify the results very much, or the addition of sufficiently high content of 140-CNF so that there was no significant deviation of the WVP value form the reference value. There were two trends, one in which the presence of fibers was not beneficial, as it only resulted in a reduction of the percentage of AG-P without these fibers compensating for it. However, beyond a certain percentage, the presence of fibers compensated for the absence of AG-P and the WVP values improved, without improving the original permeability of the A0 film, but equaling it. Since the WVP of the A0 and B1 films was very similar, the hemicellulose/pectin and 140-CNF fraction would behave similarly in terms of WVP. However, the fact that a small addition of 140-CNF was detrimental may be due to when a homogeneous network is not established through the film, the presence of the 140-CNF fraction would create zones where WVP might be higher due to a lack of entanglement between the biopolymers present.

Similar results were obtained by Hu et al. (2016), whose addition of 10 % CNF increased the WVTR value from 45.7 g/m^2·h to 74.6 g/m^2·h, while the addition of 20 % CNF decreased it. The addition of 20 and 30 % CNF decreased the WVTR value to 43.8 and 35.4 g/m^2·h. The addition of higher CNF content favored the presence of more structured layers in the material which decreased the permeability.

3.3.3. Water contact angle

The hydrophobicity of films A0, A1, A2, A3, A4, A5, and B1 was evaluated on their top side (rough, matt), being the most representative of the material as it did not contain calcium oxalate crystals. The values of water contact angle can be seen in Figure 7.C. The film with the lowest hydrophobicity was as expected A0, as it did not contain 140-CNF and therefore did not contain cellulose or lignin. The water contact angle value for A0 was 79.9°, significantly high considering that it consisted only of AG-P and glycerol. The addition of 140-CNF resulted in a considerable increase of the contact angle from 86.84° for A1 (1 % 140-CNF) to 125.8° for A5 (25 % 140-CNF). Consequently, higher 140-CNF content resulted in higher hydrophobicity. However, the B1 film formed only by 140-CNF and glycerol had a similar contact angle to the A1 film (87.9°). This shows that the presence of 140-CNF together with AG-P generates synergically higher hydrophobicity,

but that a film formed only by 140-CNF is, although more hydrophobic than a film formed only by AG-P, less hydrophobic than their mixtures in the range 5-25 %.

3.3.4. Tensile properties

Within the study of the influence of the fiber content in the films, the tensile properties of films A0, A1, A2, A3, A4, A5, y B1 were analyzed. Tensile properties are measures of the behavior under mechanical stress. The tensile stress, elongation at break, and elastic modulus (Young's modulus) were determined from the stress-strain curve. The tensile stress at maximum load indicates the maximum tensile stress that the film can withstand. Elongation at break indicates the maximum change in length of the film before it breaks. The elastic modulus is a measure of the stiffness of the film. The higher the elastic modulus, the greater the stress required for deformation. The desired properties depend on the application of the films.

Figure 8.A shows the tensile stress values at maximum load. The reference film A0 consisting only of AG-P and glycerol had a tensile stress of 3.14 MPa. The addition of fibers decreased the tensile stress value to a minimum of 1.27 MPa when 5 % 140-CNF was added. As was the case for OP and WVP, a change in the trend occurred in A4 and A5 films, where for higher values of 5 % fibers the tensile stress increased and even exceeded the reference value in A5 film (25 % 140-CNF) with 4.31 MPa. The maximum tensile stress value occurred for film B1 consisting only of 140-CNF and glycerol, reaching 7.74 MPa. It makes sense as cellulose is a stronger biopolymer than arabinogalactan (AG) or pectin. The evolution indicates that there are two regions corresponding to two tensile behaviors. In the first region (0-5 % 140-CNF) the AG-P mechanics dominates, the addition of fibers was not beneficial as it did not add strength to the film but weakened it. This may be due to the structural network was not created in the film and there were irregularities that could even generate preferential rupture zones. The addition of a sufficient amount of fibers (> 5 % 140-CNF) resulted in a change in the behavior as an increase in tensile stress with a higher percentage of fibers.

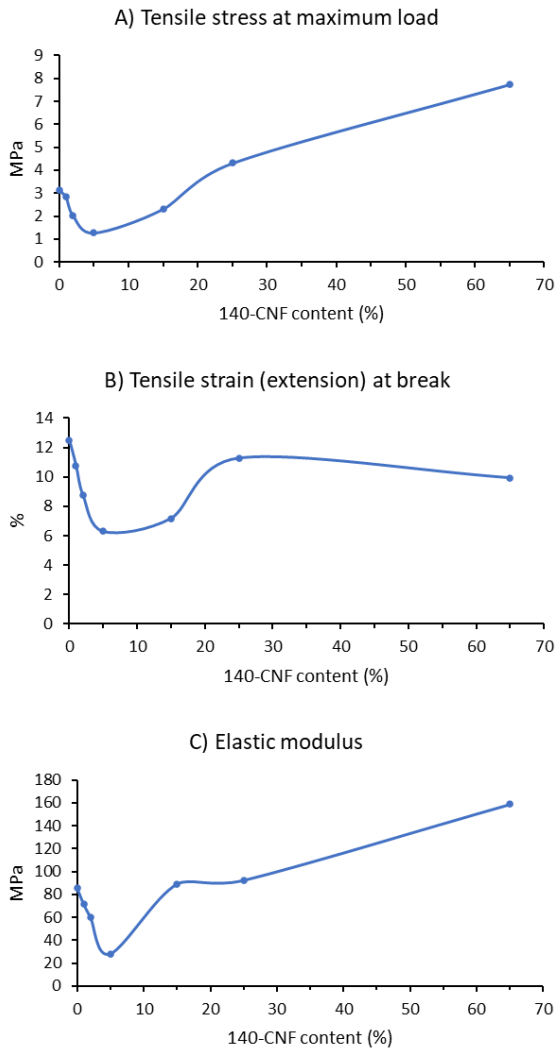


Figure 8. Tensile properties of the films A0 (0 % 140-CNF), A1 (1 % 140-CNF), A2 (2 % 140-CNF), A3 (5 % 140-CNF), A4 (15 % 140-CNF), A5 (25 % 140-CNF) and B1 (65 % 140-CNF).

Figure 8.B shows the evolution of tensile strain at rupture. Identical to the tensile stress, there were two distinct regions. In the first region where films A0, A1, A2 and A3 were found, the addition of fibers decreased the elongation percentage, making the film less stretchable. The decrease ranged from 12.49 % (A0) to 6.29 % (A3). The decrease in elongation due to the addition of 140-CNF makes sense because cellulose does not provide extensibility but strength. The decrease was also due to a small percentage of fibers failing to establish a homogeneous network that would improve the mechanical properties of the film. However, when a considerable percentage of 140-CNF was added (> 5 %), the extensibility was not so much adversely affected by the fibers. In the region of the films A4 and A5 the percentage of elongation increased recovering values close

to the reference value, but not higher (11.27 % for A5). The B1 film had an intermediate elongation between the previous ones, 9.93 %.

Figure 8.C shows the elastic modulus of films A0, A1, A2, A3, A4, A5, and B1. As in the previous cases, the addition of 140-CNF in percentages between 1-5 % resulted in a decrease of the elastic modulus with respect to the reference value, dropping from 85.57 to 27.90 MPa. This implies that the addition of a low percentage of fibers made the film less stiff, which would be related to the decrease of the tensile stress. The addition of a higher percentage of fibers in the A4 (15 % 140-CNF) and A5 (25 % 140-CNF) films allowed to reach a stiffness similar to that of the reference film with values of 88.97 MPa (A4) and 92.23 MPa (A5). The film with the lowest elasticity was B1 (65 % 140-CNF) with an elastic modulus of 158.76 MPa.

Comparing with literature, Zhang and Whistler (2004) produced films with chemically unmodified hemicelluloses and obtained values of 10-51 MPa of tensile stress, 6-12 % of elongation, and 365-1320 MPa of elastic modulus. The films were stronger than those of the present work, had similar stretchability, and were much stiffer. Liu et al. (2016) combined hemicelluloses and chitosan to make films, and achieved a tensile stress value of 14.26 MPa, closer to that of the present work but still higher. By reinforcing the hemicellulose/chitosan films with CNF, Xu et al. (2019b) obtained a tensile stress between 31.02 – 38.56 MPa and elongation between 10.07 – 15.98 %. The lower stress of the films in the present work could be associated with the type of hemicellulose, arabinogalactan, while the most common type in film formation is arabinoxylan or galactoglucomannan. Regarding the two regions that occurred in the tensile properties with respect to the fibers content, in the work of Ryu and Lee (2001) they found that with a low percentage of fibers (< 15 %) the tensile stress was dominated by the main biopolymer, the fibers acting as a network defect due to the high difference in their moduli. Beyond the critical fiber content, they found that the tensile stress started to be dominated by the fibers and the values increased. The critical point depends on the type of fibers used.

3.3.5. FTIR

The structural characterization of films A0, A1, A2, A3, A4, A5 and B1 was performed determining the FTIR spectra of their top surface (Figure A.2). The broad 3700-3000 cm^{-1} region is associated with the O-H stretching vibrations of the hydroxyl group, characteristic of lignocellulosic materials. The intensity of the films was similar except for film B1, which was lower. This makes sense as the structure is completely different, with the B1 film consisting mostly of cellulose and lignin versus the other films consisting mostly of high molecular weight AG-P.

The 3000-2800 cm^{-1} region is related to C-H stretching vibrations. This bond is present in both AG-P and CNF as it is part of the monosaccharides. The peaks 2940 and 2890 cm^{-1} were of higher intensity in films containing AG-P (A films) than in B1 film. These peaks are associated with aliphatic C-H stretching (de Souza et al., 2018), so it makes sense that in the films rich in AG-P this bond is more abundant than in the films rich in CNF and lignin.

The 1800-1500 cm^{-1} band represents carbonyl (C=O) stretching vibrations. In particular, the 1730 cm^{-1} peak is associated with the C=O methyl ester ($COOCH_3$) group present in pectins (Ben-Fadhel et al., 2020). This peak was of much higher intensity in films containing AG-P than in films not containing AG-P (B1), and the intensity was higher the higher the percentage of AG-P in the film.

The 1620 cm^{-1} peak is associated with the stretching vibration of the carbonyl group of the carboxylate ion (COO^-), present in AG and P (Sucheta et al., 2019). This peak had a similar intensity in all films.

The band between 1500-1300 cm^{-1} includes several peaks. The peaks 1420 and 1370 cm^{-1} are associated with CH_2 bending, 1330 cm^{-1} is due to ring vibration, and 1240 cm^{-1} is due to C-O stretching vibration at C6 so it is related with pyranose ring vibration (Szymanska-Chargot and Zdunek, 2013). The intensity of these peaks was higher in the AG-P films than in the B1 film. In turn, the intensity was higher for A0, A1, A2 and A3 compared to A4 and A5.

The 1200-950 cm^{-1} band contain peaks associated with polysaccharides, with the 1090 and 1020 cm^{-1} peaks standing out. The 1090 cm^{-1} peak is associated with C-O and C-C

stretching vibrations (Szymanska-Chargot and Zdunek, 2013). This peak was more marked in the films with the highest percentage of 140-CNF (B1, A4 and A5), although the intensity of the peak was lower in B1 than in the rest of the films. The 1020 cm^{-1} peak is also associated with C-O and C-C stretching vibrations (Szymanska-Chargot and Zdunek, 2013). In this case, the intensity was the same for all films containing AG-P and was lower but with a more defined peak for film B1 (65 % 140-CNF).

Finally, as in the previous case, the 917 cm^{-1} peak was identical in the films containing AG-P and of lower intensity in films B1. This peak is associated with the rocking mode of CH_3 (Muñoz-Almagro et al., 2019), abundant in AG-P.

The peaks where A4 and A5 differ from the rest of AG-P containing films could be key and relate to the difference in properties between A4 and A5.

3.4. Effect of different molecular weight and composition of the AG-P fraction

In view of the effect of the addition of 140-CNF on the properties of the films, two regions were observed. In the first region between 0-5 % 140-CNF, fibers did not provide any benefit except for a slight decrease in OP. The addition of a higher percentage of fibers between 10-25 % resulted in improved properties except for OP. This improvement was not always an improvement over the reference value of the A0 film, so the addition of 140-CNF did not seem to be beneficial beyond the increase in surface hydrophobicity. Consequently, the smallest percentage of fibers (1 % 140-CNF), whose properties are similar to those of the reference film without 140-CNF, was selected for the formation of films with different composition and molecular weight.

Films C1 and C2 were prepared for comparison with each other and with film A1. As shown in Table 3, all three films contained the same percentage of 140-CNF (1 %), the same percentage of AG-P (64 %), and the same percentage of glycerol (35 %). The difference between them was the AG-P fraction, which as shown in Table 1 was of different molecular weight and composition. The molecular weight values and the ratio of AG to total biopolymers (AG and P) were 102.75 kDa and 0.585 (C1), 80.36 kDa and 0.900 (C2), and 67.77 kDa and 0.732 (A1). To evaluate the influence of the AG-P sample on the films, OP, WVP, water contact angle, tensile properties, and FTIR spectra were

determined. Images of the films can be seen in Figure 9. The difference in color is because the AG-P fractions of C1 and C2 come from hydrothermal treatment at 140 °C, as opposed to the AG-P fraction of A1 which comes from hydrothermal treatment at 180 °C.

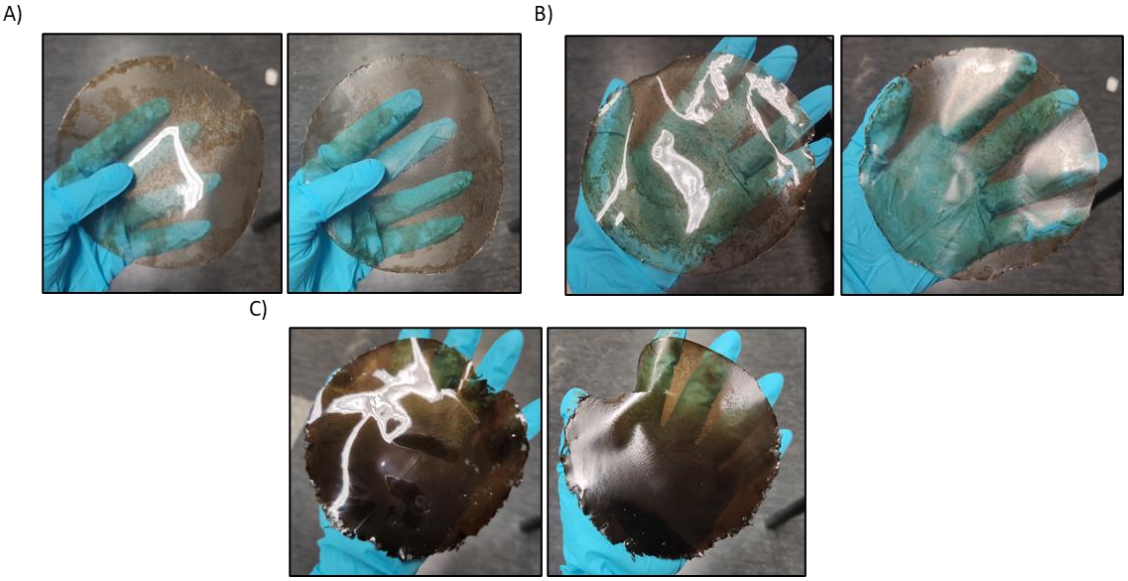


Figure 9. Images of the bottom side, top side, and surface against light background of the films C1 (102.75 kDa), C2 (80.36 kDa) and A1 (67.77 kDa).

3.4.1. Oxygen permeability (OP)

The OP values of films C1, C2 and A1 are shown in Figure 10.A. The evolution shows that the higher the molecular weight of the AG-P sample, the lower the OP. This can be explained by the fact that the higher molecular weight would imply a higher entanglement between the molecules present, which would make it more difficult for oxygen to pass through. From this point of view, the higher molecular weight film would be the most interesting. The values were between 41.14 and 48.18 $(cm^3 \cdot \mu m)/(m^2 \cdot kPa \cdot day)$.

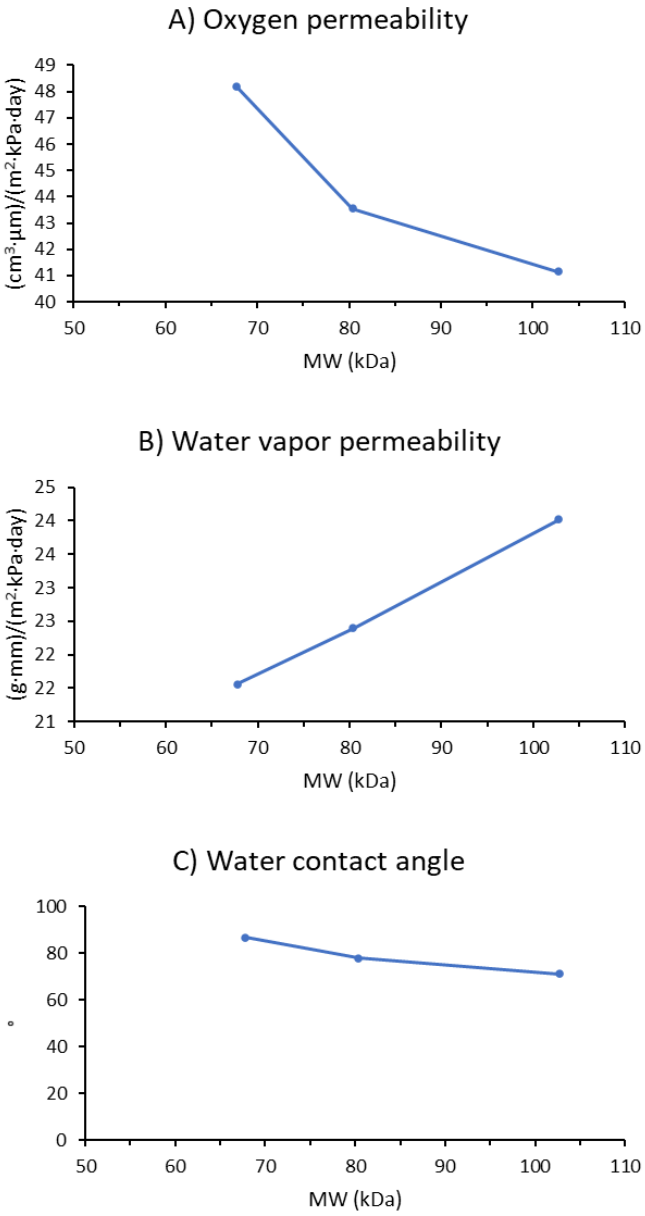


Figure 10. OP, WVP and water contact angle of the films C1 (102.75 kDa), C2 (80.36 kDa) and A1 (67.77 kDa).

3.4.2. Water vapor permeability (WVP)

The evolution of the WVP in films C1, C2 and A1 is shown in Figure 10.B. In this case, contrary to OP, a higher molecular weight resulted in a higher WVP value. This may be due to both arabinogalactan and pectin are hydrophilic and therefore cannot hinder the passage of water vapor through them despite the presence of a higher number of bonds between the monomers of the biopolymer. Despite the small number of points available for analysis, a linear dependence between WVP and molecular weight can be observed.

From the point of view of WVP, a fraction of AG-P with sufficient molecular weight to form the film but not the highest molecular weight would be interesting. The values were between 21.56 and 24.01 (g·mm)/(m^2·kPa·day).

3.4.3. Tensile properties

Films C1, C2 and A1 were studied by determining their tensile properties through tensile stress at maximum load, tensile strain (extension) at break, and elastic modulus. The analysis of the results showed that in this case not only the molecular weight influenced the tensile properties, but more importantly the composition of the AG-P fraction. The composition was defined as the ratio of arabinogalactan to total arabinogalactan and pectin, and was 0.585 (C1), 0.732 (A1) and 0.900 (C2). Given the influence of both molecular weight and composition, Figure 11.A represents the tensile stress versus the product of MW and AG/(AG+P). This product implies that a low MW value can compensate for a high AG/(AG+P) value in terms of tensile stress, and vice versa. The evolution shown in Figure 11.A is linear, with higher tensile stress for lower values of the product MW·(AG/(AG+P)) and lower for higher values of the product. Therefore, it can be established that a lower value of MW and AG/(AG+P) resulted in a higher value of tensile stress. This may be due to the higher molecular weight imply a different linearity that does not necessarily contribute to higher strength of the film, while a higher presence of pectins contributed positively to the film strength. The result values were 1.13 MPa (C2), 2.04 MPa (C1), and 2.84 MPa (A1).

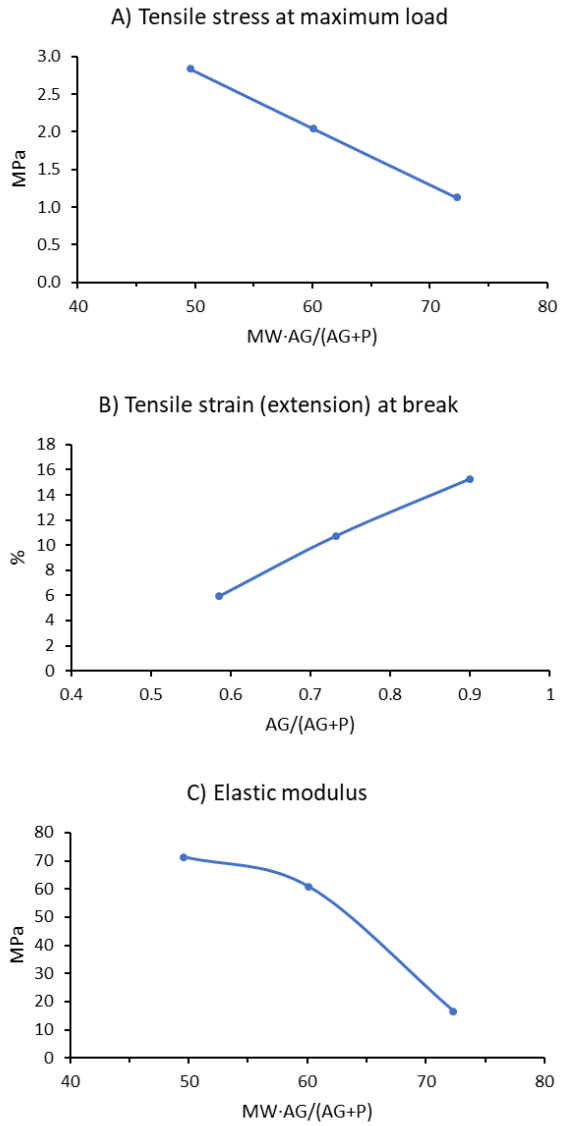


Figure 11. Tensile properties of the films C1 (102.75 kDa, AG/(AG+P):0.585), C2 (80.36 kDa, AG/(AG+P):0.900) and A1 (67.77 kDa, AG/(AG+P):0.732).

Figure 11.B shows the tensile strain of the three films. In this case, it was seen that the ratio AG/(AG+P) was the only variable with which the elongation value followed a trend. The evolution shows that a higher proportion of AG (and thus a lower presence of pectins) increased the film's stretchability. The trend was the opposite of that shown for tensile stress, and in this case, the influence of molecular weight seemed negligible. The result values were 5.92 % (C1), 10.74 % (A1), and 15.28 % (C2).

The evolution of the elastic modulus can be seen in Figure 11.C. The modulus value was plotted against the product of MW and AG/(AG+P) as both variables had a joint influence. The films were more elastic at lower molecular weight and higher presence

of pectins, and therefore stiffer at higher molecular weight and higher presence of AG. The result values were 71.32 MPa (A1), 60.80 MPa (C1), and 16.51 MPa (C2).

From a tensile point of view, if a film with higher strength is sought after, the lowest molecular weight still sufficient to form the film would be the best choice, as well as the highest possible pectin content. If a film with higher stretchability is sought after, a high AG content should be beneficial. The most elastic films would be those with higher pectin content and lower molecular weight.

As can be seen, once the molecular weight required for film production has been reached, the higher molecular weight may not always be beneficial for the type of films produced in this work.

3.4.4. FTIR

The structure of films C1, C2 and A1 was evaluated through FT-IR spectra of their top surface (Fig. A.3). The 3700-3000 cm^{-1} band represents the O-H stretching vibrations typical of lignocellulosic materials. The intensity was similar for the higher molecular weight films (C1 and C2) and higher for the lowest molecular weight film (A1), so it could be concluded that the lower the molecular weight, the lower the presence of these vibrations.

The peaks 2940 and 2890 cm^{-1}, associated with aliphatic C-H stretching vibrations (de Souza et al., 2018), had a similar intensity in the three films.

The 1730 cm^{-1} peak represents the C=O methyl ester ($COOCH_3$) group present in pectins (Ben-Fadhel et al., 2020). The intensity of the peak was very similar in the three films, although it was slightly lower in the film with the lowest pectin content (C2).

The 1620 cm^{-1} peak, associated with the stretching vibration of the carbonyl group of the carboxylate ion (COO^-), was lower for the film with the lowest pectin content (C2) and similar for the other two films.

The peaks 1420 and 1370 cm^{-1}, associated with CH_2 bending (Szymanska-Chargot and Zdunek, 2013), had a higher intensity in the film with the lowest molecular weight (A1), being very similar for the other two films.

The 1330 cm^{-1} peak, associated with ring vibration (Szymanska-Chargot and Zdunek, 2013), was also of higher intensity at lower molecular weight.

The 1240 cm^{-1} peak is associated with C-O stretching vibration at C6 (pyranose ring vibration) (Szymanska-Chargot and Zdunek, 2013), and was of markedly higher intensity and similar to each other in the two higher molecular weight films (C1 and C2), so the bond could be present in a higher proportion at higher molecular weight.

The 1090 cm^{-1} peak, associated with C-O and C-C stretching vibrations (Szymanska-Chargot and Zdunek, 2013), was slightly higher for higher molecular weight films.

The 1020 cm^{-1} peak is also associated with C-O and C-C stretching vibrations (Szymanska-Chargot and Zdunek, 2013), and its intensity was lower for the lowest molecular weight.

4. Conclusions

Biodegradable films were produced from discarded carrot fractions obtained by biorefinery techniques. The main ingredient was purified high molecular weight fractions of hemicelluloses and pectins. As an additive agent in the films, a fraction of cellulose nanofibers combined with lignin was used. This fraction was obtained through a mechanical treatment applied to the residual pulp after hydrothermal treatment. With respect to the residual pulp content (0-25 %) two regions of behavior were observed corresponding to a content < 5 % and between 5-25 %. Oxygen permeability decreased with a small percentage of fibers but recovered the reference values with a higher percentage. Water vapor permeability and tensile properties were impaired by the presence of residual pulp except when added in a percentage that allowed the formation of a homogeneous network throughout the film. Hydrophobicity was higher the higher the residual pulp content, with contact angles from 79.9° (0 %) to 125.8° (25 %). To study the influence of the hemicellulose and pectin fraction, films were made with 1 % residual pulp, molecular weights of 67.77, 80.36 and 102.75 kDa and different hemicellulose/pectin ratios. Higher molecular weight decreased oxygen permeability, increased water vapor permeability and decreased surface hydrophobicity. Tensile properties were found to have a significant dependence on the hemicellulose/pectin ratio. Tensile strength was higher at lower molecular weight and higher pectin content, while elongation was higher at higher hemicellulose content. The films had acceptable properties for food packaging applications and very high hydrophobicity, with the

advantage of being made from 100% agri-food waste and through environmentally friendly processes.

5. Acknowledgments

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7. Supplementary material

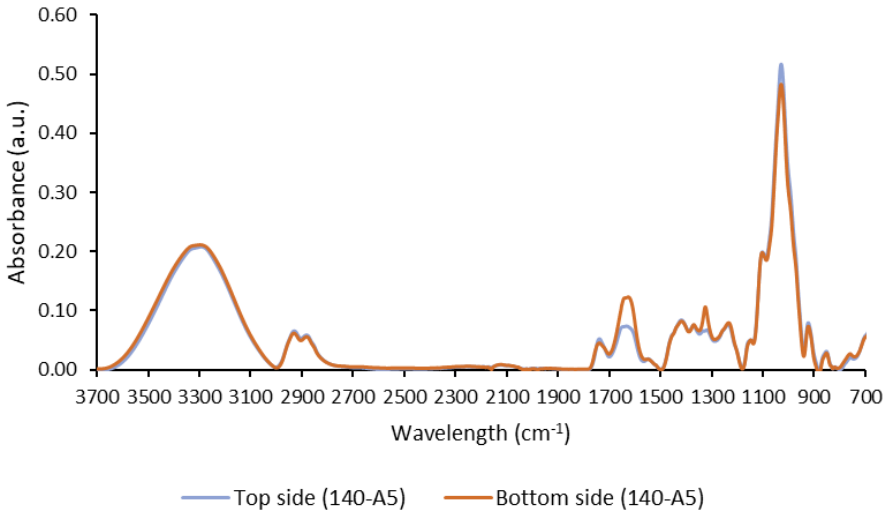

Figure A.1. FTIR specta of top and bottom sides of the film 140-A5.

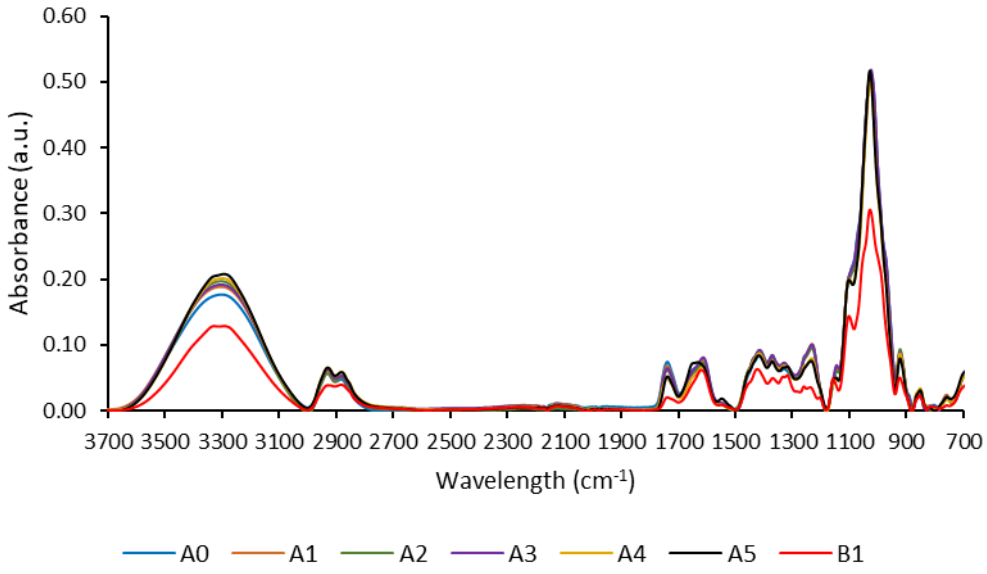

Figure A.2. FTIR spectra of the films A0 (0 % 140-CNF), A1 (1 % 140-CNF), A2 (2% 140-CNF), A3 (5% 140-CNF), A4 (15 % 140-CNF), A5 (25 % 140-CNF) and B1 (65 % 140-CNF).

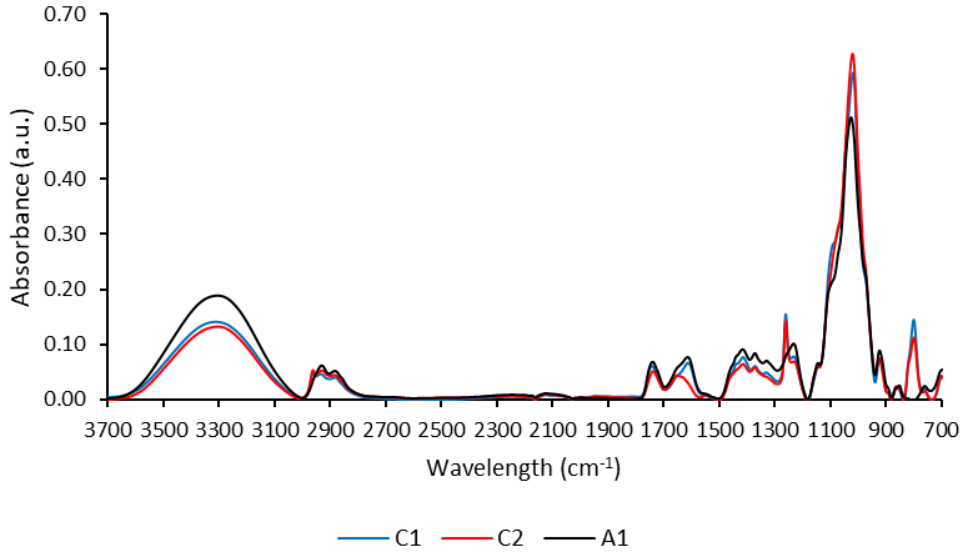

Figure A.3. FTIR spectra of the films C1 (102.75 kDa, AG/(AG+P):0.585), C2 (80.36 kDa, AG/(AG+P):0.900) and A1 (67.77 kDa, AG/(AG+P):0.732).

Conclusions

Is it possible a biorefinery at pilot-scale for agricultural and agri-food discards?

This thesis demonstrates that a pilot-scale biorefinery can be applied to agricultural and agri-food waste to produce valuable products. Specifically, we have applied pilot-scale hydrothermal treatment in cycles combined with downstream processes, e.g. ultrafiltration/diafiltration and spray drying. The processes were applied to spent coffee grounds and discarded carrots, producing intermediates such as carotenoids, sugars, hemicellulose, pectin, and lignin-containing cellulose nanofibers. These intermediates were successfully used to produce biofilms, encapsulated pigments, and fermentation products (e.g. lactic acid and ethanol).

Are spent coffee grounds suitable for a biorefinery?

Regarding Chapter 1, the application of the biorefinery concept to spent coffee grounds through its fractionation was sought after. The chemical characterization of spent coffee grounds showed that it is a biomass of interest that stands out for its high content in hexane extractives (oil) and its low content in water extractives (free sugars). This makes it an interesting material for oil valorization and subsequent extraction of the biopolymer hemicellulose. The oil was efficiently extracted with supercritical CO_2 and chemically characterized. Being extracted with a non-toxic and easily recoverable solvent, this oil may be suitable for applications in the food and pharmaceutical industry. The hydrothermal treatment applied to the defatted solid allowed the extraction of hemicelluloses (3.49 g/100 g dry material) at different temperatures with low presence of by-products. The yield was not high, which could be due to this biomass needs higher temperatures because it was previously subjected to hydrothermal treatment for the production of soluble coffee. The extracted hemicelluloses reached a high molecular weight due to the moderate temperature and the flow-through configuration of the reactor, characterized by a low liquid residence time. The conditioning of the extracts using a cascade ultrafiltration system made it possible to verify the technical viability of the separation of hemicelluloses according to their molecular weight, as well as their purification applying several diafiltration cycles. Purification through diafiltration allowed decrease by-products retentions from 45.6 % to 8.7 %. The final fractions had purities between 83.7 – 97.8 % (w/w) and average molecular weights between 1.64 and

49.73 kDa. These purified fractions are potential ingredients for multiple applications of hemicelluloses, different according to their molecular weight.

Is a highly perishable food, such as discarded carrots, suitable for a biorefinery?

The next part of the thesis was aimed at applying the biorefinery concept to discarded carrots, an agricultural and agri-food waste that is abundant in the region and that stands out for its high moisture content. The valorization started with the separation of the juice and pulp. Chapter 2 focused on the valorization of the pulp through hydrothermal treatment in a flow-through reactor system operating at different temperatures. The treatment allowed the extraction of free sugars, hemicelluloses and pectins with a high yield. Due to the abundant presence of free sugars, the purity of the biopolymers (hemicelluloses and pectins) was low, and the molecular weight distribution very wide. Due to the flow-through extraction system, hemicelluloses and pectins of very high molecular weight were obtained, higher the lower the temperature. Chemical characterization of the residual pulp remaining after hydrothermal treatment showed an abundant cellulose content (57.5 % w/w) and a considerable lignin content (15.7 % w/w). Given the multiple applications of cellulose in the generation of biomaterials, this fraction, which could be considered as a residue, was nevertheless an interesting fraction. The lignin content was seen as an interesting additive that could provide good properties to potential products.

How can carrot juice be valorized in a biorefinery?

Chapter 3 focused on the valorization of discarded carrot juice through the fractionation of its two main components: free sugars and carotenoids. The separation was done through multiple diafiltration cycles, reducing the sugar content of the carotenoid-rich fraction by 90.7 %, and the carotenoid and fiber content of the sugar-rich fraction to zero. Since this was a physical separation process, it did not require chemical agents or the application of temperature. The formulation of carotenoids was studied with gum Arabic as encapsulating agent through spray drying and freeze drying. Encapsulation by freeze drying was not adequate and the encapsulant increased degradation. Encapsulation by spray drying was efficient and reduced degradation by 51.9 % compared to the unencapsulated material. The sugar-rich fraction was interesting for its

lack of fermentation inhibitory compounds and its nutrient content. The feasibility of three types of fermentation was studied: with autochthonous microorganisms, with lactic acid bacteria and with yeasts. Fermentation with both autochthonous microorganisms and lactic acid bacteria resulted in the formation of lactic acid, while fermentation with yeast produced ethanol. The lactic acid fermentations suffered from contamination by invading yeasts, leading to co-production of ethanol. To eliminate this phenomenon, the addition of 6 % (w/v) NaCl to the medium was studied, which allowed both autochthonous microorganisms and lactic acid bacteria to produce pure lactic acid. To improve the process on a larger scale, a pH control to increase lactic acid production would be necessary.

Can biopolymers produced from hydrothermal treatment be purified?

Chapter 4 focused on the conditioning of the biopolymers hydrothermally extracted in Chapter 2 at 140, 160 and 180 °C. The three extracts were characterized chemically and in terms of their molecular weight distribution, confirming the need for conditioning. This process was performed through ultrafiltration and multiple diafiltration cycles. A cascade membrane configuration (30-10-5-1 kDa) was applied to the 140 and 160 °C extracts, and a mixed configuration (5-10-1 kDa) to the 180 °C extract. The treatment allowed increasing the concentration of certain biopolymer groups by a factor of up to 5 in the cascade configuration and by a factor of up to 16.7 in the mixed configuration. The diafiltration cycles allowed an efficient removal of free sugars (98.9-99.5 %) and by-products (94.4-99.2 %). The purity of the obtained fractions was between 73.1-100 % while the purity of the starting extracts was between 30.12-33.51 %. The use of membranes with different MWCO allowed obtaining fractions whose average molecular weight ranged from 2.59 kDa to 102.75 kDa. The higher definition of the fractions meant that the final polydispersions were in the range 1.2-4.0 versus the polydispersions of the extracts (16.2-31.6). The values of the parameters characterizing the fractions are very suitable for processing into high value-added products.

Can the hydrothermal treatment and the downstream processes be carried out on a large scale?

In view of the good results of the purified biopolymer fractions and the potential interest of the residual pulp fraction, it was decided to valorize the discarded carrot pulp on a larger scale to obtain material in sufficient quantity for processing into products. This was done in Chapter 5. In the hydrothermal treatment, instead of operating with a single reactor, more reactors were incorporated working in cycles of three reactors in series operating at the same time. A total of 7 reactors were used in each of the two experiments, performed at 140 and 180 °C. In each experiment, 11.32 kg of discarded carrots were processed compared to 1.6-2 kg when operating with a single reactor. Due to the efficient mode of operation, extraction yields were generally higher than those obtained with a single reactor. Residual pulp was recovered from each experiment for further applications. The influence of preheating or not the reactor with water at 90 °C before starting the flow-through extraction was studied. The absence of preheating resulted in greater instability due to changes in the operating pressure, but in general allowed a greater/faster extraction of the biomass components because the biomass was in direct contact with the water at the extraction temperature, without dilution. The extracts obtained in the experiments at 140 and 180 °C were recovered and treated by ultrafiltration and multiple cycles of diafiltration, which allowed obtaining purified fractions of hemicelluloses and pectins. From the 140 °C extract, fractions of molecular weight 10-30 kDa and > 30 kDa were recovered, and from the 180 °C fraction with higher autohydrolysis phenomena, fractions of molecular weight 1-10 kDa, 10-30 kDa and > 30 kDa were recovered. The diafiltration cycles were efficient and allowed the removal of 100 % of free sugars and by-products in the 140 °C extract and 98.7 % of free sugars and 99.8 % of by-products in the 180 °C extract. The five purified fractions spanned a wide range of average molecular weights (3.86-5.23-9.85-67.77-80.36 kDa), had low polydispersities (1.3-3.9) and high purities (64.5-100 % w/w). The parameter values were good, especially considering that the feeds had molecular weights, polydispersities and purities of 14.77 kDa, 19.2 and 22.2 % (140 °C) and 8.08 kDa, 18.2 and 14.9 % (180 °C). The study of membrane fouling showed that the diafiltration cycles are a stable method that can be repeated as many times as desired until the desired purity level is

reached. Drying of the purified fractions using freeze drying and spray drying allowed subsequent storage prior to use.

Are the biopolymers produced good enough to produce biofilms?

Chapter 6 focused on obtaining biodegradable films based on purified fractions of hemicelluloses and pectins obtained in Chapters 4 and 5. As an additive in the films, the effect of the residual pulps resulting from the hydrothermal treatment applied at 140 and 180 °C in Chapter 5 was studied. The residual pulp obtained at 140 °C provided greater hydrophobicity to the films than the one obtained at 180 °C, and it was therefore selected. The influence of different residual pulp contents (0-25 %) on film properties was studied, showing that in general there were two behaviors. A small residual pulp content (0-5 %) was beneficial only in slightly decreasing oxygen permeability and providing hydrophobicity but worsened other film properties. The addition of a higher percentage of residual pulp (5-25 %) allowed the film to recover values close to the reference values (film without residual pulp) and the hydrophobicity increased considerably from 79.9° to 125.8° water contact angles. A residual pulp content of 1 % was selected as the reference value because it has similar properties to the film without residual pulp but with lower oxygen permeability and higher hydrophobicity. The elaboration of films with different fractions of hemicelluloses and pectins was studied, and it was found that a minimum molecular weight is necessary for the elaboration of films. Films were only formed with the highest molecular weight fractions (67.77, 80.36 and 102.75 kDa) corresponding to the MWCO 30 kDa membrane. The higher the molecular weight of the hemicellulose and pectin fractions, the lower the oxygen permeability, the higher the water vapor permeability and the lower the hydrophobicity. The tensile properties depended mainly on the ratio of hemicelluloses/(hemicelluloses + pectins). Tensile strength was higher at lower molecular weight and higher pectin content, and elongation was higher at higher hemicelluloses content. Regarding the elastic modulus, films were more elastic at lower molecular weight and higher pectin content, while they were stiffer at higher molecular weight and higher hemicelluloses content. The best results obtained were: 41.14 $(cm^3 \cdot \mu m)/(m^2 \cdot kPa \cdot day)$ oxygen permeability, 17.64 $(g \cdot mm)/(m^2 \cdot kPa \cdot day)$ water vapor permeability, 125.8° water contact angle, 7.74 MPa tensile strength, and 15.28 % elongation. The films had

acceptable properties for food packaging, highlighting their high hydrophobicity and their predictable high biodegradability because they come from 100 % vegetable waste and no chemical modifications were necessary for their formation.

FUTURE WORK – what more can be done?

This thesis has shown that almost 100 % of a vegetable by-product can be used if the right strategy is adopted within a circular framework. The processes applied are environmentally friendly and do not require extreme chemical modifications, so that the companies would not have environmental problems and could foreseeably implement the processes in a simple way. The fact that bioplastics produced from food waste can be used in food packaging is a great environmental and economic advance.

Another option in which progress can be made is in the elaboration of films, trying to improve their properties, obtaining smart films. Some of these improvements are sensitivity to pH, temperature, or antioxidant and antibacterial capacity. These modifications could be carried out by combining them with fractions of other biomasses. For example, the carotenoid-rich fraction could be added to the films providing antioxidant properties. Other fractions rich in polyphenols could provide antibacterial properties, highly valued in food packaging. The study of the biodegradability of the biofilms produced is also recommended.

From the purified fractions of hemicelluloses and pectins, the production of alternative products such as hydrogels could be studied. These hydrogels would have interesting applications in the medical, pharmaceutical, and cosmetic industries.

An envisioned future work will include the development of biomaterials on a demo or industrial scale in agricultural companies that generate vegetable residues.

Resumen

1. Introducción

La economía mundial es fuertemente dependiente de recursos fósiles para la obtención de energía y de productos. Estos recursos se agotarán en cuestión de años pues son de carácter no renovable y su demanda es cada vez mayor debido al aumento de la población. Además de su agotamiento, la explotación de estos recursos lleva asociados problemas medioambientales relacionados con la contaminación, como por ejemplo el calentamiento global debido a la emisión de gases de efecto invernadero.

Uno de los principales productos derivado de los recursos fósiles es el plástico. Los materiales plásticos se caracterizan por unas muy buenas propiedades mecánicas y barrera, lo que los hace muy versátiles. Además, su producción está muy estudiada y es económica. Por ello son utilizados en la totalidad de los sectores industriales, entre los que destaca el envasado y en concreto el envasado de alimentos. El principal problema del plástico, además del agotamiento de la materia prima, es que es un material difícil de reciclar y no biodegradable, por lo que se acumula en el medioambiente en grandes cantidades. Dentro del problema de la acumulación destaca el problema de los microplásticos, pequeñas partículas de plástico difíciles de distinguir y por tanto fácilmente consumibles por los seres vivos de manera accidental.

Es importante encontrar una alternativa a los recursos fósiles que permita reducir los problemas medioambientales citados y en concreto el problema del plástico. La bioeconomía es un concepto que busca resolver este problema a través del establecimiento de un sistema de producción descentralizado basado en materias primas renovables y abundantes, cuyo uso no compita con otros usos relacionados con aplicaciones como la alimentación. La Unión Europea es el principal promotor de la implantación de la bioeconomía en el sistema, para lo que se sirve de diversos planes estratégicos que incluyen medidas legislativas y no legislativas aplicables a los países miembros. El asunto del plástico es uno de los problemas que forman parte de los planes de la Unión Europea que buscan proteger el medioambiente y preservar la sostenibilidad.

Del concepto de bioeconomía surge el concepto de biorrefinería. Una biorrefinería es equivalente a una refinería de petróleo, pero en lugar de partir de fuentes fósiles como

materia prima se parte de biomasa. Esta biomasa puede ser de diversos tipos, entre los que destaca la biomasa lignocelulósica por ser la fuente de carbono renovable más abundante en la Tierra. Existe una gran variabilidad en la composición de las biomasas lignocelulósicas, por lo que es importante que su fraccionamiento y demás procesos aplicados sean eficientes además de ser respetuosos con el medioambiente. Este tipo de biomasa se caracteriza por una compleja estructura cuyos principales biopolímeros son celulosa, hemicelulosa y lignina, entre otros componentes como los extractivos (azúcares libres, compuestos nitrogenados, resinas, etc.). El biopolímero pectina también puede estar presente.

Los métodos empleados en biorrefinería suelen basarse en una primera separación de componentes no estructurales como los extractivos. Los extractivos pueden ser de tipo polar o apolar, y por tanto solubles en disolventes como agua, etanol o hexano. Existen métodos de extracción alternativos basados en el uso de solventes verdes y no tóxicos, como el CO_2 supercrítico. Dependiendo del tipo de extractivo la valorización será distinta, pudiendo los compuestos bioactivos formularse para su utilización en alimentación, cosmética o farmacia; o los azúcares libres transformarse en otros compuestos químicos mediante procesos biotecnológicos.

El siguiente paso sería el fraccionamiento de los biopolímeros más fácilmente extraíbles, que son las hemicelulosas y las pectinas. Una vez separados estos biopolímeros en fase líquida, es necesario un postratamiento que permita su separación en función del peso molecular y su purificación de manera que se obtenga un biopolímero de cierta calidad. El biopolímero puede entonces emplearse para la obtención de productos de alto valor añadido, como bioplásticos. En ocasiones es interesante su secado previo para obtenerlo en fase sólida y de esta manera manipularlo mejor de cara a su almacenamiento y transformación.

El sólido de biomasa que queda tras la extracción de extractivos, hemicelulosas y pectinas es abundante en celulosa y lignina, por lo que también es un material interesante debido a las propiedades que aporta la celulosa (fortaleza, biodegradabilidad) y la lignina (hidrofobicidad, capacidad antioxidante, biodegradabilidad) pudiendo emplearse como aditivo en la fabricación de bioplásticos o de otros productos.

Dentro de la biomasa lignocelulósica, los residuos agrícolas y agroalimentarios son muy significativos a nivel global. Dentro de estos residuos destacan los derivados de la producción de alimentos, que alcanzan cifras del 35 % de la producción. Estos residuos vegetales suelen diferenciarse de otros residuos lignocelulósicos por su elevado contenido en humedad, en ocasiones próximo al 90 %. Ello hace que estos subproductos no sólo representen un problema económico para los productores sino también un problema medioambiental por su difícil gestión, pues actualmente no hay una valorización clara para ellos. Su incineración para obtener energía es ineficiente por el elevado contenido en humedad, por lo que la salida habitual es destinar un pequeño porcentaje a alimentación animal mientras que la gran mayoría se vierte en vertederos o en el campo. El vertido genera problemas por los rápidos procesos de putrefacción que tienen lugar.

Las biomasas lignocelulósicas valorizadas en la presente tesis fueron el residuo agroalimentario conocido como marro de café y el residuo agrícola y agroalimentario conocido como zanahorias descartadas o zanahorias de destrío. El marro de café es un residuo formado en el proceso de preparación de la bebida de café y sobre todo y en grandes cantidades en las industrias de café soluble. Se caracteriza por tener un contenido elevado en humedad, un porcentaje considerable de aceite, y biopolímeros propios de la biomasa lignocelulósica. Las zanahorias descartadas por su parte son un residuo generado sobre todo en el proceso de preparación de las zanahorias para su venta, donde un porcentaje cercano al 30 % son descartadas por no cumplir los estándares de forma, color y tamaño. Este residuo también se genera a nivel supermercado y en hogares al superar la fecha de expiración, o en las industrias productoras de zumo en forma de pulpa de zanahoria. Las zanahorias descartadas se caracterizan por un elevado contenido en humedad, un elevado contenido en azúcares libres (extractivos), un considerable contenido carotenoides (compuestos bioactivos con múltiples aplicaciones en industria alimentaria, farmacéutica y cosmética), y por la presencia de los biopolímeros típicos de la biomasa lignocelulósica.

2. Objetivos

El objetivo global de la tesis es la aplicación del concepto de biorrefinería a residuos de tipo agroalimentario para la valorización de todos los componentes presentes en el material, con objeto de transformarlos mediante procesos respetuosos con el medioambiente en productos de valor añadido. Este objetivo global se materializa en una serie de objetivos parciales:

1. Aplicación del concepto de biorrefinería al marro de café, fraccionándolo en (i) aceite, (ii) hidrolizado de hemicelulosas, y (iii) sólido rico en celulosa y lignina (Capítulo 1).
2. Aplicación del concepto de biorrefinería a las zanahorias descartadas, separando el zumo de la pulpa y valorizando los componentes de la pulpa (Capítulo 2).
3. Aplicación del concepto de biorrefinería al zumo de zanahorias descartadas, valorizando los carotenoides y los azúcares libres presentes (Capítulo 3).
4. Acondicionamiento de los extractos hidrotermales obtenidos a distintas temperaturas de la pulpa de zanahorias descartadas mediante ultrafiltración y diafiltración multietapa (Capítulo 4).
5. Aplicación del concepto de biorrefinería a la pulpa de zanahorias descartadas en gran escala para la obtención de fracciones purificadas en cantidad suficiente para su transformación en productos (Capítulo 5).
6. Transformación de las fracciones obtenidas a gran escala de la pulpa de zanahorias descartadas en films biodegradables con aplicación en el envasado de alimentos (Capítulo 6).

3. Resultados y discusión

3.1. Capítulo 1

El marro de café se caracterizó químicamente determinando su composición: lignina (26,90 % w/w ± 0,10), ceniza (0,60 % w/w ± 0,04), glucano (11,97 % w/w ± 0,05), manano (17,46 % w/w ± 0,09), galactano (0,58 % w/w ± 0,03), proteínas (11,11 % w/w ± 0,77), extractivos en agua (9,84 % w/w ± 0,41), extractivos en etanol (2,40 % w/w ± 0,24) y extractivos en hexano (20,00 % w/w ± 0,04).

El marro de café fue secado y sometido a extracción con CO_2 supercrítico para la retirada del aceite y compuestos apolares. Las condiciones de operación fueron de 300 bar y 45 °C, con un flujo de CO_2 supercrítico de 5 kg/h en recirculación. El motivo de retirar el aceite fue que está en un porcentaje considerable, y que su presencia dificulta la extracción de los biopolímeros. El rendimiento de extracción del aceite fue de un 14,04 % (w/w) respecto al marro de café en base seca, lo que representó un 70,2 % del rendimiento alcanzado en la extracción con hexano mediante Soxhlet. La extracción con CO_2 supercrítico es un método interesante porque es un solvente muy abundante, barato y no tóxico, y por tanto adecuado siempre que se trabaje con potenciales productos de la industria de la alimentación, cosmética o farmacia. El aceite extraído fue caracterizado químicamente determinando así su composición. Los principales lípidos fueron ácidos grasos (58,00 % w/w) y triglicéridos (32,00 % w/w). La determinación del perfil de ácidos grasos mostró un contenido del 45,1 % en ácidos grasos saturados, 11,40 % en ácidos grasos monoinsaturados y 43,4 % en ácidos grasos poliinsaturados. Los principales ácidos grasos fueron el ácido linoleico C18:2n-6 (42,1 %), ácido palmítico C16:0 (33,6 %), ácido oleico C18:1n-9 (10,47 %), ácido esteárico C18:0 (7,27 %) y ácido araquídico C20:0 (3,23 %), entre otros. De acuerdo con la bibliografía, este aceite podría utilizarse para la obtención de biodiesel. Sin embargo, se requieren estrategias que minimicen el problema derivado de los altos niveles de ácidos grasos libres, pues pueden causar neutralización del catalizar ácido que interviene en el proceso.

El sólido resultante de la extracción del aceite fue sometido a tratamiento hidrotermal para la extracción de hemicelulosas a dos temperaturas: 140 y 160 °C. La extracción se llevó a cabo en un reactor escala piloto de tipo “flow-through”. En este reactor la biomasa se carga mediante un cartucho y el agua se hace circular desde arriba hacia abajo. Al estar continuamente entrando agua fresca, el rendimiento de extracción es superior a la operación en “batch” y las hemicelulosas extraídas sufren menor autohidrólisis pues el tiempo de residencia del líquido es reducido (aproximadamente 7 min). Se obtienen por tanto hemicelulosas de un mayor peso molecular. Durante la extracción se tomaron muestras líquidas a la salida del reactor y se analizó la composición y la distribución de peso molecular.

Se determinó el rendimiento de extracción de hemicelulosa respecto a la holocelulosa (celulosa + hemicelulosa) presente en el marro de café desgrasado, alcanzándose un rendimiento máximo de 9,96 % (w/w) operando a 160 °C y de 8,22 % (w/w) operando a 140 °C. La extracción de hemicelulosas alcanzó un valor máximo de 3,49 g/100 g de materia prima en base seca operando a 160 °C. Los rendimientos moderados pudieron deberse a la presencia de aceite residual y a que la extracción de hemicelulosas en esta materia prima requiere de temperaturas más altas, pues el café ya fue sometido a una extracción previa para la producción de café soluble. Sin embargo, una temperatura de tratamiento hidrotermal muy elevada podría dar lugar a una autohidrólisis excesiva de las hemicelulosas, disminuyendo su peso molecular. El rendimiento de obtención de subproductos fue muy bajo, alcanzando un máximo de 1,21 g/100 g materia prima en base seca extrayendo a 160 °C. Ello se debe a que gran parte de los azúcares libres ya fueron extraídos en el proceso de producción de café soluble.

Respecto a la distribución de peso molecular de las hemicelulosas a la salida de la planta, el extracto obtenido a 140 °C tuvo mayor presencia del grupo de hemicelulosas de > 30 kDa que el extracto a 160 °C. En este último, tras 15 min de extracción las hemicelulosas de > 30 kDa fueron completamente autohidrolizadas a pesos moleculares inferiores. Dentro de los grupos moleculares de menor peso molecular, los más abundantes fueron los dímeros y los trímeros, seguido por el resto de los oligómeros en un orden decreciente desde tetrámeros hasta decámeros. El grupo de hemicelulosas más abundante a ambas temperaturas fue el de 1.6-5 kDa.

Los hidrolizados obtenidos del tratamiento hidrotermal fueron sometidos a un proceso de tipo “downstream” basado en una microfiltración inicial para eliminar micropartículas seguida por ultrafiltración en cascada con tres membranas de MWCO 30, 10 y 5 kDa. El volumen de la alimentación se redujo en un 80 % en cada membrana a través del permeado, logrando así concentrar las hemicelulosas retenidas. Los retenidos fueron sometidos a varias etapas de diafiltración en las que se añadió un volumen de agua igual al volumen del retenido y se hizo pasar a través de la membrana para arrastrar los productos y hemicelulosas de bajo peso molecular. Se analizó la composición y el peso molecular de los productos obtenidos de las membranas.

La reducción del volumen de la alimentación permitió concentrar hasta en un factor de 5 ciertas hemicelulosas, sobre todo en la primera membrana donde quedaron retenidas la mayoría. La formación de ciertos agregados entre las hemicelulosas es un fenómeno que pudo producirse y sería la causa de que gran parte de ellas quedaran retenidas en la primera membrana.

La aplicación de los ciclos de diafiltración a los retenidos permitió por un lado su purificación y por otro mejorar la separación de las hemicelulosas. La purificación se debió a una disminución en la retención total de subproductos desde 68,8 % a 12,5 % (140 °C) y desde 51,0 % a 19,4 % (160 °C). La separación se logró de forma que en el extracto de 160 °C los grupos de interés 5-10 kDa, 10-30 kDa y > 30 kDa se recuperaron con porcentajes de 78,9, 99,5 y 100 % mientras que los de no interés se eliminaron en un porcentaje de 57,2 %. En el extracto de 140 °C los grupos de interés 5-10 kDa, 10-30 kDa y > 30 kDa se recuperaron con porcentajes de 57,4, 85,7 y 90,6 % mientras que los grupos de no interés se eliminaron en un porcentaje de 66,2 %.

La caracterización de los seis productos finales obtenidos mostró valores de pureza entre 83,7-97,8 % y pesos moleculares medios desde 1,64 kDa hasta 49,73 kDa, mientras que las alimentaciones tuvieron purezas de 75,2 y 84,3 % y pesos moleculares de 12,79 kDa (140 °C) y 6,72 kDa (160 °C).

El sólido residual tras la extracción hidrotermal no fue caracterizado, pero es un material interesante por estar compuesto sobre todo por celulosa y lignina, junto con otros componentes de interés del marro de café. Tendría pues potenciales aplicaciones por ejemplo en la formación de biomateriales.

3.2. Capítulo 2

Al igual que el marro de café, las zanahorias descartadas fueron caracterizadas químicamente determinando así su composición: proteínas (2,26 % w/w ± 0,44), extractivos en agua (63,87 % w/w ± 1,28), extractivos en hexano (1,20 % w/w ± 0,79), celulosa (10,71 % w/w ± 0,40), hemicelulosas (8,43 % w/w ± 0,02), pectinas (5,78 % w/w ± 0,34) y lignina (7,75 % w/w ± 0,24). Los extractivos en agua fueron los componentes mayoritarios y fueron analizados. Su composición fue de un 8,01 % (w/w) ± 1,13 en pectinas, 47,28 % (w/w) ± 1,71 en sacarosa, 30,44 % (w/w) ± 0,78 en glucosa, y 14,27 %

(w/w) ± 2,06 en fructosa. La distribución de peso molecular de los extractivos en agua confirmó la presencia de monómeros, dímeros, y de un grupo de peso molecular aproximado de 2 kDa que podría atribuirse a las pectinas solubles en agua. Las hemicelulosas presentes en la pulpa fueron de tipo arabinogalactano, formadas en un 68,60 % (w/w) ± 0,18 por galactano y en un 31,40 % (w/w) ± 0,18 por arabinano. El contenido en humedad de la pulpa de zanahorias descartadas fue muy elevado (89,70 % w/w ± 1,56) por lo que se procedió a un licuado de las zanahorias para separar el zumo de la pulpa.

El presente Capítulo se centró en la valorización de la pulpa a través de tratamiento hidrotermal a distintas temperaturas (140, 160 y 180 °C) en un reactor piloto de tipo "flow-through". La extracción se llevó a cabo durante 80 min y se tomaron muestras líquidas a la salida del reactor analizando su composición y distribución de peso molecular. Los componentes extraídos fueron azúcares libres, hemicelulosas y pectinas. Los azúcares libres fueron los compuestos más abundantes en el extracto, alcanzando un rendimiento de 211,0 g/kg pulpa seca (140 °C), 189,3 g/kg pulpa seca (160 °C) y 161,9 g/kg pulpa seca (180 °C). El menor rendimiento a mayor temperatura podría asociarse a que el tiempo de precalentamiento del reactor fue mayor a mayor temperatura, extrayéndose mayor proporción de los azúcares en el precalentamiento sin recuperarse estos como parte del extracto. También podría deberse en parte a la degradación de azúcares por acción de la temperatura. La evolución del rendimiento de los azúcares libres confirmó la hidrólisis de la sacarosa dando lugar a glucosa y fructosa, siendo esta hidrólisis mayor a mayor temperatura. Mientras que extrayendo a 140 °C el rendimiento de extracción fue siempre creciente, extrayendo a 180 °C la extracción de azúcares concluyó tras los primeros 30 minutos.

Frente a los azúcares libres, la extracción de hemicelulosas y pectinas requiere de temperaturas superiores a 120 °C. Las hemicelulosas fueron extraídas como arabinogalactano de distinto peso molecular. En el caso de operar a 160 y 180 °C hubo presencia en el extracto de unidades monoméricas de arabinosa, lo que podría asociarse a una mayor autohidrólisis del arabinogalactano. El rendimiento de arabinogalactano fue de 70,45 g/kg (140 °C), 61,25 g/kg (160 °C) y 63,21 g/kg (180 °C). La liberación de las

unidades monoméricas de arabinosa contribuyó a que el rendimiento fuera menor a mayor temperatura.

Respecto a las pectinas extraídas, el rendimiento fue más del doble operando a 180 °C que a 140 y 160 °C. Ello podría deberse a la existencia de cierto tipo de pectinas que se extraerían mejor a mayor temperatura. El rendimiento fue de 11,39 g/kg pulpa seca (140 °C), 11,86 g/kg pulpa seca (160 °C) y 29,13 g/kg pulpa seca (180 °C).

Los subproductos se formaron por la degradación de azúcares, pectinas y hemicelulosas por acción del tiempo y sobre todo de la temperatura. El rendimiento máximo de subproductos fue de 14,21 g/kg pulpa seca (140 °C), 54,43 g/kg pulpa seca (160 °C) y 68,22 g/kg pulpa seca (180 °C).

Tomando como referencia el contenido en azúcares libres, hemicelulosas y pectinas de la materia prima en el reactor, los rendimientos de recuperación de estos componentes en los extractos fueron, para arabinogalactano de 82,05 % w/w (140 °C), 71,34 % w/w sin tener en cuenta y 84,42 % w/w teniendo en cuenta los monómeros de arabinosa (160 °C), y 73,62 % w/w sin tener en cuenta y 87,32 % w/w teniendo en cuenta los monómeros de arabinosa (180 °C). La recuperación de pectina respecto a la pectina presente en la materia prima fue de 19,37 % w/w (140 °C), 20,16 % w/w (160 °C) y 49,52 % w/w (180 °C). El método habitual de extracción de azúcares libres es con agua caliente a una temperatura moderada. En este trabajo la temperatura fue más alta para efectuar la extracción de los biopolímeros, pero durante la puesta en marcha hubo un periodo de precalentamiento del reactor a 90 °C y un periodo de precalentamiento desde 90 °C hasta la temperatura de operación. El extracto durante el periodo de precalentamiento no se recuperó como parte del producto por no haberse alcanzado la temperatura de operación. Es probable que una parte considerable de los azúcares fueran preextraídos en esta etapa. En consecuencia, se consideran aquí los porcentajes de recuperación sólo del extracto hidrotermal a la temperatura de operación, siendo estos de 35,26 % w/w (140 °C), 31,65 % w/w (160 °C) y 27,07 % w/w (180 °C). Esto implica que hasta un 64,74 % w/w (140 °C), 68,35 % w/w (160 °C) y 72,93 % w/w (180 °C) de los azúcares libres fueron o bien preextraídos en el precalentamiento, o bien degradados por acción de la temperatura.

La distribución de peso molecular de los extractos a la salida de la planta mostró que al comienzo de la extracción a 140 °C el grupo 1-5 kDa fue el único presente, por lo que estos serían los biopolímeros más fácilmente extraíbles del sólido. A continuación, el grupo de biopolímeros mayoritario pasó a ser el de > 30 kDa seguido por 1-5 kDa. A partir de los 40 min de extracción la disminución del grupo > 30 kDa fue marcada, mientras que la presencia de los otros grupos fue más o menos estable. A 160 °C todos los grupos moleculares fueron extraídos desde los primeros minutos, siendo mayoritario el grupo 1-5 kDa seguido por > 30 kDa. El grupo 1-5 kDa presentó dos máximos, el primero asociado a la extracción del sólido (pues también fue máxima la concentración de hemicelulosas totales) y el segundo asociado a la autohidrólisis de grupos de mayor peso molecular ya extraídos (pues no hubo máximo en la concentración total de hemicelulosas en el líquido, sino simplemente un cambio en el peso molecular). A 180 °C los biopolímeros más abundantes fueron también los de 1-5 kDa seguidos por > 30 kDa. El grupo de 1-5 kDa se extrajo más y más rápido que a menores temperaturas, alcanzando su máximo a los 10 min de comenzar la extracción. La concentración de hemicelulosas y de pectinas también mostró un marcado máximo a los 10 minutos de comenzar la extracción.

La pulpa residual resultante del tratamiento hidrotermal, en concreto del aplicado a 180 °C, fue caracterizada químicamente con objeto de determinar su composición: proteínas (0,23 % w/w ± 0,04), extractivos en agua (17,76 % w/w ± 0,35), extractivos en hexano (2,70 % w/w ± 1,75), celulosa (57,46 % w/w ± 1,78), hemicelulosas (5,61 % w/w ± 0,38), pectinas (0,50 % w/w ± 0,70), y lignina (15,74 % w/w ± 2,42). Si se compara con los rendimientos de extracción antes mencionados puede estimarse qué proporción de componentes fueron preextraídos en el precalentamiento o fueron degradados. De acuerdo con la caracterización del sólido, el rendimiento de extracción de hemicelulosas fue de un 87,86 % (w/w) y el de pectinas de un 98,49 % (w/w). Dado que las recuperaciones fueron de 73,65 % (w/w) y 49,54 % (w/w) para hemicelulosas y pectinas, el resto se estima que pudo ser degradado. Esta hipótesis concuerda con la concentración de subproductos en el extracto. Sin embargo, en el caso de los azúcares libres, la caracterización de la pulpa residual indica una extracción próxima a un 94,90 % (w/w) mientras que la recuperación fue de un 27,07 % (w/w). Dada la concentración de

subproductos obtenidos, la mayor parte de los azúcares no habrían sido degradados sino probablemente preextraídos en la etapa de precalentamiento.

3.3. Capítulo 3

Tras la separación del zumo y de la pulpa de zanahorias descartadas y la valorización de la pulpa en el Capítulo 2, en el Capítulo 3 se valorizó el zumo. Los componentes más destacados del zumo son los azúcares y los carotenoides, por lo que se procedió a su separación. El proceso se basó en múltiples ciclos de diafiltración aplicados al zumo. Dado que los carotenoides no son solubles en agua y que se encuentran formando parte de micropartículas de pulpa, no pueden atravesar la membrana y por tanto quedan en el retenido. Los azúcares libres y demás compuestos disueltos en el agua atraviesan la membrana y son obtenidos en el permeado.

El proceso comenzó con la retirada de micropartículas del zumo de tamaño superior a 50 µm. A continuación, el zumo fue diluido al 50 % para hacer su operación menos problemática de cara a ensuciamientos en la membrana, disminuyendo la concentración total de azúcares de 111,28 g/L a 55,64 g/L. Los ciclos de diafiltración se aplicaron con una membrana de MWCO 30 kDa. Durante cada ciclo se adicionó un contenido de agua igual al contenido inicial de zumo (un 50 % del volumen total teniendo en cuenta que está diluido) y se hizo pasar a través de la membrana, arrastrando de esta manera los azúcares y otros componentes disueltos y purificando la fracción rica en carotenoides. Los ciclos de diafiltración se aplicaron hasta que la concentración de azúcares tanto del retenido como de los diapermeados no disminuyó más. La concentración de azúcares se midió en tiempo real mientras se llevaba a cabo el experimento con un refractómetro medidor de grados Brix. Los sucesivos ciclos de diafiltración lograron disminuir la presencia de azúcares libres en la fracción rica en carotenoides en un 91,1 % para la sacarosa, un 90,4 % para la glucosa y un 89,7 % para la fructosa. Para ello se requirió de un volumen de agua equivalente a 7 veces el volumen de zumo.

Los diapermeados obtenidos en el arrastre de azúcares libres no fueron un subproducto, sino una fracción de interés debido a su abundante contenido en azúcares y nutrientes. La fracción rica en carotenoides fue mezclada con goma arábiga dando lugar a una suspensión en agua que se mantuvo en agitación hasta lograr un aspecto totalmente

homogéneo. Tras ello, la mezcla fue secada mediante spray con una ratio de sólido:encapsulante 1:1, y por liofilización con ratios de sólido:encapsulante de 1:0, 1:0,5, 1:1 y 1:1,5.

Durante la diafiltración se evalúo el ensuciamiento de la membrana reflejado en la evolución del flujo de permeado y de la presión transmembrana. El flujo de permeado experimentó un descenso sobre todo en el primer ciclo (desde 500,0 a 299,3 mL/m^2/min), pero después se estabilizó en valores próximos a 260 mL/m^2/min y pasó a descender de nuevo en el último ciclo hasta 179,6 mL/m^2/min. El descenso inicial del flujo de permeado estuvo acompañado de un aumento de la presión transmembrana, por lo que podría asociarse al ensuciamiento normal de la membrana. Por otro lado, el descenso del flujo de permeado del último ciclo de diafiltración no estuvo relacionado con un aumento de la presión transmembrana, por lo que podría relacionarse con el fenómeno de polarización de la concentración asociado a la formación de una torta que originaría una resistencia adicional a la transferencia de materia. Aun así, ni el ensuciamiento ni la posible polarización de la concentración impidieron la operación.

Se evaluó la degradación de carotenoides por acción del secado (spray y liofilización) y durante el almacenamiento. Se vio que el secado por spray (encapsulación 1:1) degradó un 54,7 % de los carotenoides presentes frente a una degradación en la liofilización de un 10,8 % y 19,8 % (encapsulación 1:1 y 1:1,5). Ello puede deberse a que el secado por spray se llevó a cabo a alta temperatura (150 °C) frente al secado por liofilización, además de que el sólido en el spray es sometido a contacto con un flujo de aire.

Por otro lado, la degradación durante un almacenamiento de 15 días fue de 8,9 % en el producto secado por spray (encapsulación 1:1) frente a un 18,5 % en el secado por liofilización sin encapsulación, por lo que efectivamente la encapsulación por spray protegió a los carotenoides en su almacenamiento. La encapsulación por liofilización con ratios de 1:0,5, 1:1 y 1:1,5 dio lugar a porcentajes de degradación durante el almacenamiento de 42,6 %, 38,8 % y 44,3 %. Estos valores tan elevados se debieron a que el agente encapsulante no encapsuló las micropartículas donde se encontraron los carotenoides. La causa pudo ser un fenómeno de separación de fases entre encapsulante y pigmentos que pudo apreciarse visualmente y que se daría en la etapa de congelado. Adicionalmente, la presencia del agente encapsulante resultó favorecer

la degradación de carotenoides en la liofilización, lo que podría asociarse a que cuanto más agente encapsulante estuvo presente en la muestra liofilizada menor fue el contenido en carotenoides de esta y más expuestos estuvieron al aire y la luz, pues el agente encapsulante es un material poroso, ligero y blanco. El encapsulado por spray mostró mayor estabilidad en agua dando lugar a una suspensión homogénea frente a los encapsulados por liofilización en los que se apreció la presencia de micropartículas en la suspensión acuosa.

El sólido rico en carotenoides tras la diafiltración tuvo una concentración total de 4996,4 µg/g mientras que el sólido encapsulado por spray tuvo 1131,7 µg/g. Ambos son valores elevados comparados con la bibliografía, por lo que el sólido obtenido fue un producto muy interesante de cara a potenciales aplicaciones en la industria alimentaria, cosmética o farmacéutica.

El permeado resultante de la ultrafiltración del zumo con membrana de MWCO 30 kDa fue muy buen caldo de fermentación por su elevado contenido en azúcares libres, nutrientes, y por no poseer compuestos inhibidores de la fermentación ni fibras. El permeado tuvo una concentración de azúcares de 51,13 ± 1,30 g/L en sacarosa, 23,59 ± 0,34 g/L en glucosa y 10,11 ± 0,36 g/L en fructosa. Se estudió la viabilidad de su fermentación con microorganismos autóctonos, bacterias del ácido láctico, y levaduras, buscando la obtención de ácido láctico y de etanol. Cada fermentación se llevó a cabo con y sin la adición de 6 % (w/v) NaCl, actuando este como aditivo de cara a estudiar su efecto en la prevención de la invasión por levaduras externas. Las fermentaciones se desarrollaron de forma anaerobia, con agitación y a 35 °C en recipientes de vidrio cerrados con septum y de 20 mL de volumen. Se analizó la composición química y la concentración de microorganismos en el caldo de fermentación a distintos tiempos.

Las fermentaciones dieron lugar a un consumo de azúcares de entre 92,4-97,5 % salvo en dos casos: 1) microorganismos autóctonos y NaCl en el medio, y 2) bacterias del ácido láctico y NaCl en el medio. En estos casos el consumo fue de 23,3 % y 43,8 %, respectivamente. El menor consumo pudo deberse a un marcado descenso del pH por la producción de ácido láctico. En consecuencia, si se desea continuar con la fermentación y aumentar la producción de ácido láctico sería necesario establecer un control del pH. Como era esperado, las levaduras produjeron etanol y los

microorganismos autóctonos y las bacterias del ácido láctico produjeron ácido láctico. Se formaron subproductos minoritarios como ácido fórmico y ácido acético. Tanto los microorganismos autóctonos como las bacterias de ácido láctico experimentaron una co-fermentación dando lugar a la producción de etanol. Este fenómeno pudo deberse a la invasión por parte de levaduras externas.

En los ensayos en los que se adicionó NaCl 6 % (w/v) al medio, se evitó la invasión y el ácido láctico fue el único producto presente en la fermentación tanto con microorganismos autóctonos como con bacterias del ácido láctico. La adición de 6 % (w/v) NaCl también tuvo cierto efecto en la fermentación con levaduras dando lugar a una ligera disminución en la producción de etanol y a un aumento en la formación de ácido fórmico. El ácido fórmico es un intermediario cuya concentración aumenta en ambientes de fermentación desfavorables para las levaduras. En consecuencia, la concentración de NaCl actuaría evitando la invasión por levaduras externas, pero no inhibiendo el crecimiento de las levaduras no externas, sólo creando un ambiente ligeramente desfavorable. El NaCl disminuyó ligeramente la producción de ácido láctico en comparación a cuando no se adicionó, lo que pudo deberse a efectos relacionados con el pH.

La producción final alcanzó valores máximos de 17,64 g/L para ácido láctico y 49,46 g/L para etanol. Los microorganismos autóctonos y las bacterias del ácido láctico lograron producir ácido láctico puro en concentración 6,62 ± 0,79 g/L y 9,66 ± 0,51 g/L, respectivamente. Las levaduras por efecto del NaCl redujeron su producción de etanol desde 49,46 ± 0,28 g/L hasta 44,60 ± 0,13 g/L y aumentaron la de ácido fórmico desde 1,81 ± 0,08 g/L hasta 11,91 ± 0,40 g/L. Los rendimientos máximos obtenidos fueron de 0,344 g/g para ácido láctico con microorganismos autóctonos, 0,167 g/g para ácido láctico con bacterias lácticas, y 0,441 g/g para etanol con levaduras.

3.4. Capítulo 4

El Capítulo 4 se sirvió de los tres hidrolizados obtenidos en el Capítulo 2 resultantes del tratamiento hidrotermal aplicado a la pulpa de zanahorias a 140, 160 y 180 °C. Los tres hidrolizados fueron analizados determinando su composición química y peso molecular. Los resultados mostraron una pureza de 31,25 % w/w (140 °C), 33,51 % w/w (160 °C) y

30,12 % w/w (180 °C); un peso molecular medio de 18,83 kDa (140 °C), 10,98 kDa (160 °C) y 9,02 kDa (180 °C); y una polidispersión de 31,6 (140 °C), 19,9 (160 °C) y 16,2 (180 °C). En consecuencia, fue necesario un tratamiento que permitiera obtener los biopolímeros de hemicelulosa y pectina purificados, con una baja polidispersión, de distinto peso molecular y con potenciales aplicaciones.

Los tres extractos hidrotermales fueron filtrados para eliminar micropartículas de tamaño superior a 50 µm. A continuación, los extractos hidrotermales obtenidos a 140 y 160 °C fueron tratados aplicando ultrafiltración con una configuración de membranas en cascada (30, 10, 5 y 1 kDa). Cada retenido fue a su vez sometido a múltiples ciclos de diafiltración, añadiendo en cada ciclo un volumen de agua igual al volumen del retenido y haciendo pasar este agua a través del permeado. Se aplicó un mínimo de 5 ciclos a cada retenido, y un ciclo extra por cada membrana que estuviera en posición anterior en la cascada. En el ciclo extra se reutilizó parte del agua de diafiltraciones de membranas anteriores.

El extracto hidrotermal obtenido a 180 °C fue tratado con una configuración de membranas mixta, actuando primero una membrana de 5 kDa y luego dos membranas de 10 y 1 kDa en paralelo, cada una con una de las salidas de la membrana de 5 kDa. Cada uno de los retenidos fue sometido a 5 ciclos de diafiltración, salvo el retenido de 1 kDa que además se sometió a 1 ciclo extra reutilizando agua de diafiltración de la membrana de 5 kDa.

El volumen de la alimentación se redujo un 80 % en cada membrana lo que dio lugar a la concentración de las hemicelulosas y pectinas retenidas. Con la configuración en cascada se logró concentrar ciertos grupos de biopolímeros en un factor de hasta 5 en el retenido de mayor peso molecular, mientras que con la configuración mixta se concentraron en un factor de hasta 25. Una vez aplicados los ciclos de diafiltración, la concentración de ciertos grupos disminuyó dando lugar a fracciones más definidas.

Los ciclos de diafiltración permitieron recuperar un 56,06 % (140 °C), 65,84 % (160 °C), y 60,14 % (180 °C) de las hemicelulosas de los extractos; y un 100 % (140 °C), 93,96 % (160 °C) y un 69,67 % (180 °C) de las pectinas. Por otro lado, pudieron eliminarse un

99,47 % (140 °C), 98,93 % (160 °C) y 99,11 % (180 °C) de los azúcares libres; y un 96,29 % (140 °C), 99,24 % (160 °C) y 94,35 % (180 °C) de los subproductos.

En cuanto a la separación de los biopolímeros, se pasó de amplias distribuciones de peso molecular de las alimentaciones a distribuciones mucho más definidas. En la configuración en cascada, el retenido de mayor peso molecular fue mayoritario en hemicelulosas de > 30 kDa estando estas presentes en un 66,2 % (140 °C) y 58,4 % (160 °C). Estos porcentajes fueron considerablemente altos en comparación con la alimentación: un 12,7 % (140 °C) y un 8,9 % (160 °C). Los otros grupos moleculares estuvieron presentes en orden decreciente a menor peso molecular. Los otros retenidos fueron mayoritarios en biopolímeros de pesos moleculares intermedios, destacando el retenido de menor peso molecular con un contenido en hemicelulosas del grupo 1-5 kDa en un 93,1 % (140 °C) y 68,6 % (160 °C).

En la configuración mixta aplicada al extracto de 180 °C, el producto de mayor peso molecular fue rico en hemicelulosas de > 30 kDa (61,7 %), el de peso molecular intermedio en el grupo 1-5 kDa (51,6 %) y 5-10 kDa (37,6 %), y el producto de menor peso molecular en el grupo 1-5 kDa (77,1 %).

La eficiente retirada de azúcares libres y subproductos y la separación de los grupos de distinto peso molecular dio lugar a productos purificados de muy buenas características, entre las que destacan fracciones de pesos moleculares medios de 102,8 kDa-8,06 kDa-6,20 kDa-2,59 kDa (140 °C); 59,99 kDa-11,48 kDa-6,85 kDa-4,21 kDa (160 °C); y 57,06 kDa-5,71 kDa-3,33 kDa (180 °C). Dichas fracciones tuvieron polidispersiones bajas de valores 4,0-1,6-1,7-1,2 (140 °C), 3,4-1,5-1,4-1,4 (160 °C), y 2,4-1,4-1,5 (180 °C). Las purezas de las fracciones finales fueron de 98,89 % - 100 % - 100 % - 73,06 % (140 °C), 98,50 % - 100 % - 86,61 % - 89,90 % (160 °C), y 99,74 % - 87,73 % - 96,00 % (180 °C). El análisis estructural termogravimétrico de las fracciones purificadas mostró la existencia de dos tipos de biopolímeros, siendo uno de ellos más resistente a la degradación térmica y estando presente en la fracción de mayor peso molecular. El grupo de biopolímeros que fue menos resistente a la degradación térmica estuvo en menor proporción en la fracción de mayor peso molecular cuanto mayor fue la temperatura de extracción, llegando a no estar presente en el caso de 180 °C salvo en las fracciones de peso molecular intermedio.

3.5. Capítulo 5

El Capítulo 5 se sirvió de los hallazgos obtenidos en los Capítulos 2 y 4. Se basó en el desarrollo del proceso a una mayor escala de cara a obtener fracciones purificadas de hemicelulosas/pectinas y fracciones de pulpa residual en cantidad suficiente para su transformación en productos de alto valor añadido.

El proceso partió, como en el Capítulo 2, del tratamiento hidrotermal aplicado a la pulpa de zanahorias descartadas. Se desarrollaron dos experimentos a las temperaturas extremas empleadas en el Capítulo 2: 140 y 180 °C. De cara a operar a mayor escala, en cada experimento funcionaron varios reactores en serie operando en ciclos. Cada uno de los reactores operó durante un tiempo dado en función de la temperatura de operación: 45 min a 140 °C y 30 min a 180 °C. En cada experimento se operó con un total de 7 reactores. Puesto que la planta piloto está compuesta por 5 reactores, dos de los reactores fueron utilizados en una segunda ocasión. Esta forma de operar permite el procesamiento de forma continua de tanta biomasa como se desee.

En el presente Capítulo, unos 11,32 kg de zanahorias descartadas fueron procesados en cada experimento. Como se explicó en el Capítulo 2, los reactores funcionaron en modo “flow-through”. El agua circuló de arriba hacia abajo a través de cada reactor cargado con biomasa dentro de un cartucho. Al operar en serie la corriente de salida del primer reactor entró como corriente de alimentación del segundo, y así sucesivamente. Se comenzó operando con el reactor 1, y al cabo de 15 min (140 °C) o de 10 min (180 °C) se incorporó el reactor 2. Transcurridos otros 15 min (140 °C) o 10 min (180 °C) se incorporó el reactor 3, y se mantuvieron en operación al mismo tiempo los reactores 1, 2 y 3 durante 15 min (140 °C) o 10 min (180 °C) más, haciendo un total de 45 min (140 °C) o 30 min (180 °C), siendo este el tiempo de operación asignado a cada reactor. En ese momento de desactivó el reactor 1 y se incorporó el 4. El proceso se repitió desactivando reactores cuando hubieron completado el tiempo de operación e incorporando nuevos reactores a la secuencia, operando siempre hasta 3 reactores al mismo tiempo. La toma de muestras se efectuó a la salida de cada uno de los reactores cada 15 min (140 °C) o cada 10 min (180 °C). El tiempo total de cada experimento fue de 150 min (140 °C) y de 100 min (180 °C).

Los primeros 5 reactores fueron cargados previamente con biomasa y agua, precalentada esta agua a 90 °C hasta que la planta alcanzó la temperatura y presión de operación. Este precalentamiento fue igual que el llevado a cabo en el Capítulo 2, siendo parte de los azúcares libres preextraídos en esta etapa. Sin embargo, en el Capítulo 5 al operarse con los reactores en serie este preextracto fue recuperado como parte del producto en lugar de ser desechado. A diferencia de los primeros 5 reactores, en la operación con los reactores 6 y 7 (reutilización del 1 y el 2) la biomasa se cargó sin aplicar precalentamiento con agua a 90 °C. La extracción se inició por tanto directamente a la temperatura de operación (140 o 180 °C). Se pudieron así comparar las dos formas de extracción (con y sin precalentamiento). La secuencia de finalización de cada experimento fue equivalente a la de inicio, basándose en la desactivación de reactores de forma secuencial, operando con los reactores 5, 6 y 7, luego con el 6 y el 7, y finalmente con el 7 hasta el fin de su tiempo de operación.

La operación en ciclos fue estudiada analizando la composición química y la distribución del peso molecular del extracto a la salida de cada reactor a lo largo del tiempo. La evolución de los valores de concentración mostró que el tiempo de operación asignado a cada reactor (45 min a 140 °C y 30 min a 180 °C) fue adecuado pues el extracto experimentó un aumento de concentración en los primeros minutos seguido por un descenso, indicando que la extracción retiró la mayor parte de los componentes de la biomasa. La operación a 180 °C fue bastante inestable en lo que respecta a la evolución de las concentraciones, lo que podría asociarse a una cinética acelerada frente a un tiempo de residencia del líquido en el reactor bastante reducido (aproximadamente 7 min).

Respecto a la evolución de las concentraciones a la salida de la planta, se experimentó un aumento a medida que se incorporaron reactores a la secuencia pues se pasó de operar con 1 a operar con 3. La operación con los dos últimos reactores, no sometidos a precalentamiento a 90 °C, mostró un aumento considerable de la extracción gracias a que no se dio proceso de mezcla con el descenso de temperatura y dilución asociados. Por otro lado, no precalentar los reactores con agua dio lugar a cambios importantes de presión durante el proceso de llenado que dificultaron el mantenimiento de las condiciones de operación en los valores deseados.

El grado de extracción de hemicelulosas fue de 62,82 g/kg pulpa seca (140 °C) y de 81,01 g/kg pulpa seca (180 °C), con correspondientes rendimientos de 74,5 % (140 °C) y 96,1 % (180 °C). La extracción por tanto resultó muy eficiente. En el caso de las pectinas la recuperación fue menor, llegando a valores de 5,35 g/kg pulpa seca (140 °C) y 5,22 g/kg pulpa seca (180 °C), con rendimientos de 9,2 % (140 °C) y 9,0 % (180 °C). El rendimiento de las pectinas fue menor que el que se obtuvo en el Capítulo 2, lo que podría asociarse a que en este caso el tiempo de extracción fue la mitad del empleado en el Capítulo 2 y, de acuerdo con los resultados, las pectinas se extraerían más lentamente que las hemicelulosas.

La extracción de azúcares libres alcanzó valores de 229,76 g/kg pulpa seca (140 °C) y 379,51 g/kg pulpa seca (180 °C), que corresponden a rendimientos de 39,1 % (140 °C) y 64,6 % (180 °C). Puede desprenderse que a mayor temperatura se lograron extraer los azúcares libres de forma más rápida y por tanto se extrajeron más. La degradación de los azúcares no habría sido tan significativa como en el Capítulo 2 debido a que los tiempos de extracción del Capítulo 5 fueron considerablemente inferiores. La recuperación del preextracto correspondiente al precalentamiento del reactor fue clave para alcanzar un mayor rendimiento de recuperación de azúcares libres.

Respecto a los subproductos, se alcanzó una extracción de 10,28 g/kg pulpa (140 °C) y de 39,09 g/kg pulpa (180 °C), considerablemente inferiores a los obtenidos en el Capítulo 2 gracias a un menor tiempo de operación o tiempo de residencia del sólido.

Respecto a la distribución de peso molecular a la salida de la planta, el grupo más abundante a 140 °C fue el de > 30 kDa seguido por 1-5 kDa. La extracción del grupo de > 30 kDa experimentó un máximo correspondiente a la operación con 3 reactores al mismo tiempo y otro correspondiente a la operación con los reactores 6 y 7 sin precalentamiento. La operación con los reactores 6 y 7 sin precalentamiento también dio lugar a un máximo en el grupo 1-5 kDa, más fácilmente extraíble. Los grupos de monómeros y dímeros experimentaron también un marcado máximo cuando se operó con los reactores sin precalentamiento.

En la extracción a 180 °C el grupo mayoritario fue el de 1-5 kDa seguido por > 30 kDa. La extracción de > 30 kDa experimentó de nuevo dos máximos, uno al operar con 3

reactores y otro al operar con los reactores 6 y 7 sin precalentamiento. Por su parte, el grupo 1-5 kDa experimentó varios máximos a lo largo de la extracción siendo el mayor el correspondiente a la operación con los reactores 6 y 7 sin precalentamiento. Los monómeros experimentaron varios máximos y los dímeros tuvieron su máximo más destacado al operar con los reactores 6 y 7 sin precalentamiento.

Como en el Capítulo 4, los dos extractos hidrotermales obtenidos a 140 y 180 °C fueron recogidos y tratados con un sistema de membranas. Dado que en el Capítulo 4 se vio que la configuración mixta dio lugar a una concentración mayor de las fracciones purificadas de alto peso molecular, en el Capítulo 5 se empleó una configuración mixta con las membranas. Para el tratamiento del extracto de 140 °C se empleó una membrana de 10 kDa seguida de una de 30 kDa obteniéndose como productos los biopolímeros de > 30 kDa y 10-30 kDa. El extracto de 180 °C tuvo una mayor proporción de biopolímeros de menor peso molecular que el de 140 °C por lo que la configuración empleada fue una membrana de 10 kDa seguida por dos membranas en paralelo de 30 y 1 kDa, dando lugar como productos a biopolímeros de > 30 kDa, 10-30 kDa y 1-10 kDa.

Como en el Capítulo 4, cada retenido se sometió a 5 ciclos de diafiltración con agua. En este caso no se aplicaron ciclos de diafiltración extra con agua reutilizada. En el tratamiento del extracto de 180 °C con la membrana de 30 kDa sólo se aplicaron los ciclos de diafiltración sin la ultrafiltración pues la alimentación a la membrana tuvo una concentración suficientemente alta y un valor mayor habría hecho inoperable la operación por ensuciamiento. Las dos muestras purificadas del extracto de 140 °C y las tres muestras del extracto de 180 °C fueron secadas por liofilización y por spray para su caracterización estructural y para un mejor almacenamiento de cara a su posterior transformación en productos de alto valor añadido.

La operación con las membranas dio lugar a la distribución de los azúcares libres, subproductos, hemicelulosas y pectinas en las distintas corrientes. En el tratamiento del extracto de 140 °C, la operación con la membrana de 10 kDa dio lugar a una retirada de un 83,8 % de los azúcares, 46,6 % de los subproductos, 49,8 % de las hemicelulosas y 40,7 % de las pectinas a través del permeado. Posteriormente se aplicaron los ciclos de diafiltración y la retención de hemicelulosas y pectinas disminuyó ligeramente, mientras que la retención de azúcares libres y subproductos bajó a cero. En la operación con la

membrana de 30 kDa se separó un 6,6 % de las hemicelulosas y un 23,3 % de las pectinas a través del permeado. La aplicación de los ciclos de diafiltración dio lugar a una retención del 24,9 % de las hemicelulosas extraídas y del 9,3 % de las pectinas extraídas.

En el tratamiento del extracto de 180 °C se retiraron en el permeado de la primera membrana el 77,1 % de los azúcares libres y el 84,6 % de los subproductos. Respecto a los biopolímeros, en la primera membrana quedaron retenidos un 70,1 % de las hemicelulosas y un 74,8 % de las pectinas, porcentajes considerablemente superiores a los de 140 °C. Esta diferencia podría deberse a una mayor concentración del extracto obtenido a 180 °C frente al de 140 °C (29,23 g/L versus 16,31 g/L). Tras la aplicación de los ciclos de diafiltración se eliminaron todos los subproductos retenidos, la retención de azúcares libres bajó hasta un 0,8 %, la retención de pectinas se mantuvo constante y la de hemicelulosas bajó de forma muy considerable hasta un 32,6 %. Se estableció que de forma general las pectinas estarían asociadas a hemicelulosas de alto peso molecular, y esto concuerda con que en este caso su retención se mantuviera constante.

El posterior tratamiento de diafiltración con la membrana de 30 kDa dio lugar a la separación en el grupo 10-30 kDa de un 5,8 % de las hemicelulosas, un 12,3 % de las pectinas y un 0,8 % de los azúcares. El producto de mayor peso molecular quedó entonces con un 26,8 % de las hemicelulosas y un 62,5 % de las pectinas. La gran diferencia en la retención de pectinas de alto peso molecular en el extracto de 140 y 180 °C podría deberse a que la unión ente hemicelulosas y pectinas es diferente dependiendo de la temperatura a la que se haga la extracción. Finalmente, la membrana de 1 kDa dio lugar a la eliminación del 78,1 % de los subproductos y del 58,3 % de los azúcares libres junto con un 2,1 % de las pectinas y un 16,8 % de las hemicelulosas. Los ciclos de diafiltración aplicados a la membrana de 1 kDa resultaron en un producto de 1-10 kDa reteniendo un 0,5 % de los azucares libres, un 0,2 % de los subproductos, un 4,1 % de las hemicelulosas y 15,4 % de las pectinas.

Gracias a las etapas de ultrafiltración y a los ciclos de diafiltración la distribución de peso molecular de los productos fue muy diferente a la de los extractos, lo que permitió pasar de alimentaciones de peso molecular y polidispersión 14,77 kDa y 19,2 (140 °C) y 8,08 kDa y 18,2 (180 °C) a fracciones purificadas cuyos parámetros fueron 80,36 kDa y 2,4 (140 °C, Ret-30 kDa-DF), 9,5 kDa y 2,1 (140 °C, Perm-30 kDa), 67,77 kDa y 3,8 (180 °C,

Ret-30 kDa-DF), 5,23 kDa y 1,3 (180 °C, DF-30 kDa), 3,86 kDa y 1,5 (180 °C, Ret-1 kDa-DF). Las membranas además permitieron pasar de purezas en el extracto de 22,2 % (140 °C) y 14,9 % (180 °C) a purezas de 100 % en los productos purificados de 140 °C y purezas de 100 %, 64,5 % y 66,8 % en los productos purificados de 180 °C. Los productos obtenidos fueron todos mayoritarios en hemicelulosas con porcentajes entre 62,9 – 90,0 %, salvo el de menor peso molecular del extracto de 140 °C que fue mayoritario en pectinas con un porcentaje de 51,3 %. La caracterización estructural de los productos purificados mediante termogravimetría mostró, como en el Capítulo 4, la presencia de dos grupos de biopolímeros, uno de ellos menos degradable térmicamente. Los biopolímeros más fácilmente degradables estuvieron presentes únicamente en los productos de peso molecular intermedio, mientras que los de alto peso molecular estuvieron formados por los biopolímeros más resistentes a degradación térmica.

3.6. Capítulo 6

El Capítulo 6 se desarrolló durante una estancia de 6 meses en la universidad Åbo Akademi de Turku (Finlandia). El objetivo fue la elaboración de films a partir de algunas de las fracciones purificadas de hemicelulosas y pectinas obtenidas en los Capítulos 4 y 5.

En la elaboración de los films se empleó glicerol como agente plastificador y agua como solvente. Las dos pulpas residuales obtenidas en el Capítulo 5 tras el tratamiento hidrotermal a 140 y 180 °C fueron empleadas como aditivos. La composición de las pulpas residuales fue de extractivos en agua: 19,13 % (140 °C) y 16,07 (180 °C), celulosa: 34,68 % (140 °C) y 42,82 % (180 °C), hemicelulosas: 3,43 % (140 °C) y 2,13 % (180 °C), pectinas: 1,07 % (140 °C) y bajo el límite de detección (180 °C), lignina: 21,04 % (140 °C) y 32,02 % (180 °C), y otros: 20,65 % (140 °C) y 6,96 % (180 °C). Ambas pulpas se sometieron a un tratamiento mecánico que permitió la obtención de nanofibras de celulosa combinadas con lignina, siendo estos los dos componentes mayoritarios. El tratamiento consistió en un lavado con agua, secado por liofilización, molienda en molino de bolas, hidratación y homogeneización a alta presión.

Respecto a las muestras purificadas de hemicelulosas y pectinas, se emplearon 6 muestras de pesos moleculares entre 3,86 y 102,75 kDa, y sólo las tres muestras de

mayor peso molecular permitieron la formación de films. Estas tres muestras estuvieron caracterizadas por los parámetros de peso molecular medio (MW), polidispersión (PD), pureza, ratio arabinogalactano respecto al total de arabinogalactano y pectinas (AG/(AG+P)), y ratio de galactano respecto al arabinano (G/A). El valor de los parámetros para las muestras utilizadas fue, para la muestra 1: 67,77 kDa (MW), 3,8 (PD), 100 % w/w (pureza), 0,732 (AG/(AG+P)), 1,63 (G/A); para la muestra 2: 80,36 kDa (MW), 2,4 (PD), 100 % w/w (pureza), 0,900 (AG/(AG+P)), 1,84 (G/A); y para la muestra 3: 102,75 (MW), 4,0 (PD), 98,9 % w/w (pureza), 0,585 (AG/(AG+P)), 1,74 (G/A).

La preparación de los films se basó en la técnica de mezclado, en la que a un volumen determinado de suspensión de pulpa residual homogeneizada se añadió la cantidad adecuada de fracción de hemicelulosas y pectinas. Esta mezcla se mantuvo en agitación y calentamiento durante 3 h a 40-50 °C, y después en agitación toda la noche a temperatura ambiente. A continuación, se añadió la cantidad adecuada de glicerol y se mantuvo en agitación a 40-50 °C durante 3 horas, tras lo que la mezcla fue vertida sobre una placa de Petri. El secado se llevó a cabo en dos etapas, primero en un horno con ventilación a 40 °C durante 6 horas, y luego en una sala acondicionada a 23 °C y 50 % de humedad relativa.

Las pulpas residuales antes y después del tratamiento fueron caracterizadas a través de imágenes con microscopio óptico y microscopio de transmisión electrónica. En ellas se vio que, sin aplicar tratamiento de homogeneización, las nanofibras de celulosa en suspensión formaron agregados. Tras el tratamiento de homogeneización a alta presión los agregados desaparecieron y el material se distribuyó homogéneamente como fibras de diámetro de dimensión nanométrica y longitud de micrómetros. La lignina pudo apreciarse en forma de micropartículas más homogéneamente distribuidas tras el tratamiento.

De cara a comparar ambas pulpas residuales se elaboraron los films 140-A5 y 180-A5, con un contenido de fracción de arabinogalactano y pectinas de 40 %, 25 % de pulpa residual, y 35 % de glicerol. El film 140-A5 se elaboró con pulpa residual proveniente de la extracción a 140 °C y el film 180-A5 con pulpa de la extracción a 180 °C. El contenido de glicerol fue constante en todos los films del Capítulo. La fracción de hemicelulosas y pectinas fue la correspondiente a un peso molecular de 67,77 kDa. De ambos films se

evaluó la permeabilidad del vapor de agua, ángulo de contacto del agua, y la estructura a través de FTIR y SEM.

La medida de permeabilidad del vapor de agua fue de 24,14 (g·mm)/(m^2·kPa·day) para 140-A5 and 24,35 (g·mm)/(m^2·kPa·day) para 180-A5. En consecuencia, no se apreciaron diferencias sustanciales entre ambas pulpas residuales. Respecto al ángulo de contacto del agua, se midieron ambas superficies de ambos films. La superficie inferior, aquella en contacto con la placa de Petri, resultó ser pulida y brillante frente a la superficie superior, en contacto con el ambiente, que resultó ser rugosa y mate. La superficie inferior fue muy poco hidrófoba frente a la superficie superior que fue altamente hidrófoba. Los ángulos de contacto de las superficies inferiores fueron 53,34° (140-A5) y 64,55° (180-A5). Las superficies superiores tuvieron ángulos de contacto de 116,57° (140-A5) y 91,02° (180-A5). La diferencia en la rugosidad pudo ser una de las causas de la diferencia en la hidrofobicidad. Las imágenes SEM obtenidas de ambas superficies de los films mostraron la rugosidad de la superficie superior y especialmente la presencia de cristales en la superficie inferior con una morfología igual a la del oxalato de calcio. Mediante FTIR se estudió la estructura de ambas caras de los films y se confirmó la presencia de cristales de oxalato cálcico en la cara inferior. Estos cristales provendrían de las zanahorias pues en otros estudios se ha mostrado su presencia en distintos vegetales.

Dado que la única diferencia entre ambas pulpas fue la hidrofobicidad, se seleccionó la de pulpa residual de 140 °C para la elaboración de los siguientes films, manteniendo la fracción de arabinogalactano y pectinas (67,77 kDa). De cara a estudiar la influencia del contenido de pulpa residual (PR) en los films basados en arabinogalactano y pectinas (AG-P), se prepararon 7 films con la siguiente composición: A0 (65 % AG-P, 0 % PR), A1 (64 % AG-P, 1 % PR), A2 (63 % AG-P, 2 % PR), A3 (60 % AG-P, 5 % PR), A4 (50 % AG-P, 15 % PR), A5 (40 % AG-P, 25 % PR), y B1 (0 % AG-P, 65 % PR). La caracterización de los films se hizo a través de la permeabilidad del oxígeno, permeabilidad del vapor de agua, ángulo de contacto del agua, propiedades tensiles, y estructura mediante FTIR.

Respecto a la permeabilidad del oxígeno, el film con menor permeabilidad fue A1 (1 % PR) con un valor de 48,18 (cm^3STP·µm)/(m^2·kPa·day). El film A0 (0 % PR) tuvo una permeabilidad de 67,73 (cm^3STP·µm)/(m^2·kPa·day), por lo que un pequeño porcentaje

de PR resultó beneficioso de cara a reducir el paso de oxígeno. Según se aumentó el porcentaje la permeabilidad continuó siendo más baja que la del film de referencia (A0) pero aumentó a mayor contenido de PR. Para un contenido > 5 % en PR (films A4 y A5) la presencia de PR ya no disminuyó la permeabilidad del oxígeno y se alcanzó un valor similar al film A0 de referencia, 73,65 ($cm^3STP \cdot \mu m)/(m^2 \cdot kPa \cdot day$). El film B1, formado únicamente por PR, tuvo la permeabilidad más alta de todas, 239,83 ($cm^3STP \cdot \mu m)/(m^2 \cdot kPa \cdot day$). La PR es por tanto una peor barrera frente al oxígeno que la fracción de hemicelulosa y pectina. Sin embargo, se observó cierta sinergia entre ambas fracciones cuando la PR estuvo en un porcentaje < 5 %.

Respecto a la permeabilidad del vapor de agua, el valor de referencia para el film A0 fue de 17,77 $(g \cdot mm)/(m^2 \cdot kPa \cdot day)$. La adición de PR en un porcentaje de 5 % incrementó el valor de permeabilidad ligeramente, 27,56 $(g \cdot mm)/(m^2 \cdot kPa \cdot day)$. En los films A4 y A5, con mayor contenido en PR, la WVP volvió a descender hasta valores de 22,62 $(g \cdot mm)/(m^2 \cdot kPa \cdot day)$. El film B1, formado únicamente por pulpa residual, tuvo una WVP muy similar a la del film A0 de referencia. La barrera frente al vapor de agua de las fracciones AG-P y PR resultó ser similar, pero la adición de PR en un pequeño porcentaje pudo ser perjudicial por no distribuirse homogéneamente en la estructura y por tanto sólo implicar una disminución de la fracción de AG-P que no fue compensada por el pequeño porcentaje de PR.

En lo que respecta al ángulo de contacto del agua, este se evaluó en la superficie superior de los films. El film con la menor hidrofobicidad fue el de referencia A0 (0 % PR) con un valor de 79,9°. La adición de PR dio lugar a un aumento de la hidrofobicidad hasta un máximo de 125,8° en el film A5 (25 % PR). Sorprendentemente, el film B1 formado únicamente por PR no fue el más hidrófobo, pues tuvo un ángulo de contacto de 87,9°. Esto mostró que hubo cierto efecto sinérgico entre la fracción AG-P y PR, y a mayores la hidrofobicidad fue mayor a mayor contenido en PR.

La evaluación de las propiedades tensiles se hizo a través de la medida del esfuerzo tensil, la elongación tensil y el módulo elástico. En la medida de estas propiedades también se observaron dos regiones de comportamiento como en las otras propiedades. Para un contenido de PR < 5 %, la PR actuó disminuyendo el esfuerzo tensil, mientras que una vez que se superó el 5 % a mayor contenido de PR mayor esfuerzo tensil. El

valor máximo correspondió al film B1 formado únicamente por PR. La presencia de PR en baja concentración no llegó a crear una red homogénea en el film y por lo tanto no resultó beneficiosa, pudieron generarse zonas de ruptura preferencial.

En la elongación tensil ocurrió que para un contenido < 5 % de PR, la PR actúo disminuyendo la elongación, lo cual es lógico pues la celulosa suele proporcionar fuerza y no elongación. Superado el 5 % de PR, la PR actuó mejorando la elongación y en el film A5 se llegó a alcanzar un valor cercano al del film de referencia A0.

El módulo elástico disminuyó por la adición de PR cuando se añadió en un porcentaje inferior al 5 %. La adición de PR en mayor porcentaje dio lugar a valores de módulo elástico similares al film de referencia. El film B1 tuvo la menor elasticidad (modulo elástico más alto).

Visto que la presencia de PR en general no mejoró las propiedades de los films salvo por la hidrofobicidad y por la permeabilidad del oxígeno, los siguientes films se prepararon con un contenido en PR de un 1 %. De cara a estudiar la influencia del peso molecular y de la composición de la fracción de AG-P, se elaboraron dos films más que se compararon con el film A1: C1 (MW: 102,75 kDa, 58,5 % AG, 41,5 % P) y C2 (80,36 kDa, 90,0 % AG, 10,0 % P). Los films con las fracciones de AG-P de peso molecular menor (9,85 kDa, 5,23 kDa y 3,86 kDa) no pudieron ser retirados de la placa de Petri sin romperse, resultando no válidos.

En los films A1, C1 y C2 la permeabilidad del oxígeno resultó ser menor a mayor peso molecular, lo que podría deberse a un mayor entrelazamiento entre las moléculas que dificultaría el paso del oxígeno. El valor mínimo fue de 41,14 $(cm^3 \cdot \mu m)/(m^2 \cdot kPa \cdot day)$.

La permeabilidad del vapor de agua fue por el contrario mayor a mayor peso molecular. Esto pudo deberse a que el arabinogalactano y las pectinas son hidrófilos y por tanto un mayor peso molecular daría lugar a mayor número de enlaces entre las moléculas que los forman, pero no necesariamente a una mayor repulsión al agua. El valor mínimo fue de 21,56 $(g \cdot mm)/(m^2 \cdot kPa \cdot day)$.

El ángulo de contacto resultó ser menor a mayor peso molecular, disminuyendo desde 86.8° (67,77 kDa) hasta 71,1° (102,48 kDa). Ello podría deberse a una mayor interacción

entre la lignina y la fracción AG-P cuando el peso molecular es elevado, disminuyendo quizá la hidrofobicidad de la lignina.

La medida de las propiedades tensiles mostró que no sólo dependieron del peso molecular sino también del porcentaje de arabinogalactano y de pectina presente en el film. A mayor peso molecular, menor fue el esfuerzo tensil. Por el contrario, a mayor porcentaje de pectinas en el film mayor esfuerzo tensil. El máximo valor fue de 2,84 MPa. En el caso de la elongación, sólo dependió de la composición del film y no tanto del peso molecular, viéndose que a mayor porcentaje de arabinogalactano mayor porcentaje de elongación. El valor máximo fue 15,28 %. El módulo elástico fue menor a mayor peso molecular, así como a mayor presencia de arabinogalactano. Los films más elásticos fueron los de menor peso molecular y mayor presencia de pectinas, mientras que los films más rígidos fueron los de mayor peso molecular y mayor presencia de arabinogalactano. Los valores de módulo elástico estuvieron entre 16,51 – 71,32 MPa.

4. Conclusiones

4.1. Capítulo 1

Tras la caracterización química del marro de café se planteó como estrategia la extracción del aceite y la posterior extracción de las hemicelulosas. El aceite fue retirado de forma eficiente a través de CO_2 supercrítico, solvente no tóxico y barato. El aceite fue caracterizado químicamente lo que permitiría la selección de una potencial aplicación a nivel industrial. El tratamiento hidrotermal aplicado al marro de café desgrasado permitió extraer las hemicelulosas a distintas temperaturas. El rendimiento no fue elevado (3.49 g/100 g materia prima seca), lo que podría atribuirse a que esta biomasa necesita temperaturas más altas pues ya fue sometida a un tratamiento hidrotermal anterior para la producción de café soluble. Sin embargo, las hemicelulosas extraídas tuvieron un peso molecular alto gracias a esa moderada temperatura y a la configuración “flow-though” del reactor. La pureza del extracto fue considerablemente alta gracias a la baja degradación y a la baja presencia de componentes no estructurales (extractivos en agua), pues la mayor parte de estos extractivos ya fueron retirados en el proceso de preparación del café soluble. El acondicionamiento de los extractos mediante un sistema de membranas en cascada aplicando ultrafiltración y múltiples

ciclos de diafiltración permitió verificar la viabilidad técnica del fraccionamiento en función del peso molecular. La retención de subproductos disminuyó gracias a los ciclos de diafiltración de 45.6 a 8.7 %. Las purezas finales de las fracciones estuvieron en el rango 83.7 – 97.8 % (w/w) y los pesos moleculares medios entre 1.64 – 49.73 kDa. Su purificación mediante la aplicación de varios ciclos de diafiltración fue efectiva. Las fracciones purificadas resultaron potenciales ingredientes para múltiples aplicaciones propias de las hemicelulosas en función de su peso molecular.

4.2. Capítulo 2

La pulpa de zanahorias descartadas se caracterizó químicamente. Al contrario del marro de café, el material tuvo un gran porcentaje de extractivos en agua (azúcares libres). Debido a su elevado contenido en humedad el primer paso fue la separación de la pulpa y del zumo de cara a aplicar una estrategia de valorización diferente a cada fracción. La valorización de la pulpa a través de tratamiento hidrotermal en un sistema de reactor en “flow-through” a distintas temperaturas permitió un alto rendimiento de extracción de azúcares libres, hemicelulosas y pectinas con valores de hasta 211,0 g/kg pulpa seca, 70,45 g/kg pulpa seca y 29,13 g/kg pulpa seca, respectivamente. Debido a la abundante presencia de azúcares libres, la pureza de los biopolímeros (hemicelulosas y pectinas) fue baja y la distribución de peso molecular muy amplia. Gracias al sistema de extracción en “flow-through” se obtuvieron hemicelulosas y pectinas de peso molecular muy alto, mayor cuando menor fue la temperatura del tratamiento. La caracterización química de la pulpa residual que quedó tras el tratamiento hidrotermal mostró un abundante contenido en celulosa (58 % w/w) y con un contenido considerable en lignina (16 % w/w). Dadas las múltiples aplicaciones de la celulosa en la generación de biomateriales, esta fracción que podría considerarse como un residuo fue sin embargo una fracción interesante. Además, el contenido de lignina se vio como un aditivo interesante que podría aportar buenas propiedades a los potenciales productos.

4.3. Capítulo 3

El proceso de separación de los azucares libres y carotenoides del zumo permitió valorizar de forma independiente ambos componentes. La separación a través de múltiples ciclos de diafiltración utilizó agua como único agente. La fracción rica en

carotenoides experimentó una reducción en el contenido de azúcares del 90,7 % a través de los ciclos, por lo que se incrementó considerablemente su pureza. La fracción rica en azúcares alcanzó una concentración de 84,83 g/L y la rica en carotenoides un contenido en carotenoides totales de 4996,4 μg/g. La formulación de carotenoides fue estudiada con goma arábiga a través de secado por spray y liofilización. El estudio demostró que la encapsulación por liofilización, en el caso de este material en particular, no fue efectiva pues durante la congelación tuvo lugar una separación del agente encapsulante y las micropartículas de pulpa. La distribución no homogénea del encapsulante no sólo no protegió al material frente a la degradación, sino que acentuó esta degradación. El encapsulado por spray fue eficiente reduciendo la degradación en un 51,9 % comparado con la degradación del material sin encapsular. La fracción rica en azúcares fue un material interesante de cara a procesos biotecnológicos. Se estudió la viabilidad de la fermentación con microorganismos autóctonos, adicionando bacterias lácticas, y adicionando levaduras. La fermentación con microorganismos autóctonos y con bacterias lácticas dio lugar a la producción de ácido láctico (hasta 9,66 ± 0,51 g/L), mientras que la fermentación con levaduras produjo etanol (hasta 49,46 ± 0,28 g/L). La fermentación con microorganismos autóctonos y bacterias lácticas sufrió contaminación por levaduras por lo que tuvo lugar una co-producción de etanol. De cara a eliminar este fenómeno, se estudió la adición de 6 % (w/v) NaCl como aditivo al medio y ello permitió que tanto con microorganismos autóctonos como con bacterias lácticas se produjera ácido láctico como único producto. De cara a mejorar el proceso y operar a una mayor escala, se vio necesario llevar a cabo un control del pH para conseguir aumentar el consumo de sustrato en las fermentaciones a ácido láctico, y de esta manera también aumentar la producción de ácido láctico.

4.4. Capítulo 4

En la valorización de la pulpa se extrajeron los biopolímeros (hemicelulosas y pectinas) hidrotermalmente a 140, 160 y 180 °C. Los tres extractos fueron caracterizados químicamente y en función de su distribución de peso molecular, confirmando la necesidad de un tratamiento de acondicionamiento que se llevó a cabo a través de ultrafiltración y múltiples ciclos de diafiltración con membranas. Se empleó una configuración en cascada (30-10-5-1 kDa) con los extractos de 140 y 160 °C, y una

configuración mixta (5-10-1 kDa) con el extracto de 180 °C. El tratamiento permitió aumentar la concentración de ciertos grupos de biopolímeros en un factor de hasta 5 en la configuración en cascada y en un factor de hasta 16,7 en la configuración mixta. Los ciclos de diafiltración fueron clave en la purificación, logrando retirar un 98,9-99,5 % de los azúcares libres y un 94,4-99,2 % de los subproductos. La pureza de las fracciones obtenidas se encontró entre 73,1-100 % mientras que la pureza de partida de los extractos fue de entre 30,12-33,51 %. Las fracciones purificadas tuvieron pesos moleculares medios desde 2,59 kDa hasta 102,75 kDa. La mayor definición de las fracciones hizo que las polidispersiones finales estuvieron en el rango 1,2-4,0 frente a las polidispersiones de la alimentación entre 16,2-31,6. Las fracciones de mayor peso molecular fueron más resistentes a degradación térmica de acuerdo con los análisis termogravimétricos. Se considera pues que el valor de los parámetros obtenidos fue muy adecuado para la transformación de los biopolímeros en productos de alto valor añadido.

4.5. Capítulo 5

En vista de los resultados obtenidos, se decidió valorizar la pulpa a una mayor escala de cara a obtener fracciones en cantidad suficiente para su transformación en productos. En el proceso de extracción se comenzó con un único reactor y después se fueron incorporando nuevos reactores. Cada reactor operó un tiempo dado en función de la temperatura, pasando a desactivarse después y a introducirse un nuevo reactor en la secuencia. Hasta 3 reactores estuvieron operando en serie al mismo tiempo, y en total operaron 7 reactores en cada experimento. El proceso a gran escala se llevó a cabo a 140 y 180 °C, y se pudieron procesar unos 11,32 kg de zanahorias descartadas en cada experimento. Debido al eficiente modo de operación, los rendimientos de extracción fueron en general superiores a los obtenidos con un solo reactor, con extracciones de hasta 379,51 g/kg pulpa seca (azúcares libres), 81,01 g/kg pulpa seca (hemicelulosa arabinogalactano) y 5,35 g/kg pulpa seca (pectina homogalacturonano). De cada experimento se recuperó la pulpa residual para posteriores aplicaciones. Los extractos hidrotermales fueron recuperados y tratados mediante varias ultrafiltraciones y ciclos de diafiltración. Los ciclos de diafiltración fueron eficientes pues permitieron retirar el 100 % de los azúcares libres y subproductos de la extracción a 140 °C y el 98,7 % de los

azúcares libres y 99,8 % de los subproductos de la extracción a 180 °C. Se obtuvieron un total de cinco fracciones purificadas que abarcaron un amplio rango de pesos moleculares medios: 3,86 – 5,23 – 9,85 – 67,77 – 80,36 kDa, tuvieron polidispersiones bajas entre 1,3 – 3,9, y purezas altas entre 64,5 – 100 %. Los valores de los parámetros fueron buenos, más teniendo en cuenta que las alimentaciones tuvieron pesos moleculares, polidispersiones y purezas de 14,77 kDa, 19,2 y 22,2 % (140 °C) y 8,08 kDa, 18,2 y 14,9 % (180 °C). El secado de las fracciones purificadas mediante liofilización y spray permitió su almacenamiento para un posterior uso.

4.6. Capítulo 6

Se produjeron films biodegradables a partir de las fracciones purificadas de hemicelulosas y pectinas obtenidas en los Capítulos 4 y 5. Como aditivo en los films se estudiaron las fracciones de pulpa residual resultantes del tratamiento hidrotermal a 140 y 180 °C aplicado a la pulpa de zanahorias descartadas en el Capítulo 5. Las pulpas residuales fueron sometidas a un tratamiento mecánico basado en molienda y homogeneización a alta presión que permitió la obtención de nanofibras de celulosa combinadas con lignina. La pulpa residual del tratamiento a 140 °C aportó la mayor hidrofobicidad a los films, por lo que fue seleccionada para un mayor estudio. Su adición en un porcentaje bajo (0-5 %) resultó beneficiosa disminuyendo la permeabilidad del oxígeno y aumentando la hidrofobicidad, pero empeoró otras propiedades del film. Sin embargo, su adición en mayor contenido (5-25 %) permitió al film recuperar la permeabilidad del vapor de agua y las propiedades tensiles que tenía el film de referencia (sin pulpa residual), teniendo además una alta hidrofobicidad pasando de ángulos de contacto del agua de 79,9° en el film de referencia a 125,8° (25 % pulpa residual). Se estudió la influencia de la composición y del peso molecular (67,77, 80,36, 102,75 kDa) de las fracciones de hemicelulosas y pectinas en las propiedades de los films conteniendo un 1 % de pulpa residual. A mayor peso molecular disminuyó la permeabilidad del oxígeno, aumentó la del vapor de agua y disminuyó la hidrofobicidad. El esfuerzo tensil fue mayor a menor peso molecular y a mayor contenido en pectinas, y la elongación fue mayor a mayor contenido en hemicelulosas. Se obtuvieron films más elásticos a menor peso molecular y mayor presencia de pectinas, y fueron más rígidos a mayor peso molecular y mayor presencia de hemicelulosas. Las propiedades de los films

fueron aceptables para envasado de alimentos, destacando su alta hidrofobicidad y su buena biodegradabilidad gracias a la ausencia de modificaciones químicas y gracias a su origen 100 % de un residuo agroalimentario.

5. Trabajo futuro

Como trabajo futuro se contempla la elaboración de biomateriales a escala industrial en empresas agrícolas que generen residuos vegetales. Los procesos aplicados en la presente tesis son respetuosos con el medioambiente y prácticamente no requieren de modificaciones químicas por lo que no implicarían problemas medioambientales a las empresas y podrían ser sencillos de implementar. El hecho de que bioplásticos fabricadas a partir de residuos alimentarios puedas ser empleados en el envasado alimentario es una gran apuesta desde el punto de vista medioambiental y económico en el ámbito de la bioeconomía circular.

Asimismo, puede profundizarse en la elaboración de los films tratando de mejorar sus propiedades mediante la combinación con fracciones de otras biomasas, pudiendo ciertas fracciones aportar propiedades interesantes. Por ejemplo, la fracción obtenida de los carotenoides podría ser adicionada a los films aportando propiedades antioxidantes. Otras fracciones ricas en polifenoles podrían aportar propiedades antibacterianas a los films, muy valoradas en el envasado de alimentos. El estudio de la biodegradabilidad de estos films sería recomendable.

Con las fracciones purificadas de hemicelulosas y pectinas podría estudiarse la obtención de productos alternativos a los films, como por ejemplo los hidrogeles. Estos hidrogeles tendían interesantes aplicaciones en la industria médica, farmacéutica y cosmética.

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About the author

Marta Ramos Andrés (Valladolid, Spain, 1993) started her bachelor's and master's degree studies in chemical engineering in 2011 at the University of Valladolid, graduating in 2017. During her studies she obtained the extraordinary end-of-degree (2015) and end-of-master's degree (2017) prizes awarded by University of Valladolid. During her bachelor studies she obtained a collaboration grant from the national government that allowed her to do research in biotechnology applied to the transformation of industrial by-products. During his master's studies she did an international stay at the Instituto Superior Tecnico in Lisbon (Portugal) focused on the study of mass transfer in ultrafiltration membranes through simulation using Computational Fluid Dynamics. In 2016 she obtained a grant for doctoral and teaching training from the Spanish government, starting her PhD thesis at the University of Valladolid.

Her doctoral thesis focused on the development of the biorefinery concept applied to two agri-food biomasses abundant in the region, spent coffee grounds and discarded carrots. For the valorization of these materials, Marta performed a chemical characterization that allowed the most appropriate strategy to be applied. Extraction, concentration, separation, purification, drying, encapsulation, and fermentation processes were then applied to achieve the fractionation of the biomasses. The processes used were environmentally friendly and were mostly applied on a pilot scale. The strategy employed allowed full valorization of the discarded carrots without discarding any part of the material. During 2020/21, Marta performed an international doctoral stay at Johan Gadolin Process Chemistry Center at Åbo Akademi University in Turku (Finland), with the aim of producing biodegradable films from the purified biopolymer fractions obtained in Valladolid. The films were the main product of the PhD thesis, characterized by the fact that they are 100% biomass-based and do not require chemical modifications for their formation. These films would be good candidates as substitutes for single-use plastics in food packaging. Other products obtained from the thesis were spent coffee oil, bioethanol, lactic acid, and encapsulated carotenoids.

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TEACHING EXPERIENCE

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- Environmental and Process Technology (Bachelor in Electronic and Automatic Engineering, first course).
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